CBER CLINICAL REVIEW OF STUDIES SUBMITTED IN SUPPORT OF LICENSURE OF PROQUAD™

1 PROQUAD: General Information

1.1 Medical Officers' Review

1.1.1	**BLA #:**	STN 125108
1.1.2	**Related IND #:**	IND 7068
1.1.3	**Reviewer Name and Division**:	Judy Beeler, M.D., DVP Philip Krause, M.D. DVP
1.1.4	**Submission Received by FDA**:	August 31, 2004
1.1.5	**Review Completed**:	August 26, 2005

1.2 Product

1.2.1	**Proper Name**:	Measles, Mumps, Rubella and Varicella (Oka/Merck) Virus Vaccine Live
1.2.2	**Proposed Trade Name**:	ProQuad™
1.2.3	**Product Formulation**:	0.5 mL per dose
	Vaccine Virus Strains	
	Measles:	Ender's attenuated Edmonston Minimum: 3.0 $\log_{10}$ $TCID_{50}$/dose Maximum:----------
	Mumps:	Jeryl Lynn (B level) Minimum: 4.3 $\log_{10}TCID_{50}$/dose Maximum:----------
	Rubella	Wistar RA 27/3 Minimum: 3.0 $\log_{10}$ $TCID_{50}$/dose Maximum:----------
	Varicella	Oka/Merck Minimum: 3.99 $\log_{10}$ PFU/dose Maximum: ----------
	Adjuvants: Preservatives:	None None
	Cell Substrates:	Measles and mumps strains are grown in chick embryo fibroblast cell culture; rubella vaccine virus is grown in WI-38

		human diploid lung fibroblast cells; varicella vaccine virus is grown in MRC-5 human diploid lung fibroblast cell cultures.
	Media constituents:	Sucrose, < 21 mg Hydrolyzed gelatin, 11 mg Sodium chloride, 2.4 mg Sorbitol, 1.8 mg Monosodium L-glutamate, 0.40 mg Potassium phosphate monobasic, 72 mcg Potassium phosphate dibasic,-- mcg Sodium phosphate dibasic, 0.34 mg Human albumin, 0.31 mg Sodium bicarbonate, 0.17 mg Potassium chloride, 60 mcg Residual MRC-5 cellular DNA and protein Neomycin, <16 mcg Bovine calf serum, 0.5 mcg
	1.2.4 **Chemical Name, Structure**:	Not applicable
1.3	**Applicant:**	Merck and Co. Whitehouse Station, NJ, USA 08889
1.4	**Pharmacological Category:**	Biologic, Vaccine; sterile lyophilized preparation of live attenuated vaccine strains of measles (rubeola), mumps, rubella (German measles) and varicella (chickenpox) viruses.
1.5	**Proposed Indication(s):**	For simultaneous vaccination against measles, mumps, rubella, and varicella in children 12 months to 12 years of age. May be used in children 12 months to 12 years of age if a first or second dose of measles, mumps and/or rubella vaccine is to be administered.
1.6	**Proposed Populations**:	Indicated for use in healthy children 12 months to 12 years of age.
1.7	**Dosage Form**:	Single 0.5 mL dose, lyophilized vaccine stored frozen at –15 C or colder. It is supplied with sterile water for reconstitution.
	Route of Administration:	Subcutaneous injection

1.8 **Important Related Products:**

MMRII™	(Merck and Co.)
VARIVAX™	(Merck and Co.)
Attenuvax	(Merck and Co.)
Mumpsvax	(Merck and Co.)
Rubeovax	(Merck and Co.)
Meruvax	(Merck and Co.)

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3 Executive Summary

In the pre-vaccine era, measles, mumps, rubella, and varicella were common childhood diseases. Although most acute infections with these viruses are not severe, serious complications and deaths have been reported as a consequence of these infections. Measles may cause pneumonia and encephalitis; mumps infections have been associated with aseptic meningitis, deafness and orchitis; rubella infection during pregnancy may cause congenital rubella syndrome in infants of infected mothers and many of these infants suffer from severe mental retardation, deafness, cataracts and cardiac abnormalities. Wild type varicella infection can be associated with bacterial superinfection, pneumonia, encephalitis and Reye's syndrome. Vaccination against measles, mumps, and rubella using monovalent vaccines (first licensed in 1963, 1967, and 1969, respectively) and or trivalent combinations of measles, mumps and rubella vaccine viruses (licensed since 1971) has led to a >99% reduction in these diseases in Finland, Sweden and the US. A modest reduction in the number of varicella cases has also been seen since licensure of monovalent varicella vaccine in 1995 in the US and this difference has been attributed to both lower varicella immunization rates when compared to those achieved for measles, mumps, and rubella containing vaccines as well as lower vaccine efficacy. Merck and Co. has developed a quadrivalent vaccine, ProQuad™ that can be used to vaccinate against these four viruses simultaneously. Use of this vaccine may increase varicella vaccination rates and further decrease varicella disease in the US. Another potential benefit of using the quadrivalent combination vaccine is a reduction in injection site reactions as one injection is used instead of two.

3.1 Summary of Clinical Findings

3.1.1 Brief Overview of Clinical Program:

The principal objective was to demonstrate that ProQuad™ was as immunogenic as concomitant administration with MMRII™ and VARIVAX™ in children 12 months of age and older up to 12 years of age and that immunization with ProQuad™ was generally well tolerated when compared to the reactogenicity profile seen after concomitant immunization with the component licensed vaccines.

Five clinical trials using an early formulation of ProQuad™ were conducted in more than 1600 children in the 1980's and early 1990's. These studies indicated that ProQuad was generally well tolerated. Likewise, immune responses to the measles, mumps, and rubella components were comparable to those seen after immunization with MMRII™, however, the immune responses to the varicella component were sub-optimal. The poor varicella antibody responses seen in children immunized with the quadrivalent vaccine were thought to be due to viral interference from the measles component. Subsequently, ProQuad™ was formulated to contain varicella virus at higher potencies and clinical trials performed to determine if one or two doses of these formulations could elicit antibody responses that were similar to those seen following immunization with MMRII™ and VARIVAX™ given concurrently but at separate injection sites.

The clinical program to support licensure of ProQuad™ consisted of 5 randomized controlled clinical trials conducted in the United States. These studies included:

Table 3.1. Summary of Clinical Studies Submitted in Support of ProQuad Licensure

Study 009:	Pilot Study (Proof of Concept): A study to compare the safety, tolerability and immunogenicity of one versus two doses of ProQuad™ formulated to contain varicella virus at a higher potency (4.81 pfu log10 varicella virus/dose) with concomitant administration of MMRII™ and VARIVAX™ in healthy children.
Study 011:	Dose-Ranging/Dose-Selection Study: A dose ranging study in healthy children comparing immunogenicity of three formulations of ProQuad™ containing high (4.25 log10 pfu/dose), medium (3.97 log10 pfu/dose) and low (3.48 log10 pfu/dose) doses of varicella virus to MMRII™ given concomitantly with Varicella Vaccine manufactured by a similar method.
Study 012:	Lot-to-Lot Consistency Study: Multicenter study comparing safety and tolerability of three lots of Proquad™ with MMRII™ and VARIVAX™ in healthy children.
Study 013:	Concomitant Vaccination Study: Multicenter study comparing safety tolerability and immunogenicity of ProQuad given concomitantly with Tripedia and Comvax vs. sequentially with ProQuad™ followed by Tripedia and Comvax.
Study 014:	Immunogencity of ProQuad™ in healthy children 4 to 6 years old.

Table 3.2. Summary of Enrollment by Study and Vaccine Group (One Dose)

Study	**Ages**	**Number of Participants Vaccinated**			
		ProQuad™	ProQuad™ >3.9 pfu log10/dose	MMRII™ + VARIVAX™ *	MMRII™
009	12-23 months	323	323	157	NA
011	11-23 months	1161	774	390	NA
012	12-23 months	2915	2915	1012	NA
013	11-16 months	1434	1434	479	NA
Subtotal	11-23 months	**5833**	**5446**	**2038**	
014	4-6 years	399	399	195	205
Totals		**6232**	**5845**	**2233**	**205**

*The FDA approved changes in the process for manufacturing VARIVAX during the study period (the new vaccine was called Process Upgrade Varicella Vaccine, or PUVV). . ProQuad was compared with currently licensed vaccine in each of these studies

Table 3.3. Studies Evaluating Two Doses of ProQuad™ Given at Least 3 Months Apart

Study	Ages	Number vaccinated	
		ProQuad™ 2 doses	**ProQuad™ Two doses >3.97 pfu log_{10}/dose**
009	12-22 months	310	310
011	11-23 months	1161	725
012	12-23 months	0	0
013	11-16 months	0	0
Subtotal	11-23 months	**1471**	**1035**
014	4-6 years	0	0
Totals		**1471**	**1035**

3.1.2 Efficacy

Clinical efficacy of measles, mumps, rubella, and varicella vaccine strains were shown previously in a series of clinical trials using each monovalent vaccine. No formal clinical efficacy trial was conducted with ProQuad™. Efficacy of ProQuad™ was inferred using immunological assays to demonstrate that vaccine specific antibody responses for each vaccine antigen (measles, mumps, rubella, and varicella) following a single dose of ProQuad™ were not inferior to those seen following immunization with licensed MMRII™ and VARIVAX™ given concurrently at separate sites.

Statistical analyses of vaccine immunogenicity was assessed in the per protocol population seronegative for antibody pre-vaccination for each respective vaccine antigen. Immunogenicity was evaluated using virus specific ELISAs developed using viruses that were wild-type or low-passage strains or using virus antigens derived from these strains. The assays were designed and validated at Merck Research Laboratories. The cut-offs for each assay were chosen to detect levels of antibodies that had been previously associated with protection in other studies. For example, a positive measles seroresponse correlated with $\geq$207.5 milli-International Units per milliliter (mIU/mL) (in studies 009 and 011) or with $\geq$120mIU/mL (in studies 012, 013 and 014) of measles antibody. An international standard for mumps antibody does not exist. A positive mumps seroresponse correlated with $\geq$ 10 mumps ELISA antibody units/mL. A positive rubella seroresponse correlated with $\geq$10 IU rubella antibody/mL, while a positive varicella seroresponse correlated with $\geq$ 5 gpELISA units/mL in individuals who had <1.25 gpELISA units/mL antibody pre-vaccination.

Following a single dose of ProQuad™ (N=5446), the vaccine response rates in seronegative individuals were 97.4% for measles, 95.8 to 98.8% for mumps, 98.5% for rubella and 91.2% for varicella. Children in study 012 who responded to ProQuad™ were also followed for one year after vaccination to assess

antibody persistence as well as rates of clinical cases of measles, mumps, rubella, and varicella reported post-vaccination. The results indicated that antibody persisted for at least one year against all 4 antigens in >96% of responders; measles antibody was present in 99.1%, mumps antibody in 96.2%, rubella antibody in 99.5% and varicella antibody detected in 97.5% at one year post-immunization. Also, in the year after immunization in this study there were no cases of measles, mumps, or rubella reported in children immunized with ProQuad™ or with MMRII™ and VARIVAX™. Fourteen of 2497 or 0.6% of individuals immunized with ProQuad™ reported chickenpox while 0.7% or 6/858 of those immunized with MMRII™ plus VARIVAX™ reported breakthrough cases. In both vaccine groups the cases of chickenpox were mild with fewer than 50 varicella lesions per case.

ProQuad™ was also used to immunize children 4 to 6 years of age (N=399) who had been previously immunized with MMRII™ and VARIVAX™ and immunogenicity compared to that seen after either a second dose of MMRII™ plus placebo or a second dose of MMRII™ plus VARIVAX™ given concomitantly at separate sites (Study 014). Seroprevalence rates after a second dose of ProQuad™ were 99.4% for measles, 99.9% for mumps, 98.3% for rubella and 99.4% for varicella. The geometric mean titers (GMTs) for measles, mumps and rubella antibodies were increased about 2-fold, 6 weeks after vaccination in all groups. Following ProQuad™ immunization, varicella antibody GMTs were increased about 13-fold over the pre-vaccination titer while varicella responses in the group immunized with a second dose of VARIVAX™ increased about 8.7-fold. This study indicated that ProQuad™ might be used in place of MMRII™ and VARIVAX™ if a second dose of measles, mumps and rubella containing vaccine is to be administered.

Using ProQuad™ formulated to contain varicella virus at a potency of >3.97pfu log10/dose, the above 5 randomized, controlled clinical trials demonstrated that measles, mumps, rubella and varicella antibody responses following ProQuad™ immunization were similar to those seen following immunization with MMRII™ and VARIVAX™ given concomitantly at separate sites based on statistical tests of non-inferiority. Antibody responses were evaluated in the following ways: First, vaccine specific response rates were compared (studies 011, 012, 013 and 014) using a 5 percentage point margin for the difference in measles, mumps and rubella responses rates and a 10 percentage margin for varicella (because varicella antibody responses are more variable). In study 009 similar comparisons were made but, as it was a pilot study, wider margins (10% for measles, mumps and rubella and 15% for varicella) were allowed. Second, geometric mean titers for measles, mumps, rubella and varicella antibody responses were compared (studies 011, 012, 013 and 014). In studies 011, 012 and 014, a < 1.5-fold difference was allowed for comparisons of GMTs between ProQuad™ immunized vs. MMRII™ and VARIVAX™ immunized children for each vaccine antigen while in study 013, a 2-fold difference in GMTs was allowed. Third, the antibody seroresponse rates for each vaccine antigen were evaluated to see if they met the following acceptability criteria: seroresponse rates had to be at least ≥90% for measles, mumps, and rubella and ≥76% for varicella (studies 012 and 013). These acceptance criteria are consistent with the

minimum seroresponse rates historically determined during the original licensure of monovalent vaccines for measles, mumps, rubella, and varicella.

ProQuad™ was not evaluated in children 6 years to 12 years of age, however, there is no reason to believe that immune responses or reactogenicity seen in healthy children 6 to 12 years old would differ significantly from those seen following a first dose of vaccine in children 12 to 23 months of age or following a second dose of a measles, mumps, rubella and varicella containing vaccine given between 4 to 6 years of age.

3.1.3 Safety

Safety data for ProQuad™ is derived from the 4 randomized clinical trials in 12 to 23 month old infants given a primary dose of vaccine and from 1 study in children 4 to 6 years of age who had been previously immunized with MMRII™ and VARIVAX™.

The numbers of subjects immunized by treatment groups and by study are listed in Table 1 above.

Safety data for 4497 children 12 to 23 months of age given ProQuad™ containing varicella virus potencies >3.97 log10 PFU/ dose (the minimum clinically acceptable dose) were compared to the safety data for 2038 children immunized with MMRII™ and VARIVAX™. The rates of overall clinical adverse experiences, injection site reactions, systemic clinical adverse experiences, and serious clinical experiences were compared.

Safety follow-up was obtained for ~98% in both groups. The percent of subjects reporting one or more clinical adverse experiences was comparable between ProQuad™ recipients and children immunized with MMRII™ and VARIVAX™ (81.5% and 79.6%, respectively).

The number reporting injection site reactions (pain/tenderness/soreness, erythema, swelling, rash at the injection site) was significantly lower for ProQuad™ recipients that for children immunized with MMRII™ and VARIVAX™ (31.3% versus 34.6%, respectively). However, erythema occurred significantly more frequently at ProQuad™ injection sites than at the VARIVAX™ injection site (14.5% versus 12.4%, respectively). Rashes were also reported more frequently at the ProQuad™ injection site than at either the MMRII™ injection site or the VARIVAX injection site (2.4% versus 0.5% and 1.4% respectively). The rates of erythema and rash seen following ProQuad™ immunization are within the rates reported for VARIVAX previously, and all differences seen in injection site reactions in the pivotal studies comparing ProQuad™ with MMRII™ and VARIVAX™ were small.

The number of children reporting systemic adverse experiences following vaccination with ProQuad™ was higher than the number reporting after MMRII™ and VARIVAX™ (76.1% versus 72.3%, respectively). The only systemic clinical

adverse experiences that that were reported at a higher rate in ProQuad™ recipients were fever (37.2% versus 31.5%, respectively) upper respiratory tract infection (23.5% versus 20.7%), and measles-like rash (3.2% versus 2.2%, respectively). The onset of upper respiratory tract infections reported after immunization in both vaccine groups was randomly distributed over the 42 days after immunization and did not cluster around the time of measles-like rash. The difference seen in the reporting rates in each vaccine group for upper respiratory tract infections when data across the studies were combined was small and not significantly different. Fever and measles-like rashes were the only systemic AEs occurring significantly more frequently in ProQuad™ recipients than in children immunized with MMRII™ and VARIVAX™. Most ProQuad™ related fevers were of short duration (mean of 1.7 days) and 61% of the fevers reported were judged to be mild. Measles-like rashes occurred at a statistically higher rate after ProQuad™ immunization than after MMRII™ and VARIVAX™ but the rates in both groups was low (3.0% versus 2.1%, respectively). The duration of rashes was similar in both groups (5 to 6 days) and ~58% of the rashes in each group were described as mild. Varicella like rashes occurred with similar frequency in both groups, i.e., in 2.4% of those immunized with ProQuad™ and in 2.5% of those immunized with MMRII™ and VARIVAX™.

High fever in children in this age group is a risk factor for febrile seizures. The rate of febrile seizures in ProQuad™ recipients over the 4 studies in children 12 to 23 months old was comparable to the rate seen after MMRII™ and VARIVAX™ immunization (0.2% versus 0.4%, respectively), however, the studies were not designed or powered to detect an increase in the frequency of this low frequency adverse reaction (see Post-marketing Studies, Section 13.2).

Following a second dose of ProQuad™ in children 12 to 23 months of age the rate of reporting of clinical adverse reactions was decreased compared to reporting rates after Dose 1 with 16.1% reporting injection site reactions and 65.5% reporting systemic clinical adverse reactions. Fevers were reported in 26.3% and measles-like rash in 0.7%.

Following ProQuad™ immunization at 4 to 6 years of age in children previously immunized with MMRII™ and VARIVAX™, the rates of reporting of clinical adverse reactions were similar to those seen after a second dose of MMRII™ and VARIVAX™. Following ProQuad™ plus placebo immunization, 56.2% reported injection site reactions while 51.3% reported injection site reactions after MMRII™ and VARIVAX™. Similarly, 54.7% reported one or more systemic adverse reactions after ProQuad™ while 59.1% reported systemic AEs after MMRII™ and VARIVAX™.

3.1.4 Dosing Regimen and Administration

A single 0.5-mL dose is recommended for healthy children 12 months through 12 years of age. The vaccine is lyophilized and stored frozen. It is reconstituted with sterile water that is supplied in a separate vial with the vaccine; reconstituted vaccine is used immediately. ProQuad™ is administered by subcutaneous injection.

In the pivotal studies submitted in support of licensure, immunogenicity and safety of a single dose of ProQuad was evaluated in children 12 months to 23 months of age (N=5833) by assessing antibody responses 6 weeks after vaccination.

ProQuad™ was also evaluated in children 4 to 6 years of age (N=399) previously immunized with MMRII™ and VARIVAX™. Based on this study, ProQuad™ may be used instead of MMRII™ if a second dose of measles, mumps and rubella vaccine is to be administered.

Although the varicella gpELISA GMT following ProQuad™ administration in Study 014 was 1.49 fold higher than the gpELISA GMT in the children immunized with two doses of MMRII™ + VARIVAX™, there has not been a direct comparison of varicella immune responses following two doses of ProQuad™ with responses seen in children immunized with two doses of VARIVAX™ using the same interval between immunizations. For this reason, ProQuad™ is not currently indicated as a substitute for VARIVAX™ if a second dose of varicella vaccine is to be administered (see Post-marketing Studies, Section 13.2).

The rationale for the minimum and maximum potencies for each vaccine strain included in ProQuad™ is discussed in the Review of the Manufacture of ProQuad™ (see Labeling Potency Rationale, Section IV.C, page 71). The minimum or end expiry potency for measles, mumps and rubella are the same as the minimum potencies used for MMRII™ and these potencies were determined in previous clinical trials. The titers used reflect 2 to 500-fold increase over the human ID_{100} dose [infectious dose inducing seroconversion in 100% of naïve individuals] identified in dose ranging studies performed with the monovalent vaccines. For example, the ID_{100} dose for Enders-Edmonston measles vaccine was 20 $TCID_{50}$/dose; the ID_{100} dose for Jeryl-Lynn was 317 $TCID_{50}$/dose and the ID_{100} dose for RA 27/3 rubella vaccine virus was ~500 $TCID_{50}$/dose. Maximum potencies for measles, mumps and rubella were determined based on a review of reactogenicity data seen with the use of licensed vaccines of various potencies in 8 clinical trials and a review of WAES adverse experience data compiled over several years and reflect the highest maximum titers released. There is an additional theoretical concern for measles virus vaccines. Previously, Schwarz and Edmonston Zagreb measles vaccines used at potencies greater than 4.5 log_{10} $TCID_{50}$/dose were associated with increased mortality in vaccinated girls. For this reason, the maximum dose for measles vaccine at release was set at -- log_{10} $TCID_{50}$/dose. The minimum or end expiry potency of the varicella component of ProQuad™ was determined in Study 011 and reflects the ID_{76} dose; the maximum potency for varicella vaccine reflects the highest potency of ProQuad™ used in a pivotal efficacy study (Study 009, --- log_{10} PFU/dose).

3.1.5 Drug-Drug Interactions:

At least one month should elapse between administration of measles, mumps, and rubella containing vaccines and ProQuad™ administration because there is a concern that serum interferons elicited in response to the first vaccine may inhibit response to a live virus vaccine given later.

Likewise, at least 3 months should elapse between doses of varicella containing vaccines including ProQuad™ when it is used as the first and/or second dose in a series.

Immunoglobulins may also interfere with the immune response to live virus vaccines and may inhibit the immune response to ProQuad™ if administered concomitantly. Vaccination should be deferred for 3 months or more following blood or plasma transfusions or administration of immune globulins. If Varicella Zoster Immune Globulin, VZIG, is administered, then at least 5 months should elapse before immunizing with ProQuad™. Immunoglobulins, including VZIG, should not be given for 1 month after ProQuad™ immunization unless the benefit of its use outweighs the benefit of ProQuad™ immunization.

Reye's syndrome has been reported following the use of salicylates during wild-type varicella infection. ProQuad™ recipients should avoid the use of salicylates for 6 weeks after ProQuad™ immunization.

ProQuad™ may be used in individuals using topical steroids, low dose corticosteroids for asthma prophylaxis or replacement therapy for Addison's disease. ProQuad™ should not be given to individuals receiving immunosuppressive doses of corticosteroids or other immunosuppressive drugs.

Measles, mumps and rubella vaccines may depress tuberculin skin sensitivity for 4 to 6 weeks after immunization and it is likely that ProQuad™ may do the same. If a tuberculin test is to be done, it should be administered simultaneously with ProQuad™ or 4 to 6 weeks later.

In Study 013, immunogenicity and safety of ProQuad™ given concomitantly with Tripedia and COMVAX was compared to that seen following immunization in 2 control groups of children: 1) those immunized with ProQuad™ followed by immunization with Tripedia and COMVAX 42 days later or, 2) children immunized with MMRII™ plus VARIVAX™ followed by COMVAX and Tripedia 42 days later. Immune responses to ProQuad™ antigens were similar in all groups indicating the concomitant immunization with Comvax and Tripedia did not interfere with the antibody responses to measles, mumps, rubella, or varicella. Likewise, the antibody responses to each of the antigens in COMVAX (*Hemophilus influenza* type b polyribosyl phosphate, PRP, and Hepatitis B surface antigen) were similar in the concomitant and non-concomitant groups. Antibody responses to tetanus and diphtheria antigens in Tripedia vaccine were also similar between groups. In contrast, antibody responses to the two pertussis antigens in Tripedia, filamentous hemagglutinin and pertactin, were more than 15 percentage points

lower in children given vaccines concomitantly. Because of this failure, a conclusion of a similar immune response for the concomitant group compare to the nonconcomitant group could not be made. Based on this study, ProQuad™ and COMVAX may be administered concomitantly however ProQuad™ and Tripedia may not.

3.1.6 Special Populations:

ProQuad™ has not been evaluated for use in special populations. It is contraindicated in individuals with known hypersensitivity to any of the components (with exceptions permitted for some individuals with egg allergy and for those with contact hypersensitivity to neomycin). Persons with a history of immediate hypersensitivity reactions subsequent to egg ingestion may be at enhanced risk of immediate-type hypersensitivity reactions after receiving vaccines containing traces of chick embryo antigen. Such individuals may be vaccinated with extreme caution and adequate treatment should be readily available should a reaction occur. Children with egg allergy are at low risk for anaphylactic reactions to measles and mumps containing vaccines and skin testing is not predictive of reactions to MMRII™.

ProQuad™ is contraindicated in those who are immunosuppressed due to disease or medications including but not limited to leukemia, lymphoma, blood dyscrasias, or neoplasms affecting the bone marrow or lymphatic system, as well as those with primary and acquired immunodeficiency states. ProQuad™ is not indicated for use in children infected with HIV. ProQuad™ should not be administered to children with a family history of immunodeficiency until the immune status to the recipient is known.

ProQuad™ is contraindicated in individuals with active untreated tuberculosis. ProQuad™ is not indicated for use in individuals 13 years of age or older. It is not indicated for use in females in the childbearing age group and is not indicated for use in pregnant females or for females in the postpartum period. ProQuad™ has not been evaluated in the elderly and has not been used in studies for the prevention of shingles.

ProQuad™ should not be administered to individuals who have contact with high-risk populations such as immunocompromised individuals, varicella susceptible pregnant women or newborns of mothers who are varicella susceptible due to the possibility of transmission of varicella vaccine virus to these individuals.

3.1.7 Conclusions and Recommendations

The data support the use of a single dose of ProQuad™ for immunization of healthy children 12 month to 12 years of age in place of MMRII and VARIVAX.

ProQuad™ may be used in healthy children 12 months through 12 years of age if a second dose of measles, mumps and rubella containing vaccine is to be administered.

The rate of elevated fevers ≥ 102 F is significantly higher in ProQuad™ recipients than in children immunized with MMRII™ and VARIVAX™ (37.3% versus 31.6%, respectively) but the fevers are generally mild and of short duration.

Measles-like rashes were also reported significantly more frequently after ProQuad™ immunization (3.2%) than after MMRII™ and VARIVAX™ (2.2%).

The majority of fevers and measles-like rashes occurred 5 to 12 days after immunization coincident with the majority of fevers and rashes occurring after MMRII™ and VARIVAX™.

Post-marketing studies will address the following:

Additional studies may be performed to provide data in support of concomitant immunization with other vaccines:

1. Safety and immunogenicity of ProQuad™ given concomitantly with Prevnar.
2. Safety and immunogenicity of ProQuad™ given concomitantly with VAQTA™.
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4 Significant Findings from Other Review Disciplines

4.1 **Statistical Review**:

See Dr. Sang Ahnn's review of the statistical analyses used to evaluate immunogenicity and safety data obtained from the clinical studies submitted in support of ProQuad licensure.

4.2 **Chemistry Manufacturing Controls** (CMC):

See Mr. Steven Rubin's review for details of ProQuad™ manufacture and testing.

4.3 **Animal Pharmacology/Toxicology**:

ProQuad™ has not been evaluated in animal toxicology tests. Validated animals models of human disease do not exist for measles, mumps, rubella or varicella viruses, hence animal pharmacology studies have not been performed.

Measles, mumps and varicella seed viruses have previously been tested in monkey neurovirulence tests and were found to be not virulent. These results were reviewed previously and were not re-reviewed as part of this submission.

5 Clinical and Regulatory Background

5.1 Diseases Studied and Available Interventions

Measles is caused by a paramyxovirus of the genus *Morbillivirus* and is transmitted from person-to-person by aerosolized infectious droplets. The clinical presentation is characterized by prodromal fever, conjunctivitis, coryza, cough, and Koplik spots. Subsequently, a maculopapular rash often appears, spreads from the head to the entire body and fades within 4 to 7 days. Measles can result in otitis media, pneumonia, encephalitis, and death. Human IG (immunoglobulin) may be used for passive immunoprophylaxis in high-risk populations. There are no drugs or anti-viral agents approved for the treatment of measles infection.

Mumps is caused by a paramyxovirus of the genus *Rubulavirus* and is spread via the respiratory route. The clinical presentation is characterized by swelling of one or more salivary glands, which may be preceded by nonspecific symptoms, including fever, lymphadenopathy, headache, malaise, myalgia, and anorexia. Mumps can result in deafness, orchitis, pancreatitis, meningitis, encephalitis, and death. No alternative therapies are available.

Rubella is caused by a togavirus of the genus *Rubivirus* and is spread via infectious droplets shed from the respiratory secretions of infected individuals to susceptible individuals. Nonspecific signs and symptoms including transient erythematous and sometimes pruritic rash, postauricular or suboccipital lymphadenopathy, and low-grade fever characterize the clinical presentation. The most important consequences of rubella are the miscarriages, stillbirths, fetal anomalies, and therapeutic abortions, associated with Congenital Rubella Syndrome (CRS). No alternative therapies are available.

Varicella is caused by varicella-zoster virus (VZV), a herpes virus. The clinical presentation of varicella is characterized by fever, malaise, and a generalized rash. The rash is usually pruritic and consists of 300 to 500 maculopapular lesions that progress to vesicles, and crust over the course of several days. The skin lesions are generally concentrated on the face, head, and trunk. Varicella may be associated with serious and life-threatening complications including bacterial superinfection of skin lesions with S*taphylococcus aureus or Streptococcus pyogenes,* viral or bacterial pneumonia, septic shock, secondary bacterial arthritis, fasciitis, cerebella ataxia, and encephalitis. Varicella zoster immunoglobulin may be used in high-risk individuals to provide pre and /or post exposure prophylaxis. Severe varicella infection may be treated with acyclovir.

Monovalent measles vaccine was introduced in the United States (U.S.) in 1963; a combined measles, mumps, and rubella vaccine (M-M-R™) was introduced in 1971. M-M-R™II, which contains a different strain of rubella vaccine virus, RA27/3, was introduced in 1979 and is currently the only licensed measles, mumps, and rubella vaccine in the United States and it is also licensed in some other countries. The vaccine is generally well tolerated, immunogenic, and highly efficacious. The vaccine has been shown to be highly effective in reducing the incidence of measles, mumps, and rubella in the United States and other countries. VARIVAX™ was introduced in the United States in 1995 and in 22 other countries over the past 9 years. The vaccine has been shown to

be generally well tolerated, immunogenic, and efficacious. Routine use of VARIVAX™ in the United States has resulted in a substantial reduction in the overall incidence of varicella.

5.2 Important Information from Related INDs and BLAs/NDAs, Including Marketed Products

Quadrivalent measles, mumps, rubella and varicella vaccines were evaluated under IND 7068.

INDs for MMRII™:	IND ----	STN101069-5061
	IND -----	STN101069-5068
INDs for Varivax™:	IND ---	STN 103552-5079
INDs for Zoster vaccine:	IND ---	

Merck manufactures the following US licensed vaccines that contain the identical measles, mumps, rubella and/or varicella vaccine strains as are found in ProQuad:

MMRII™	(Merck and Co.)
VARIVAX™	(Merck and Co.)
Attenuvax	(Merck and Co.)
Mumpsvax	(Merck and Co.)
Rubeovax	(Merck and Co.)
Meruvax	(Merck and Co)

Since licensure of MMRII™ in 1978 more than ---------- doses have been distributed worldwide.

Since licensure of VARIVAX™ in 1995, over ---------- doses have been distributed worldwide.

5.3 Previous Human Experience with the Product or Related Products/Foreign Experience:

ProQuad™ has not been licensed previously. It is not licensed outside of the United States.

5.4 **Regulatory Background Information** (FDA-Sponsor Meetings, Advisory Committee Meetings, Commitments)

Listed below is the history of regulatory communications between the FDA and Merck for IND 7068 that directly relate to this BLA. Dr. Herbert Smith, Chair of the BLA Committee and Regulatory Reviewer for IND 7068, prepared this summary. The referenced documents include Merck submissions to the IND or BLA, records of FDA and Sponsor meetings, telephone conference call memorandums, and specific commitments agreed on by FDA and Merck.

March 18, 1997	IND Acknowledgement of Receipt Letter Original Submission dated February 28, 1997, and Received on March 3, 1997
June 6, 1997	Telephone Conference Call Memorandum In this telephone conference call Merck committed to conduct Protocol 008 with ProQuad wherein ---------- was used for ---------- to prepare the varicella component.
May 8, 1998	CBER Letter Advice and information request related to Protocol 009. CBER requested that Merck provide information to support a second dose of MMRII vaccine prior to the usual booster dose at 4 - 6 years. CBER also requested information for the ELISA assays used to assess serological responses to ProQuad. CBER also requested that Merck used statistically validated methods to demonstrate no significant differences between the distribution of the titers obtained for each of the components of MMRII and VARIVAX and each of the components of ProQuad.
November 12, 1998	Telephone Conference Call Memorandum This telephone conference call with the sponsor dealt with statistical methods used to demonstrate non-inferiority of ProQuad vs. MMRII and VARIVAX. CBER concurred with the proposed methodology.
October 26, 1999	CBER Letter This letter requested that Merck address the use of the ELISA to assess mumps seroconversion rates and mean geometric titers. CBER also requested additional information regarding a post-vaccination adverse event.
November 22, 1999	Telephone Conference Call Memorandum Denial of end-of-phase 2/3 meeting due to inadequate documentation for a PDUFA2 meeting.
November 24, 1999	Telephone Conference Call Memorandum Request from sponsor for guidance regarding the submission of a Type B meeting request.
January 21, 2000	Telephone Conference Call Memorandum ----------
January 26, 2000	Meeting End of Phase2/ pre-Phase 3 meeting with Merck to discuss pivotal efficacy studies for licensure.
January 31, 2000	Telephone Conference Call Memorandum Request for 30day review extension. Reference to February 17, 2000 telephone conference call memorandum.
February 17, 2000	Telephone Conference Call Memorandum The telephone conversation memorandum summarized the end-of-Phase 2/pre Phase 3 meeting conducted with Merck on January 26, 2000. This meeting was requested by Merck to discuss their Phase 3 clinical development plans. Merck requested guidance on the following items: **a. Concurrence with overall clinical development plan and design of phase 3 studies.** *CBER recommended 3000 - 5000 subjects with dose and formulation*

	intended for licensure. CBER requested clarification of the proposed indication for the use of ProQuad in children ------ months and in children---- years of age for a second dose of MMRII using ProQuad. CBER indicated that Protocol 014 was inadequate to support this indication. CBER indicated that Merck evaluate ProQuad with each component at release potencies that will exceed the expected potency at end-expiry. Protocol 013 should use ProQuad vaccine with the same release potency as intended for licensure. CBER requested the determination of seroconversion rates and geometric mean antibody titers in each of the proposed studies. CBER requested stratification of baseline serostatus to evaluate vaccine safety. CBER requested that criterion to establish similarity of ProQuad to MMRII and VARIVAX allow no more than 5% difference for measles, mumps and rubella responses and no more than 10% difference for varicella responses. CBER requested that Merck evaluate PBMCs from vaccinated children with rash by RT-PCR to assess measles vaccine replication. CBER requested that Merck evaluate concomitant administration of IPV in the recommended 4-dose schedule for IPV. **b. Concurrence with the total number of subjects for safety and immunogenicity and safety.** *CBER did not concur with study subject number (see above).* **c. Concurrence with the criteria for establishing immunological equivalence between ProQuad and MMRII and VARIVAX.** *CBER concurred with criteria for establishing immunological equivalence between ProQuad vs. MMRII and VARIVAX.* **d. Concurrence with the plan for the determination of the minimum clinically acceptable dose of varicella in ProQuad.** *CBER concurred with the plan for the determination of the minimum clinically acceptable dose of varicella vaccine in ProQuad.* **e. Agreement with the use of the ELISA assay used to measure measles, mumps, and rubella, and the gpELISA to measure responses to varicella.** *CBER requested that Merck utilize a wild-type mumps virus strain in their ELISA assay and provide a correlation to ---------- ----------. CBER requested that Merck provide data to validate each assay.*
March 7, 2000	Telephone Conference Call Memorandum Merck requested guidance related to the initiation of Protocol 012, specifically, information regarding an increase in sample size, and the use of CBER recommended equivalence margins.
June 23, 2000	CBER Letter CBER requested that Merck perform PBRT on the WI-38 Master Cell Bank (used for the production of rubella vaccine). CBER requested that Merck perform polymerase based reverse transcriptase assays (PBRT) on all four (measles, mumps, rubella and varicella) Master Virus Seed Stocks. CBER concurred with Merck's plan to assess adventitious agent contamination of ProQuad vaccine.
June 26, 2000	CBER Letter Comments on the lot consistency Protocol 012.

	1. Dose-ranging study design proposed by Merck imposes a risk that Merck may be unable to demonstrate similarity across lots. 2. CBER is concerned that the potency of lowest varicella dose of vaccine may fall below the specified lower limit over the course of the study. 3. CBER is concerned that the proposed sub-study to collect blood sample days 10 -17 post-vaccination may decrease the ability to obtain 42 day blood samples. 4. CBER requested that subject randomization be stratified by study center. 5. Clarification to avoid lot-by-center confounding. 6. CBER requested that Merck provide the statistical method that will be used to assess similarity of seroconversion rates. 7. CBER agreed that immunogenicity for the varicella component of ProQuad would use a cut-off of ≥5 gpELISA and use 76% response in the initially seronegative population as a measure of acceptability. 8. Procedure to assess possible non-similarity of lots used in the study. 9. CBER concurred with the proposed per protocol analysis. 10. Recommendation to assess covariance with days since vaccination along with lot number and center as co-variables to provide precision in assessment of seroconversion rates and GMTs. 11. CBER requested a description of the method that will be used to determine consistency of safety across clinical consistency lots. 12. CBER provided recommendations regarding the proposed interim study analysis.
June 27, 2000	CBER Letter Merck requested clarification of some of the conclusions from the summary dated February 15, 2000 for the end-of-Phase 2/ pre-Phase 3 meeting conducted on January 26, 2000. 1. CBER concurred with the description of the proposed indication and product profile. 2. Merck indicated their intention to increase study numbers as recommended by CBER. Merck indicated that they intended to use only ProQuad formulated to contain varicella doses >14,500 PFU (4.2 log10) in this study. Merck increased the study subject numbers from 975 to 2550 in protocol 012. 3. Merck also proposed to increase study numbers of subjects 4-6 years of age to 350 in study 014. 4. Merck agreed to release ProQuad formulated to contain mumps vaccine virus to support a minimum end-expiry titer of >4.3 log10 PFU/dose. 5. The primary immunogenicity analysis for varicella responses will consider the ability of immunization to induce titers in previously seronegative individuals of ≥5 gpELISA antibody units/mL. Merck concurred with CBER's request to assess both seroconversion rates and GMTs. Merck also concurred with the proposal for stratification of subjects and analysis of study results based on baseline serostatus. 6. Merck agreed with CBER's recommendations to adjust the statistical criterion for similarity of ProQuad with MMRII and VARIVAX. 7. CBER agreed with Merck's proposal to collect PBMCs from

	vaccinated children with measles like rash in protocols 012 and 013 and to evaluate the samples using RT-PCR. CBER also concurred with Merck's proposal to assess GMTs to measles in children with measles-like rash versus those without rash. 8. Concurrence with proposal to assess concomitant administration of IPV and ProQuad in children 12 – 18 months of age. 9. CBER concurred with Merck's proposal to assess immunological equivalence between immunization with ProQuad vs. MMRII, and VARIVAX. 10. CBER concurred with Merck's proposal to determine the minimum clinically acceptable dose of varicella in ProQuad. 11. CBER concurred with Merck's proposal to use a wild-type mumps strain in the ELISA and to action response if the assay performs differently. Concurrence that CBER will not require the establishment of a correlation between wild-type neutralization assay and the newly developed ELISA assay using a wild-type mumps strain.
July 6, 2000	Telephone Conference Call Memorandum Merck requested clarification of the ------ assay that will be used to assess vaccine master seeds of ProQuad.
July 26, 2000	Telephone Conference Call Memorandum Merck requested clarification of comments communicated in CBER letters dated June 26, and June 27, 2000.
September 5, 2000	CBER Letter CBER provided Merck with clarification of the retrovirus testing requirements discussed in the CBER letter of June 23, 2000.
September 7, 2000	CBER Letter Further questions to Merck pertaining to the ELISAs used to determine antibody responses to measles and rubella.
September 9, 2000	Telephone Conference Call Memorandum Request of correct titer of varicella in ProQuad used in protocol 014. Request for information on retention vials and evaluation of potency or stability at study end. Request for clarification on serological assays for rubella and measles.
November 27, 2000	Record of Internal Meeting Merck intends to submit a BLA application for ProQuad in late fourth quarter of 2001. The vaccine is intended for use in children 12 months of age or older for the indication of primary prevention of measles, mumps, rubella, and varicella. The new combination vaccine will use the existing bulk manufacturing processes for licensed MMRII and VARIVAX. This meeting was requested by Merck to discuss issues relating to the following CMC areas 1) Raw Materials Sourcing, 2) Adventitious Agent Screening, 3) Process Validation, 4) Bulk Stability, 5) Final Product Stability, 6) Mumps Potency, 7) Facility Qualification and Environmental Monitoring. The meeting was facilitated by discussion of specific questions relating to these topics as presented in the pre-meeting materials.

	1. Does CBER concur that the proposed BLA content of available documentation for animal-derived raw materials, as summarized in the background document, meets regulatory requirements for licensure of ProQuad? CBER concurred with the available sourcing documentation of animal-derived raw materials used in the manufacture of measles, mumps, and rubella vaccines. CBER did not concur that sufficient documentation had been provided for animal-derived raw materials used in the manufacture of varicella virus vaccine. Consistent with current policy CBER will publicly disclose the vaccine type, trade name, and manufacturer for any product that utilizes implicated bovine-derived products or bovine-derived materials for which the source country is not known until adequate documentation is received. Merck indicated that they would provide additional documentation for CBER review. **2. Does CBER concur that adventitious agent testing for ProQuad satisfy testing requirements for licensure of ProQuad?** The committee generally agreed with the proposal for adventitious agent testing for the MMRII and varicella vaccines as proposed in the pre-meeting materials. Prior to providing Merck a final decision on the adequacy of the proposed adventitious agent testing CBER requested additional time to review ICH and WHO guidelines to ensure that the adventitious agent characterization proposed for ProQuad is consistent with these guidelines (See, "Requirements for Use of Animal Cells as In Vitro Substrates for the Production of Biologicals" and "Guidance on Quality of Biotechnological /Biological Products: Derivation and Characterization of Cell Substrates Used for the Production of Biotechnological/Biological Products".) Merck agreed to further discussion of this issue with CBER staff to resolve any remaining issues. CBER and Merck agreed to schedule a meeting early next year to discuss ongoing PBRT evaluation of the WI-38 cell bank used for production of rubella virus vaccine, and master seeds for rubella, and varicella vaccines. **3. Does CBER concur that the approach for process validation of the measles, mumps, and rubella bulk vaccine manufacturing processes is adequate for licensure of ProQuad?** CBER generally concurred with the identification of the Critical Process Parameters and Critical Quality Attributes for the processes used to manufacture ProQuad. CBER requested that data be submitted for review that supports the chosen acceptance criteria and product specifications used in the manufacture of ProQuad. CBER indicated that this data could be submitted in summary form but that it should include a statistical analysis with the range of values and a calculation of error. Merck agreed to provide summary data for aseptic processing validation for the most recent qualification of the measles, mumps, rubella, and varicella vaccine manufacturing processes**.**

	4. Does CBER concur that the proposed bulk stability data are sufficient to support licensure of ProQuad? CBER agreed with the conceptual approach to the assessment of bulk stability Merck provided in the pre-meeting materials. **5. Does CBER concur with the current plans for generating stability data for the final formulated product in the BLA? In particular, is the modified stability protocol for generating varicella potency data acceptable, and is the amount of data to be provided adequate to support 18month expiry dating of the product?** CBER agreed with the conceptual approach to the assessment stability for the final formulated product. CBER agreed that the modified stability protocol for generating varicella potency data is acceptable. CBER agreed that the amount of data projected to be available for the BLA submission is acceptable but that the acceptability will depend on the quality of the data collected and availability at the time of submission. Merck agreed to provide the results of reconstitution stability studies in the final container. **6. Does CBER concur with the approach for overcoming the process loss in mumps potency in the final product by increasing the mumps input for ProQuad as necessary to maintain a target release mumps potency of --- $\log_{10}TCID_{50}$ and a minimum release of --- $\log_{10}TCID_{50}$, in order to ensure a minimum claimed mumps potency of 4.3 $\log_{10}TCID_{50}$ per dose at expiry?** CBER stated that the proposed minimum release criteria of --- $\log_{10}TCID_{50}$ for the mumps potency in the final product is actually the upper bound of the 95% confidence interval for the minimum claimed mumps potency at expiry 4.3 $\log_{10}TCID_{50}$ and thus cannot be the minimum release criterion as well. Merck indicated that recent data suggest that this confidence interval is narrower and with an average annual potency loss of ---- log10 per year, they will be able to achieve the proposed expiry titer. CBER requested that the data supporting this claim be submitted for review. **7. Does CBER concur that the proposed BLA content with regard to environmental control monitoring data is sufficient to support licensure of ProQuad? Specifically, does CBER agree that a summary of historical performance data for a period of six months for each department and for each of the approximately --- monitoring sites of the existing classified rooms and utilities associated with ProQuad manufacturing, pooling and filling is sufficient?**

	CBER generally concurs with Merck's proposal for environmental control monitoring.
December 18, 2000	Telephone Conference Call Memorandum This memo summarizes the above pre-BLA meeting with sponsor. Items listed above for November 27, 2000 were communicated to the sponsor.
February 7, 2001	Telephone Conference Call Memorandum - This telephone conference call was requested by Merck to further clarify PBRT requirements for ProQuad product licensure. Merck described their efforts for direct testing of viral seeds for rubella, measles, mumps and varicella. CBER concurred with Merck's approach and indicated that these proposed studies satisfied the requirements identified in the letter issued by CBER regarding ---------- testing. - Merck indicated that co-cultivation studies on the WI-38 WCB would include ----------. The assay will be performed after -- passages and -- weeks of culture followed by ---- testing at the ---------- - CBER requested clarification on whether Merck proposed a direct ---- on varicella vaccine. Merck indicated that the test would be performed on ----------. The testing will include ----------. Varicella is cell associated and the experiment should work out well. - Merck wanted clarification of whether the relevant ICH requirements would satisfy CBER. CBER indicated that the ICH documents did not appear to deviate from CBER recommendations. CBER indicated that the identity testing described in the ICH document would not need to be performed, namely, ----------, and that a different method could be chosen to demonstrate identity. - Merck described -------- test methods for the WI-38 and MRC-5 cells. They will perform -- passages with appropriate positive controls. Merck expects to see millions of --- units. CBER recommended the use of non-cultivated spikes to address this issue. The ---- assay uses ---------- that will enable the detection of 1000 molecules. CBER recommended that Merck have ---------- run a dilution series to titrate ---------- in the ---- assay.

	- Merck wanted to know whether the use of bovine serum was an issue for this product. CBER recommended an evaluation similar to that performed for the rotavirus vaccine. - For the gpELISA cutoff to designate vaccine responders a cutoff of 1.25 pre-vaccination and ≥5.0 gpELISA units/mL post vaccination was considered appropriate by CBER. Merck agreed with CBER recommendations.
August 13, 2001	CBER Letter In this letter CBER provided comments on outstanding issues from the December 18, 2000 meeting with Merck and meeting minutes issued on December 19, 2000. - CBER concurred that sufficient documentation was provided for animal derived raw materials used in the manufacture of VARIVAX. CBER concurred that products in which implicated or unknown sourced materials are limited to Viral Master Seeds or earlier passages, Viral Master Seed will not need to be re-derived due to its extensive clinical and manufacturing experience. CBER indicated that it does not intend to list products for which the unknown sourced materials are limited to Master Seeds or earlier in a publicly available publication or the FDA website. CBER is providing information on request for products in which implicated or unknown sourced materials were used to derive Viral Master Seeds or earlier passages. - CBER noted that Merck ---------- - CBER review of the relevant ICH and WHO guidance documents did not identify additional adventitious agents testing requirements necessary for the BLA submission beyond those currently proposed by Merck and agreed to by CBER. However CBER advised Merck that these guidance documents are updated periodically and that recommendations may change in future revisions. - CBER confirmed that the testing requirements for ingredients of animal origin (9CFR 113.53) used for production of biological products will not be applied to pre-Master seeds used in the

	manufacture of ProQuad. CBER noted that testing for bovine adventitious agents as described in 9CFR 113.53 must be performed on ingredients of animal origin used to establish master and working virus seeds for the manufacture ProQuad, but such testing will not be required for the pre-Master viral seed used for the manufacture of ProQuad due to the extensive clinical and manufacturing experience wit these materials. - CBER commented on specifications and acceptance criteria for the measles, mumps, and rubella manufacturing processes and the summary data for aseptic processing validation for the most recent qualification of the measles, mumps, rubella, and varicella manufacturing processes. - CBER requested that Merck provide historical data to support the declared values for the acceptance criteria for measles harvest titers, the specification for measles target pool titer, the specification for mumps harvest titer, the mumps target pool titer, and indicated that the specified acceptance criteria need to reflect current fill potency. - CBER noted that a single challenge is being performed ------- to represent the measles and mumps manufacturing processes as opposed to one --------- challenge for the mumps process and one ---------- challenge for the measles process. CBER agreed that simulating the measles process for the media challenge represents a worst-case scenario; however, media challenges should be performed in ----------. As the purpose of the -------- media challenge is to re-qualify the aseptic processes that take place subsequent to the final sterilization step, the challenge should capture all elements of these processes, including equipment and personnel. Performing a media challenge in only --------- facilities where these processes take place fails to meet these goals. - CBER noted the submission of final stability protocols in response to a CBER request during the February 7, 2001, telephone conference call. CBER requested clarification of whether reconstitution potencies will be calculated as part of the stability program. - CBER noted the submission of potency and degradation analysis summary tables for minimum release specifications for mumps in ProQuad™ (Frozen) for various expiry times. The stability data include the results from 8 lots of vaccine stored frozen at –15ºC in a frost-free freezer for 12 and -- months. CBER requested that Merck comment on the use of months post date of manufacture to calculate degradation estimates as opposed to the use of time

	under storage at –15ºC. CBER noted that these two time frames may not be equivalent, as the product is stored at –20ºC prior to initiation of the stability study.
September 13, 2001	CBER Letter CBER requested clarifications of Merck proposed revisions to protocol 012 including the definition of the 1year post vaccination interval, revaccination of seronegative subjects, and clarification of testing of measles rash isolation samples by RT-PCR.
February 13, 2002	Telephone Conference Call Memorandum CBER request to revaccinate subjects in protocol 012 that failed to seroconvert after primary vaccination with ProQuad.
March 12, 2002	CBER Letter CBER requested information for a mumps lot below the current release specification. CBER requested that Merck acknowledge that this lot will have a mumps potency of at least 4.3 log10 TCID50 per dose.
March 21, 2002	Telephone Conference Call Memorandum CBER concurred with the use of a --- mIU/ml cutoff value for the measles component of ProQuad as a reasonable and more conservative cutoff than the previously recommended --- mIU/ml. Communicated contents of CBER letter dated May 15, 2002.
May 7, 2002	CBER Letter Special FDAMA 113 Request for Protocols
May 15, 2002	CBER Letter CBER concurred with the use of a --- mIU/ml cutoff value for the measles component of ProQuad as a reasonable and more conservative cutoff than the previously recommended --- mIU/ml. CBER noted that this cutoff should only be applied to future studies and to studies that have been unblinded.
May 12, 2003	Telephone Conference Call Memorandum CBER requested clarification of ELISA to detect rubella antibodies and comparison of the legacy assay and the modified rubella assay.
June 23, 2003	CBER Letter CBER notes Merck's proposal to change measles cutoff from ----- to ---------- mIU/ml as initially described in the February 8, 2002 submission to the IND and in response to the CBER letter of May 15, 2002.
August 11, 2004	CBER Letter CBER requested clarification for the Merck proposal to modify the gpELISA for measurement of varicella antibody
June 2, 2005	CBER Letter CBER provided comments on the proposed post-marketing studies for ProQuad. The proposals for post-marketing studies for ProQuad included a Post-Licensure Evaluation of the Short-Term Safety of ProQuad™, and Protocol 019 entitled, "An Open, Randomized, Multi-center Study of the Safety, Tolerability, and Immunogenicity of ProQuad™ Given Concomitantly with a Fourth Dose of PREVNAR™ and Third Dose of IPOL™ in Healthy Children 12 to 15 Months of Age." The following comments were communicated to Merck: - CBER recommends supplementing the safety database for a

	second dose of ProQuad. A total safety database of 3000 children who received ProQuad as a second dose in the key target group of ages 15-23 months of age is recommended. Please discuss your plans to collect additional safety data in this age group (see also comment 4). This section determined not to be releasable

	Two pages determined not to be releasable

6 Clinical Data Sources, Review Strategy, and Data Integrity

6.1 Material Reviewed

6.1.1 BLA Volume Numbers Which Serve as a Basis for the Clinical Review

We reviewed the following electronic documents:

- STN 125108, original application,
  - Volume 2, Common Technical Document
    - 2.2 Introduction
    - 2.5 Clinical Overview
    - 2. 7 Clinical Summary
      - Summary of Clinical Efficacy, 2.7.3
      - Summary of Clinical Safety, 2.7.4
      - Synopses of Individual Studies, 2.7.6
  - Volume 5, Clinical Study Reports
- Amendment 2, Safety Report
- Amendment 4, Response to CBER request for Clinical Information
- Amendment 9, Analysis of complete dataset for Tripedia serological data, Study 013.
- Amendment 17, information pertaining to Varicella expiry titer
- Amendment 19, additional information pertaining to Financial Disclosures
- Amendment 20, brief synopsis of proposed Post-marketing studies.

6.1.2 Literature Reviewed:

Arbeter AM, Starr SE, Plotkin SA. Varicella vaccine studies in healthy children and adults. Pediatrics 1986; 78, (Suppl):748-56.

Black S, Shinefield H, Ray P, Lewis E, Hansen J, Schwalbe J, et al. Post-marketing evaluation of the safety and effectiveness of varicella vaccine. Pediatr Infect Dis J 1999; 18(12):1041-6.

Kuter BJ, Weibel RE, Guess H, Matthews H, Morton DH, Neff BJ, et al. Oka/Merck varicella vaccine in healthy children: final report of a 2-year efficacy study and 7-year follow-up studies. Vaccine 1991;9(9):643-7.

Li S, Chan ISF, Matthews H, Heyse JF, Chan CY, Kuter BJ, et al. Inverse relationship between six week post-vaccination varicella antibody response to vaccine and likelihood of long term breakthrough infection. Pediatr Infect Dis J 2002; 21(4):337-42.

Shinefield H, Black S, Morozumi P, Froehlich H, Bergen R, Lavetter A, et al. Safety and immunogenicity of concomitant separate administration of MMRII vaccine and VARIVAX (OKA/Merck varicella vaccine) vs. injections of MMRII and VARIVAX given six weeks apart. Pediatric Research 1995; 37, 4 part 2, 188A

Vessey SJR, Chan CY, Kuter BJ, Kaplan KM, Waters M, Kutzler DP, et al. Childhood vaccination against varicella: persistence of antibody, duration of protection, and vaccine efficacy. J Pediatr 2001;139(2):297-304.

Watson B, Piercy S, Soppas D, Browngoehl K, Warner M, Isganitis K, et al. The effect of decreasing amounts of live virus, while antigen content remains constant, on immunogenicity of Oka/Merck varicella vaccine. J Infect Dis 1993;168(6):13

Weibel RE, Neff BJ, Kuter BJ, Guess H, Rothenberger CA, Fitzgerald AJ, et al. Live attentuated varicella virus vaccine: efficacy trial in healthy children. N Engl J Med 1984;310:1409-15.

USA Circular: VARIVAX [Varicella Virus Vaccine Live (Oka/Merck)].: 2001.

Gershon AA, Takahashi M, White CJ. Varicella Vaccine. In: Plotkin SA, Orenstein WA, eds. Vaccines. 3 ed. Philadelphia: W.B. Saunders Company, 1999:475-507.

Seward JF, Watson BM, Peterson CL, Mascola L, Pelosi JW, Zhang JX, et al. Varicella disease after introduction of varicella vaccine in the United States, 1995-2000. JAMA 2002;287(5):606-11.

Centers for Disease Control and Prevention. Decline in annual incidence of varicella—selected states, 1990-2001. MMWR 2003; 52(37):884-5.

Memo to Ukwu H from Morsy M: BB-IND 7068: MMRV Pre-phase III meeting minutes received from CBER 2-15-00, 16-Feb-2000.

Wharton M, Fehrs L, Cochi SL, Stroup N. Health impact of varicella in the 1980's [Abstract #1138]. ICAAC; 1990Thirtieth Interscience Conference on Antimicrobial Agents and Chemotherapy; 1990

Finger R, Hughes JP, Meade BJ, Pelletier AR, and Palmer CT. Age-specific incidence of chickenpox. Public Health Rep 1994;109(6):750-5.

Kuter B, Matthews H, Shinefield H, Black S, Dennehy P, Watson B, et al. Ten year follow-up of healthy children who received one or two injections of varicella vaccine. Pediatr Infect Dis J 2004;23(2):132-7.

Gershon AA. Varicella Vaccine—Are Two Doses Better Than One? [editorials]. N Engl J Med 2002;347(24):1962-3.

Hardy I, Gershon AA, Steinberg SP, LaRussa P, and the Varicella Vaccine Collaborative Study Group. The incidence of zoster after immunization with live attenuated varicella vaccine: a study in children with leukemia. N Engl J Med 1991; 325(22): 1545-50.

Anonymous. General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep 1994; 43(RR-1):1-38.

Committee on Infectious Diseases, American Academy of Pediatrics. In: Pickering LK, Peter G, Baker CJ, et al., eds. 2000 red book: report of the committee on infectious diseases. 25th ed. Elk Grove Village, IL: American Academy of Pediatrics

Galil K, Lee B, Strine T, Carraher C, Baughman AL, Eaton M, et al. Outbreak of varicella at a day-care center despite vaccination. N Engl J Med 2002;347(24):1909-15.

Clements DA, Moreira SP, Coplan PM, Bland CL, Walter EB. Post-licensure study of varicella vaccine effectiveness in a day-care setting. Pediatr Infect Dis J 1999;18(12):1047-50.

Vazquez M, LaRussa PS, Gershon AA, Steinberg SP, Freudigman K, Shapiro ED. The effectiveness of the varicella vaccine in clinical practice. N Engl J Med 2001;344(13):955-60.

Li S, Chan ISF, Matthews H, Heyse JF, Chan CY, Kuter BJ, et al. Inverse relationship between six week post-vaccination varicella antibody response to vaccine and likelihood of long term breakthrough infection. Pediatr Infect Dis J 2002; 21(4): 337-42

6.1.3 Post-Marketing Experience:

ProQuad™ has not been previously licensed in any country.

6.2 Tables of Clinical Studies:

See Appendix A, Tables of Clinical Studies.

6.3 Review Strategy:

The Case Study Reports for the 5 pivotal studies identified above were reviewed including studies 009, 011, 012, 013, and 014. All summaries of immunogenicity data in seronegative and seropositive children were reviewed. All safety data was also reviewed from each of the 5 clinical trials, including all reports using ProQuad™ formulated to contain varicella at the various potencies listed above. However, the overview of ProQuad™ safety reflects the summary experience using vaccine formulated to contain at least 3.97 log10 PFU varicella /dose as that was defined as the minimal clinically acceptable dose.

6.4 Good Clinical Practices and BioResearch Monitoring

CBER's BioResearch Monitoring Program for ProQuad visited 3 clinical sites. Items noted during these inspections are summarized in the report from Debra Bower, OCBQ, DIS, BMB and are excerpted and discussed below:

Study 009: This was a pilot study and there were no issues identified regarding the conduct of this study that would compromise the ability to evaluate immunogenicity or safety data derived from it.

Study 011: There were no issues identified regarding the conduct of this study that would compromise the ability to evaluate immunogenicity or safety data derived from it.

Study 012: There were no issues identified regarding the conduct of this study that would compromise the ability to evaluate immunogenicity or safety data derived from it. The team performing the CBER FDA Bioresearch and Monitoring (BiMo) Inspection of one of the Study 012 sites reported some discrepancies between adverse reactions noted on the case report form and those provided by the sponsor in line listings for 25 subjects. The most frequently noted discrepancy was the omission of the intensity of local injection site reactions. The discrepant reports were observed for 18 children immunized with ProQuad™ and 8 children immunized with MMRII™ plus VARIVAX™. Dr. Black's study site implicated by the inspection was one of 40 in this study and the discrepancies for these subjects reflect < 1% of the total subjects reporting any adverse reaction (25/3258) for the entire study. Thus, these discrepancies are not expected to have an impact on the ability to interpret safety data from this study.

Study 013: One issue was identified related to the conduct of this study that might impact on the interpretation of the immunogenicity results. Children enrolled in the arm of this study immunized concomitantly with ProQuad™, Tripedia, and Comvax were between 12 and 15 months of age. Tripedia is licensed for immunization of children as a fourth dose at 15 months of age or older. The young age at the time of the Tripedia booster dose in this study may have contributed to the significantly lower antibody responses to pertussis antigens seen in this group. The FDA BiMo inspection also identified an issue related to the conduct of the study that was not in compliance with Good Clinical Practices but did not otherwise compromise the ability to analyze immunogenicity or safety data. At one study site (Dr. Coury, Columbus, Ohio, N=199 subjects at this site), an addendum was added to the Informed Consent Form regarding new information about possible adverse events associated with the 4th dose of Tripedia. This addendum was not presented to the parents and signed until well after subjects were enrolled and/or received the 4th dose of Tripedia. In addition, at one of Dr. Coury's clinics, Olentangy Pediatrics, the COMVAX and TRIPEDIA vaccines were stored in a refrigerator that had temperatures recorded between 0 and –5 C even though they are not to be stored frozen while vaccine at the Westerville pediatric site was in a refrigerator that had a recorded temperature of 32 F. See the review of Study 013 in section 8 that describes the exclusion of the immunogenicity data from children who received compromised vaccine. Because their data was excluded from the immunogenicity analysis, the overall results are not compromised. Also, seven subjects were enrolled even though they did not follow the randomization protocol precisely. These subjects were enrolled and given an allocation number even though it was subsequently determined that they were not eligible to receive vaccine on that day due to a minor illness or recent receipt of a non-study vaccine. They returned two weeks later and were immunized without being randomized again. Although this does not follow the study design it would not be expected to compromise the quality of the immunogenicity or safety data for the study.

Study 014: There were no issues identified regarding the conduct of this study that would compromise the ability to evaluate immunogenicity or safety data derived from it. There were equivalent numbers of protocol deviations in each vaccine group that resulted in data being excluded from the analysis and these data are described in more detail in Section 8 under in the review of Study 014. The CBER, FDA BiMo inspection of Dr. Keith Reisinger, (Pittsburgh, Pa., enrolled 65 subjects), revealed that one subject received VARIVAX™ from a lot that had expired 4 days previously; One subject randomized to receive MMRII™ and VARIVAX™ received vaccines derived from lots that were intended for other studies. Two subjects received VARIVAX™ prior to 12 months of age and another received OPV within 30 days of receipt of the study vaccine. These protocol violations were noted by the sponsor and are described under Protocol

Deviations section in the review of Study 014. The immunogenicity data from these subjects were excluded from the immunogenicity analysis. Numerous discrepancies in the vaccine accountability record were noted but for the most part, these reflected mistakes in recording vial numbers and not in the type of vaccine administered.

6.5 Financial Disclosures:

As required under the regulation Financial Disclosure by Clinical Investigators, Merck requested that each investigator complete questionnaires related to their financial interests in Merck. There were 2047 investigators and sub-investigators participating in the 5 studies in support of licensure of ProQuad. Of these, 1937 filed the required form and reported no significant financial interest in Merck. 91 investigators did not file reports despite multiple attempts by Merck to obtain the required information because they were no longer at that site (N=78) or because they failed to return the form (N=20). 19 investigators returned forms and identified significant equity interest in Merck and of these 11 were Principal Investigators. None of the investigators received payments based on the outcome of the study and none of the investigators had proprietary interest in ProQuad™ or in Merck. A Table listing the investigators with significant financial interest in Merck, the Protocols they participated in and their study sites along with the number of subjects these investigators enrolled are listed in Table 6.1 below.

Table 6.1 Listing of Principal Investigators with Significant Financial Interest in Merck

Investigator	Protocol/Site	Subjects Enrolled n	Percent of Total Subjects Enrolled per Study	
Reisinger, Keith S.			TOTAL	%
	009-001	160	480	**33.3**
	011-001	154	1559	**9.9**
	013-004	180	1915	**9.4**
	014-004	272	802	**33.9**
Marshall, Gary S.				
	011-003	56	1559	**3.6**
	013-003	22	1915	**1.2**
	014-005	64	802	**8.0**
Watson, Barbara M.				
	011-010	82	1559	**5.3**
	013-006	101	1915	**5.3**
Anderson, Edwin L.				
	011-011	12	1559	**0.8**
Dennehy, Penelope H.				
	012-001	48	3928	**1.2**
Clements, Dennis Alfred, III				
	012-006	111	3928	**2.8**
	013-030	11	1915	**0.6**
Lieberman, Jay M.				
	012-026	247	3928	**6.3**
King, Stephen				
	012-043	24	3928	**0.6**
	013-039	28	1915	**1.5**
Kuiken, Ben C.				
	013-016	67	1915	**3.5**
Azimi, Parvin H.				
	013-053	6	1915	**0.3**
Barone, Stephen R.				
	014-007	6	802	**0.7**

With the exception of Dr. Reisinger who enrolled about 1/3 of the children in study 009 and 014, the remaining PI's with significant financial interest in Merck enrolled <10% of the total in any individual clinical study. In the case of Dr. Reisinger, Study 009 was a pilot study to conducted to help identify a dose of varicella that could be used successfully in combination with measles, mumps and rubella and was not a pivotal study; likewise, Study 014 demonstrated that ProQuad could be used in place of MMRII in children 4 to 6 years of age and was not a pivotal study for primary immunization with this vaccine.

The children enrolled in the studies performed in support of licensure of ProQuad™ were randomized by centrally generated computerized schedules and it is unlikely that investigators with significant financial interests could have altered the results of the trial

by providing biased results. Not all studies were blinded with regard to vaccine assignment. However, all samples were blinded at the time serological assays were performed. Merck manufactured all measles, mumps, rubella, and varicella vaccines compared in these studies. There was no obvious financial incentive to favor one product over the other.

7 Human Pharmacology

7.1 Immunogenicity:

In clinical efficacy studies, seroconversion in response to vaccination against measles, mumps, rubella and varicella paralleled protection against these diseases. In studies performed in support of the licensure of ProQuad™ the presence of detectable antibody was assessed by an appropriately sensitive enzyme linked immunosorbent assay (ELISA) for measles, mumps, and rubella, and by gpELISA for varicella antibody.

Based on testing of a limited number of sera (n=107) there was good agreement between the measles ELISA and measles --- assay. Two sera gave equivocal results in ELISA. When these two samples were removed from the analysis, the overall agreement between assays was 100% and including these two samples (one sample was --- negative and one sample was --- positive), the overall agreement was 98.1%. The positive predictive value of the measles ELISA with regards to measles --- was 98% and sensitivity was 98.3%. The cut-off for measles seropositivity was set at ---- mIU/mL, ---- ----mIU/mL and in some summaries of data, a cut-off of --- mIU measles antibody/mL was used.

Mumps ELISAs were performed using the vaccine strain for antigen or later using low passage Jeryl Lynn mumps virus as antigen. There was a good correlation between the results of both ELISA assays irrespective of the antigen used. Results of testing by mumps ELISA were compared to results obtained in mumps ----------. 469 subjects positive in ELISA were also positive by ---------- assay (titer > 1:32) giving a positive predictive value of 94.2%. With regard to seroconversion, the overall agreement between the two assays was 93.4%. Of the 1023 sera tested, there were 98 discordant samples. Of these, 29 were positive by ELISA and negative by ---- while 69 were positive by ---- and negative by ELISA indicating that the ELISA identified fewer responders and was the more conservative test. The seropositive cut-off for the mumps ELISA was --- mumps antibody units/mL.

The specificity of the rubella ELISA versus rubella --- was 95.8% and the sensitivity was 100% based on testing 258 sera. The cut-off for seropositivity in the rubella ELISA was ---- IU rubella antibody/mL.

The purpose of the varicella glycoprotein enzyme-linked immunosorbent assay (gpELISA) was to detect IgG antibody to varicella-zoster virus (VZV) before and after vaccination with VZV-containing vaccine(s). This method detects antibodies to VZV glycoproteins (gp), which have been lectin affinity-purified from MRC-5 cells infected with the KMcC strain of VZV. The assay and the purification of the VZV gp from VZV-infected cells have been described ----------
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Serum sample titers determined by gpELISA correlate with neutralizing antibody titers (Krah, DL, Cho I, Schofield T, et al. Comparison of gpELISA and neutralizing antibody responses to Oka/Merck live varicella vaccine in children and adults. Vaccine 1997 15(1):61-64.) and with protective efficacy (White CJ, Kuter BJ, Ngai A, et al. Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. Pediatr Infect Dis J 1992 11(1):19-23.).

Results for the assay are reported as concentration of antibody in gpELISA units/mL. The negative control used for this assay was an individual human serum at a dilution of 1:50, found to be negative for anti-VZV. The high positive marker was a VZV-antibody-positive serum, diluted 1:15,000, which gave a response in the assay at the upper end of the standard curve. The low positive marker was a VZV-antibody-positive serum diluted 1:50,000, which gave a response in the assay at the lower end of the standard curve. A VZV-antibody-positive individual human serum was used to generate a standard curve (range of 0.625 to 20 gpELISA units/mL).

Prior to June-2001, the standard curve was approximated using a quadratic function fit to the 0.625 to 20 gpELISA units/mL concentration range of the standard. Since June-2001, the standard curve has been approximated using the four-parameter weighted logistic regression function. A statistical analysis comparing the two fit procedures showed that the quadratic and logistic processing methods yield similar titers (generally within 3%) when interpolating from the 0.625 to 20 gpELISA units/mL region of the standard curve.

During the validation, the limit of detection (LOD) was mathematically determined to be 0.3 gpELISA units/mL. However, because no standard concentrations below 0.625 gpELISA units/mL are run in the assay, the LOD is reported as <0.625 gpELISA units/mL. The quantifiable range of the assay is 0.625 to 20 gpELISA units/mL. Dilutability is defined as the attribute of a standard curve assay whereby it is demonstrated that a test sample can be diluted through a series, yielding equivalent titers across that series. The assay is dilutable for samples tested in the 1:500 to 1:40,000 dilution range. The precision of the assay for a sample titer was 11%. There was no statistical evidence of increased variability in test sample results due to different analysts performing the assay. The gpELISA assay is acceptable for use in the immunogenicity endpoints of the studies performed.

7.2 Pharmacology:

Measles vaccine virus:

The genetic sequence of the Enders-Edmonston measles vaccine strain is known, however the mutations responsible for attenuation are not. Measles-like rashes after vaccination indicate that viremia presumably occurs following local replication at the site of inoculation. However, vaccine virus was not isolated from the blood of monkeys studied early in the development of measles vaccine and has not been isolated from normal healthy humans after vaccination.

Molecular evaluation of measles-like rashes during ProQuad™ studies:

Because fever 5 to 12 days after immunization and measles-like rashes were reported more frequently after ProQuad™ than after MMRII™ and VARIVAX™ immunization and because measles antibody titers were higher after ProQuad™, there was a hypothesis that measles viremia occurred more frequently or at higher levels in children given ProQuad™ than in children immunized with MMRII™. In order to test this hypothesis, blood samples were obtained from a subset of children immunized with ProQuad who reported measles like rashes as well as from control children immunized with ProQuad who did not have rash and from children immunized with MMRII™ and VARIVAX™. Blood samples were evaluated by RT-PCR for measles virus genome as part of a sub-study in protocols 012 and 013. A total of 193 blood samples were tested by measles RT-PCR. ---------- Samples were collected between 5 and 44 days after immunization with the majority of control (no rash) and rash samples collected between 10 and 17 days after immunization. 58 samples were from subjects with measles-like rashes (45 from ProQuad™ vaccinees and 13 from subjects immunized with MMRII™ and VARIVAX™) and 135 samples were from RT-PCR control subjects who did not have rashes (69 were from ProQuad™ recipients and 66 from children immunized with MMRII™ and VARIVAX™). The sensitivity of the RT-PCR test was --- copies of measles virus genome or -- TCID50. Only 3 samples gave a positive result. Of these 3 samples, 2 were from control children who did not have rashes and these samples were collected 12 days after immunization; 1 sample was from a child with measles like rash collected 14 days after immunization. All 3 samples were from children immunized with ProQuad™. In retrospect, this study was unlikely to detect measles viremia because viremia is rarely detectable once the rash has appeared. However, these data suggest, that if viremia occurs following ProQuad™ immunization, it is at a very low level and of a brief duration.

There is no evidence that infectious vaccine virus is shed after immunization and transmission of measles vaccine virus from vaccinated individuals to close contacts has not been documented. Measles vaccine virus may induce elevated levels of serum interferons after immunization for up to 17 days. To avoid interference with vaccine take, it is generally advised that one month elapse between immunization with measles containing vaccines and vaccination with other live viral vaccines. Measles vaccine virus may induce a period of immunosuppression for about one month after vaccination.

Mumps vaccine virus:

The Jeryl Lynn strain of mumps vaccine virus is a ----------.
Viremia after vaccination has not been documented although it presumably occurs rarely as cases of parotitis, pancreatitis, and orchitis that occur in temporal association with vaccination are thought to be due to vaccine virus replication. Throat samples collected from 71 mumps naïve children immunized with Jeryl Lynn vaccine virus on days 8,10,14,17 and 21 after vaccination were evaluated for vaccine virus shedding. Tissue culture monolayers did not reveal mumps virus cpe after seven days in culture and subculture onto fresh cells did not reveal the presence of mumps virus or any hemadsorbing virus after an additional incubation period. Transmission to susceptible contacts has not been reported.

Rubella vaccine virus:

The genome of rubella RA27/3 virus has been sequenced but the attenuating mutations are not known. Vaccine virus viremia may occur 7 to 11 days after immunization with RA 27/3 vaccine virus. Pharyngeal excretion of low amounts of vaccine virus (~10 PFU per swab) occurs between 7 to 21 days after vaccination with peak excretion around day 11 in a high percentage of vaccinees. Although most vaccinees shed vaccine virus following immunization, studies have not documented spread to seronegative close contacts or to rubella susceptible family members. Infectious rubella vaccine virus may also be shed in breast milk of mothers immunized in the postpartum period and be transmitted to and infect their nursing newborns. Studies in rubella susceptible women immunized with the RA 27/3 strain 7 to 10 days prior to elective abortion failed to show any evidence of vaccine virus infection in the placenta or fetus. One published report describes vaccine virus infection in an infant born to a mother vaccinated in early pregnancy; this infant excreted vaccine virus at birth but did not have any of the physical stigmata associated with congenital rubella infection.

Varicella vaccine virus:

The Oka strain of varicella virus was attenuated by serial passage in cell culture. The genome of the Oka strain of varicella-zoster virus has been sequenced, but the attenuating mutations are not known. Diffuse varicella-like rashes have been described after immunization in a few percent of vaccinees, suggesting the vaccine strain is capable of causing a viremia. Transmission of vaccine virus to susceptible seronegatives is rare, but has been demonstrated. Shedding of vaccine virus is more likely in vaccinees with rashes. However, the tendency of wild-type varicella to be shed at the highest levels prior to evidence of rash suggests that the vaccine strain may behave similarly; thus, the absence of a rash may not necessarily indicate the absence of shedding. Although varicella vaccine has been given accidentally during pregnancy, there are no reports of vaccine virus transmission to a fetus. However, the ability of the wild-type virus to cause a congenital varicella syndrome suggests that vaccine should not deliberately be used during pregnancy due to the theoretical risk of fetal transmission.

Molecular evaluation of varicella-like rashes during ProQuad™ studies

In some cases, ---------- was employed to determine whether varicella-like rashes were due to wild-type or vaccine strain varicella virus. The ---------- assay that was used identified ---------- ---------- that have been shown to definitively distinguish vaccine from wild-type VZV strains.

This assay has been employed in various varicella-related vaccine studies. For example, in protocol 045, studying Process Upgrade Varicella Vaccine, a disseminated rash soon after immunization was shown to be wild-type varicella, while a zoster-like rash was confirmed to be the vaccine strain.

In Study 009, comparing ProQuad with MMRII plus VARIVAX, 4 subjects who received ProQuad and no subjects who received MMRII plus VARIVAX provided samples from rashes that resembled varicella. Unfortunately, 3 of the samples were inadequate. The fourth sample did not confirm the presence either of vaccine or wild-type varicella strains in the rash.

In Study 011, ----- was also intended for use in studying varicella-like rashes, but almost all of these samples were inadequate for study. The single valid sample from the ProQuad group was negative, while the single valid sample from the control group was positive for vaccine strain. ---- also was used to study an adverse reaction in study 011, of a child who developed a rash together with signs of pneumococcal bacteremia. In this case, the sample was inadequate, and thus the presence of either wild-type or vaccine strain varicella as a co-factor could not be ruled in or out.

In Study 012, 2 of 4 samples from children with varicella-like rashes were negative for both vaccine strain and wild-type VZV, and the remaining 2 samples were inadequate. One subject in the control group had a varicella-like rash with the vaccine virus, while a case of zoster in a ProQuad recipient was positive for wild-type VZV.

In Study 013, a single sample from the control group was positive for vaccine strain VZV, while none of 3 samples (two of which were inadequate) were positive from children who received ProQuad.

Thus, overall, ---- studies of rashes post-vaccination were not very helpful in further characterizing the cause of varicella-like rashes among vaccinees. This is not considered to be a major issue, because the overall incidence of varicella-like rashes among ProQuad recipients was not higher than that among the control groups, or higher than historical data would suggest would be expected with an Oka/Merck containing vaccine.

7.3 Pharmacokinetics

See information provided above in 7.2 for data concerning viremia and shedding.

8 Clinical Studies

8.1 Trial # 009

A Pilot Study to Compare the Safety, Tolerability, and Immunogenicity of Measles, Mumps, Rubella, and Varicella (MMRV) Vaccine with Concomitant Administration of Currently Licensed VARIVAX and MMRII in Healthy Children.

8.1.1 Objective/Rationale:

The primary objective was to determine if one or two doses of ProQuad could elicit a similar immune response to varicella as the concomitant administration of 1 dose of currently licensed VARIVAX and MMRII and to assess the safety and tolerability of ProQuad after 1 and 2 doses. Low varicella seroconversion rates were observed in previous studies with ProQuad formulated to contain 3500-4800 PFU of varicella /dose which was attributed to viral interference between varicella virus and measles vaccine virus. Therefore, ProQuad in this study was formulated to contain varicella at a dose of 40,000 PFU (or $4.6\log_{10}$/0.5mL, target titer, actual titer was 4.81 $\log_{10}$ PFU/dose) in an attempt to increase antibody responses to that component. Prior experience also suggested that 2 doses of varicella vaccine given 3 months apart significantly increased gpELISA varicella antibody titers 6 weeks after the second dose, so this study was designed to compare immunogenicity of 1 vs. 2 doses of ProQuad.

8.1.2 Design Overview:

Partially blinded, multi-center (two study sites) randomized study in two groups of healthy children who received ProQuad plus placebo on Day 0 followed by ProQuad at Day 90 (Group A) or MMRII and VARIVAX on Day 0 only (Group B). Parents or legal guardians provided informed consent and subjects were randomized and vaccinated on Study Day 0 and then followed for 42 days for adverse reactions. After the 42 day follow-up period was completed, subjects vaccinated with ProQuad in Group A were contacted by the un-blinded study person and requested to return on approximately Day 90 for a second dose of ProQuad. These children were followed for an additional 42 days after the second dose. The person assigning the allocation number, reconstituting the vaccine and drawing the vaccine into the syringe was not blinded to group assignment. Syringes were labeled with the subject's allocation number and initials and delivered to a blinded study person for administration. Parents, guardians, children, study personnel administering the vaccine and performing follow-up for adverse events as well as all persons performing serological assays were blinded to group assignments. The IRB at each site reviewed and approved the clinical protocol and approved the Informed Consent Form used to enroll subjects in this study. Serum samples were obtained prior to each dose of vaccine and 6 weeks after vaccination. An overview of the study design is shown in Table 8.1.1.

Planned enrollment was for 480 children starting on **March 24, 1998**. Enrollment ended on **January 5, 1999**. Subjects who provided serum samples were offered revaccination with any component of the vaccine to which they did not respond.

Table 8.1.1 Overview of the study design:

Time	Group A (ProQuad + Placebo)	Group B (MMRII + Varivax)
Day 0	History/consent/eligibility Obtain pre-vaccine serum sample. Administer vaccine and placebo. Provide vaccination report cards.	History/consent/eligibility Obtain pre-vaccine serum sample. Administer vaccines. Provide vaccination report cards.
Days 7,14, 21	First 10 vaccinees: telephone calls for serious AEs	First 5 vaccinees: telephone calls for serious AEs
Day 0-42	Parents and guardians perform follow-up for Adverse Reactions	Parents and guardians perform follow-up for Adverse Reactions
Day 42	Obtain post vaccination serum sample. Collect and review vaccination report cards Collect information on exposure to measles, mumps rubella or varicella	Obtain post vaccination serum sample. Collect and review vaccination report cards Collect information on exposure to measles, mumps rubella or varicella
>Day 42 < Day 90	Inform subjects of need to return at Day 90 for second dose of ProQuad	-
Day 90	Administer second dose of ProQuad and distribute VRC.	-
Days 90-132	Perform follow-up for AEs	-
Day 132	Obtain post vaccination #2 serum sample Collect and review vaccination report cards Collect information on exposure to measles, mumps rubella and varicella.	-

8.1.2.1 **Randomization:**

Children were randomized 2:1 into Group A or Group B on Day 0 at the time of enrollment and immediately after informed consent had been given. Subjects were randomly assigned to one of the two treatment groups using an allocation table supplied by Merck to each site. Allocation schedules were in blocks of 100. Un-blinded study personnel sequentially assigned allocation numbers and were also responsible for reconstituting vaccine. Allocation numbers were not reassigned for any reason.

8.1.2.2 **Interim analyses:**

Interim analyses were performed to assess the adequacy of the sample size and to assure that the pre-vaccine varicella seropositivity rate did not exceed 20%. Varicella seropositivity rates were assessed at ~25, 50 and 75% enrollment.

8.1.2.3 **Study Population:**

The vaccines were evaluated in healthy children, 12-23 months of age who met the following criteria:

8.1.2.3.1 Inclusion criteria:

- Good health
- 12-23 months of age
- Negative history for varicella, shingles, measles, mumps and rubella

8.1.2.3.2 Exclusion criteria:

- Previous receipt of measles, mumps rubella or varicella vaccine either alone or in any combination.
- Immune impairment or deficiency, neoplastic disease, depressed immunity from steroid or other therapy
- History of anaphylactic reaction to neomycin
- History of anaphylactic or other immediate allergic reactions subsequent to egg ingestion.
- Any exposure to measles, mumps, rubella, varicella or shingles in the 4 weeks prior to each vaccination involving:
    - Continuous household contact
    - Playmate contact > 1 hour indoors
    - Hospital contact in the same room or prolonged face-to-face contact
    - Contact with a newborn whose mother had chickenpox 5 days or less prior to delivery or within 48 hours of delivery.
- Vaccination with an inactivated vaccine within 14 days prior to receipt of each dose of vaccine or scheduled within 42 days thereafter.
- Vaccination with a live virus vaccine within 30 days of a dose of the study vaccine or scheduled within 42 days thereafter.
- Immune globulin or any blood products administered 3 months prior to or within 2 months after each vaccination.
- Any contraindications to either MMRII or VARIVAX as stated in the package circulars.
- Any condition that in the opinion of the investigator might interfere with the evaluation of the study objectives.
- It was recommended that subjects not receive salicylates during the 6 weeks after vaccination because aspirin use in children with varicella has been associated with Reye's syndrome.

8.1.2.3.3 Subjects were discontinued from the study if they developed an anaphylactic reaction after vaccine administration or if they developed varicella, measles, mumps, or rubella prior to the administration of the study vaccine. Subjects who received other vaccines or blood products before serologic follow-up samples were obtained were not necessarily discontinued from the study but their serology data may have been excluded from the group analyses.

8.1.3 Products used:

Products used in this protocol were manufactured by Merck. All clinical materials were supplied in 0.7mL single-dose vials. Study vaccines were re-supplied as needed throughout the study on a site-by-site basis. Doses were administered on Day 0, the day of entry into the study. The lot numbers and release potency of each vaccine lot used in this study are listed in Table 8.1.2.

Table 8.1.2 Vaccine lot numbers and potency

Group	Vaccine	Lot Number	Fill Number	Bulk Number	Potency/ 0.5mL dose	Vol. ML	Route
A	ProQuad	1530/WD478	----	[-------]	3.63 4.90 $log_{10}TCID_{50}$ 3.87 $log_{10}TCID_{50}$ 4.81 $log_{10}PFU$	0.5	Subcutaneous
	Placebo	1510/WD458	----	----------	N/A	0.5	Subcutaneous
B	VARIVAX	0690E	----	-------- --------	3.5 $log_{10}PFU$	0.5	Subcutaneous
	MMRII	0034H	----	[-------]	3.9 $log_{10}TCID_{50}$ 4.9 $log_{10}TCID_{50}$ 4.0 $log_{10}TCID_{50}$	0.5	Subcutaneous
		0958H	----	[-------]	3.6 $log_{10}TCID_{50}$ 5.1 $log_{10}TCID_{50}$ 3.9 $log_{10}TCID_{50}$	0.5	Subcutaneous
A & B	Diluent	0864H	N/A	N/A	N/A	N/A	N/A
		1658E	N/A	N/A	N/A	N/A	N/A

*PGS is phosphate, glutamate, and sorbitol stabilizer. It is reconstituted using the sterile diluent.
** Diluent: sterile water for injection.
N/A: not applicable.

8.1.4 Study Objectives:

8.1.4.1 Primary Hypothesis, Immunogenicity:

The immune response to varicella (as measured by percent of subjects with glycoprotein enzyme-linked immunosorbent assay [gpELISA] titers ≥5 units) at 6 weeks following either 1 or 2 doses of MMRV (~40,000 plaque-forming units [PFU] of varicella/0.5-mL dose) will be similar to the response following the concomitant administration of 1 dose of the currently licensed VARIVAX™ and MMR™ II.

8.1.4.2 Primary Hypothesis, Safety:

There will be no vaccine-related serious adverse experiences in the MMRV (~40,000 PFU of varicella/0.5-mL dose) treatment group.

8.1.4.3 Secondary Hypothesis (1):

The administration of 1 dose of MMRV (~40,000 PFU of varicella/0.5-mL dose) will elicit similar seroconversion rates to measles, mumps, and

rubella at 6 weeks post-vaccination as the concomitant administration of 1 dose of the currently licensed VARIVAX™ and M-M-R™ II.

8.1.4.4 Secondary Hypothesis (2):

The administration of 2 doses of MMRV (~40,000 PFU of varicella/0.5-mLdose) ~90 days apart will elicit a better immune response to varicella (as measured by the percent of subjects with gpELISA titers ≥5 units) at 6 weeks after dose 2 than that attained at 6 weeks following the administration of 1 dose of MMRV (~40,000 PFU of varicella /0.5-mL dose).

8.1.4.5 Study Endpoints:

Immunogenicity endpoints were measured using immunological assays that specifically measured IgG antibody responses to each vaccine virus. **Safety endpoints** were assessed using the Vaccination Report Card that was completed by each subject's parent or legal guardian.

8.1.4.5.1 **Detection of Measles IgG Antibody (ELISA):**

The measles ELISA used measles antigen purchased from ----------
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----------The limit of detection of this assay was determined to be 2.13 measles antibody units and the quantifiable range was 2.13 to 136.15 measles antibody units. The assay precision was 23%. Samples were considered to be seronegative if they were below the optical density (OD) cut-off and samples were considered to be seropositive if they had ≥21.3 ELISA antibody units (equivalent to 207.8mIU measles antibody/mL). To convert ELISA units to milli-International Units, (mIU), the titer was divided by 0.1025.

8.1.4.5.2 **Detection of Mumps IgG Antibody (ELISA):**

Mumps virus antigen used for this assay was produced at MRL. The mumps antigen was ----------
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----------. The quantity of anti-mumps IgG was determined by comparing the response in the test sample to the standard curve. The cut-off was determined by running 10 replicates of the negative control serum. The assay cut-off was equivalent to the mean O.D. +0.15 for the 10 assays on the negative control serum where 0.15 was 3 S.D. above the mean of a panel of known mumps negative sera. Samples with ODs less than or equal to the cut-off were serostatus negative and assigned a titer of < 2.0 antibody units. Samples with OD values greater than the cut-off were quantified using the standard curve. The quantifiable range was 2.0 to 40 mumps antibody (Ab) units/mL. Sera whose titers exceeded this range were re-analyzed at greater dilutions until an

endpoint titer was obtained. The negative control for the assay was a pool of human sera known to be mumps negative. The low positive control was a pool of human sera while the high positive was also a pool of human sera. A single mumps positive serum was used to generate the standard curve. The standard curve data were fit using a quadratic polynomial. Samples with low titers measured 1.85 fold lower at the lowest dilution tested while pools with medium and high titers showed no evidence of lack of dilutability. The precision of the assay was 14%.

8.1.4.5.3 Detection of Rubella IgG (ELISA):

Inactivated rubella antigen purchased from ------------------------------ -- -- -- -- ------------------------- The cut-off for the assay was established as follows: the mean OD value for 10 known rubella negative control sera plus 5 times the S.D. of the negative controls was determined. Samples with OD values less than this cut-off value were considered to be rubella seronegative and were assigned a value of <10 Ab units. Rubella antibody positive samples were quantitated relative to the standard curve. The negative control for this assay was a single human serum known to be negative for rubella antibody. The low positive and high positive controls were the WHO International Standard diluted to 40 and 160 mIU/mL. The WHO reference serum was also used to generate the standard curve. Standard curve data were fit using a quadratic polynomial. The LOD was 0.91 rubella antibody units/mL. The quantifiable range of the assay was 1-32 antibody units /mL. There was no evidence of significant dilution bias and the overall assay variability was 22.4%. A pre-vaccination sample was considered to be seronegative if it was below the OD cut-off and a post-vaccination sample was considered to be seropositive if it contained ≥12.8 ELISA antibody units (=10 IU/mL). To convert to International Units the ELISA titer was divided by 1.28.

8.1.4.5.4 Varicella IgG gp ELISA antibody

The purpose of the glycoprotein enzyme-linked immunosorbent assay (gpELISA) was to detect IgG antibody to varicella-zoster virus (VZV) before and after vaccination with VZV-containing vaccine(s). This method detects antibodies to VZV glycoproteins (gp), which have been lectin affinity-purified from MRC-5 cells infected with the KMcC strain of VZV. The assay and the purification of the VZV gp from VZV-infected cells have been described -- -- -- -- -- -- --

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Serum sample titers determined by gpELISA correlate with neutralizing antibody titers (Krah, DL, Cho I, Schofield T, et al. Comparison of gpELISA and neutralizing antibody responses to Oka/Merck live varicella vaccine in children and adults. Vaccine 1997 15(1):61-64.) and with protective efficacy (White CJ, Kuter BJ, Ngai A, et al. Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. Pediatr Infect Dis J 1992 11(1):19-23.).

Results for the assay are reported as concentration of antibody in gpELISA units/mL. The negative control used for this assay was an individual human serum at a dilution of 1:50, found to be negative for anti-VZV. The high positive marker was a VZV-antibody-positive serum, diluted 1:15,000, which gave a response in the assay at the upper end of the standard curve. The low positive marker was a VZV-antibody-positive serum diluted 1:50,000, which gave a response in the assay at the lower end of the standard curve. A VZV-antibody-positive individual human serum was used to generate a standard curve (range of 0.625 to 20 gpELISA units/mL).

Prior to June-2001, the standard curve was approximated using a quadratic function fit to the 0.625 to 20 gpELISA units/mL concentration range of the standard. Since June-2001, the standard curve has been approximated using the four-parameter weighted logistic regression function. A statistical analysis comparing the two fit procedures showed that the quadratic and logistic processing methods yield similar titers (generally within 3%) when interpolating from the 0.625 to 20 gpELISA units/mL region of the standard curve.

During the validation, the limit of detection (LOD) was mathematically determined to be 0.3 gpELISA units/mL. However, because no standard concentrations below 0.625 gpELISA units/mL are run in the assay, the LOD is reported as <0.625 gpELISA units/mL. The quantifiable range of the assay is 0.625 to 20 gpELISA units/mL. Dilutability is defined as the attribute of a standard curve assay whereby it is demonstrated that a test sample can be diluted through a series, yielding equivalent titers across that series. The assay is dilutable for samples tested in the 1:500 to 1:40,000 dilution range. The precision of the assay for a sample titer was 11%. There was no statistical evidence of increased variability in test sample results due to different analysts performing the assay.

8.1.4.6 Changes in the Conduct of the Study:

8.1.4.6.1 CBER asked Merck to evaluate the measles serology using 120mIU/mL as a sero-protective cut-off. However, the Limit of Detection (LOD) for the Measles ELISA was 207.8mIU/mL so this cut-off was used in lieu of 120mIU/mL.

8.1.4.6.2 CBER asked Merck to evaluate rubella serology using 10IU/mL as a sero-protective cut-off.

8.1.4.6.3 The planned analysis of missing data was not conducted because so little data was actually missing.

8.1.4.6.4 At CBER's request, the primary varicella immunogenicity analysis was performed on subjects with a baseline antibody titer < 1.25 gpELISA units instead of on subjects with baseline titers < 5gpELISA units.

8.1.5 Surveillance

8.1.5.1 Merck Research Labs (MRL) conducts its own Quality Assurance and Quality Control Program and surveillance included on-site monitoring of investigators, on site and in-house review of clinical data and resultant databases, review of the clinical study reports and summary documents.

8.1.5.2 No formal interim analysis was performed. Pre-vaccination varicella seropositivity rates were monitored at approximately 25, 50 and 75% enrollment in order to insure that sufficient numbers of susceptible children in each group were enrolled for the primary analysis. If varicella seropositivity rates rose above 35% then the power of the study would drop below 80% and additional subjects would have been recruited into the study.

8.1.5.3 Active surveillance for cases of measles, mumps, rubella and varicella in the community was not done although parents and guardians of children enrolled in this study were asked about any known exposures or diagnosis of these diseases.

8.1.5.4 Follow-up visits for safety assessments and serology were as follows:

Parents or guardians filled out the Vaccination Report Cards for 42 days after each vaccination. They were required to note local and systemic adverse reactions and record temperatures for 42 days. They were to contact study personnel immediately if any serious adverse reactions were noted. Study personnel evaluated all children with rash immediately upon notification. Varicella-like lesions were cultured and samples tested by ----- or blood samples obtained from children with measles-like rashes for measles specific RT-PCR after additional informed consent was obtained from the parent/guardian.

Blinded study personnel provided follow-up and collected information regarding the adverse reactions.

8.1.6 Statistical considerations:

8.1.6.1 The primary purpose of the study was to show that 1 or 2 doses of ProQuad would elicit an immune response to varicella that was similar to that seen in children immunized with MMRII and VARIVAX given concomitantly. Two, one-sided, non-inferiority tests were performed to show that the percent of initially seronegative subjects with post-vaccination varicella antibody titers ≥5 gpELISA units in subjects immunized with ProQuad were similar to the percent who achieved this level when immunized with MMRII and VARIVAX given concomitantly. Because this was a pilot study, similarity was defined as < 15percentage point decrease in seroconversion rates. The significance level was adjusted for multiplicity at the one-sided, α=0.025 level. Rejection of the null hypothesis led to the conclusion that the varicella response rates were similar in the two groups. The primary immunogenicity analysis was based on the per-protocol population who were initially seronegative for varicella antibody based on the OD cut-off.

It was expected that 80% of the subjects enrolled would be seronegative for varicella antibody and 10% would be lost to follow-up after each vaccination. The target enrollment of 320 subjects immunized with ProQuad would yield 224 evaluable subjects after the first dose and 192 evaluable subjects after the second dose. With 160 subjects enrolled in the MMRII plus VARIVAX arm, 112 subjects would be evaluable for comparison. The expected response rate in the control group was 85%, and assuming that the response rates would be similar, the study had 94% power to rule out a decrease of 15% points between the treatment and control group, i.e., the 95% confidence interval for the difference in proportions [treatment-control] would not include a decrease of 15 percentage points or more.

The proportion of subjects with post-vaccination varicella antibody titers greater than or equal to 5 gpELISA units was adjusted by study center.

8.1.6.2 No serious vaccine related AEs were expected in this study. If none were observed in 320 subjects, there was a 95% probability that the true rate was <0.9%.

In order to control the overall significance level at the one-sided alpha= 0.025 level, and allow for multiple comparisons, a modified Bonferroni approach with a stepped Hochberg adjustment was used.

8.1.6.3 The primary endpoint for safety was the incidence of vaccine related serious adverse experiences. In addition, for adverse reactions occurring in at least 1% of subjects in any treatment group, the risk difference and 95% confidence interval for the risk difference were compared.

8.1.6.4 The secondary immunogenicity analysis for measles, mumps, and rubella responses rates consisted of 3, one-sided, non-inferiority tests to demonstrate that the post-vaccination responses after one dose of ProQuad were similar to the responses after one dose of MMRII. An equivalence margin of 10% was allowed because this was a pilot study. Rejection of the null hypothesis allowed the conclusion that the immune responses to the vaccines were similar. The primary immunogenicity analysis for measles, mumps and rubella were based on the comparison of immune responses in the per-protocol population of subjects who were initially seronegative to the respective antigen.

The statistical criteria required that the seroconversion rates for measles, mumps, and rubella in the MMRV (ProQuad) group will be no more than 10 percentage points lower than in the control group, i.e., the 95% confidence interval for the difference in seroconversion rates [treatment-control] will not include a decrease of 10 percentage points or more. The expected rate in the control group was 95% for each component.

The proportion of subjects with post-vaccination measles, mumps, and rubella antibody was adjusted for study center.

8.1.6.5 The second immunogenicity analysis was to show the superiority of the varicella immune response after 2 doses of ProQuad compared to the response seen after a single dose. The comparison was based on a one-sided paired difference test at the α0.025 level. This analysis was based on the per-protocol population who were initially seronegative based on the OD cut-offs as described below and who also had antibody measurements after Dose 1 and Dose 2.

Assuming there would be 192 evaluable subjects after a second dose of ProQuad and 112 subjects after MMRII plus VARIVAX, this study had 92% power to rule out a decrease of 15 percentage points or more between treatment and control group at alpha= 0.025 (one-sided) using the method of Farrington and Manning. The comparison of the paired difference in two proportions used a version of McNemar's test to stratify by study center.

A sensitivity analysis was planned to assess the potential impact of differential loss to follow-up for the Post-Dose 2 responses. However, the dropout rate was lower than expected. After Dose 1, subjects who were lost to follow-up were assumed to have post-vaccination varicella antibody titers of < 5gpELISA units.

8.1.6.6 GMTs and corresponding 95% confidence intervals were calculated for varicella, measles, mumps, and rubella responses by treatment group. Fold differences between treatment groups were also compared as well as the fold rise in varicella antibody between dose 1 and 6 weeks after dose 2 using ANOVA.

8.1.7 Results

8.1.7.1 Populations enrolled/analyzed

8.1.7.1.1 The study was conducted at two study sites in the United States. Drs. Keith Reisinger (Pittsburgh, Pa., N = 160) and Steven Black (Oakland, California, N= 320) were the Principal Investigators

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8.1.7.1.2 480 subjects were enrolled (323 in Group A and 157 in Group B) with 456 (95%) completing the study. 303/323 (93.8%) completed the study in ProQuad Group A vs. 153/157 (97.5%) in MMRII + VARIVAX Group B. Reasons for dropouts were similar in each group and are listed in the Table 8.1.3 below:

Table 8.1.3: Enrollment and Study Dropouts by Vaccine Group:

	ProQuad+ Placebo followed by ProQuad		**MMRII + VARIVAX**	
	N=323		N=157	
	N	(%)	N	(%)
Male, age (months)	150 (12-22)		70 (12-19)	
Female, age (months)	173 (12-22)		87(12-19)	
Vaccinated at				
Visit 1	323	(100)	157	(100)
Visit 2	310	(96.0)		
Completed	303	(93.8)	153	(97.5)
Discontinued	20	(6.2)	4	(2.5)
Clinical AEs	1	(0.3)	0	
Laboratory AE	0		0	
Deviation from protocol	2	(0.6)	0	
Refused to participate	10	(3.1)	3	(1.9)
Lost to follow up	7	(2.2)	1	(0.6)

8.1.7.1.3 Protocol deviations that resulted in data being excluded from the primary immunogenicity analysis after dose 1 included: blood samples obtained outside of the acceptable day range, subject lost to follow-up, subject refused further participation, blood sample was difficult to obtain, baseline sample was missing. Protocol deviations that occurred in the post-dose 2 follow-up period included: blood sample outside of the acceptable day range, subject lost to follow-up, subject refused further participation, baseline sample was missing or invalid, quantity not sufficient, subject was participating in another clinical trial or received another live viral vaccine during the study period.

8.1.7.1.4 No subjects were prematurely un-blinded during the 42 days of follow-up after administration of dose #1. The

study was not blinded during or after administration of dose 2.

8.1.7.1.5 The primary analysis of immunogenicity was based on the per-protocol population and only subjects who were initially seronegative for the vaccine antigen were evaluated. Serostatus for each vaccine antigen at baseline is listed below in Table 8.1.4:

Table 8.1.4: Serostatus for each vaccine antigen at baseline (pre-vaccination)

	Exclusion for Corresponding Vaccine Components/Dose 1							
	Varicella		**Measles**		**Mumps**		**Rubella**	
	Group A	**Group B**	**Group A**	**Group B**	**Group A**	**Group B**	**Group A**	**Group B**
Subjects vaccinated at visit 1	323	157	323	157	323	157	323	157
Subjects included	250	128	302	145	295	150	304	153
Subjects excluded	73	29	21	12	28	7	19	4
Subjects initially seropositive	**60**	**25**	**5**	**8**	**11**	**3**	**2**	**0**
	Exclusion for Corresponding Vaccine Components/Dose 2							
Subjects vaccinated at visit 2	310	NA	310	NA	310	NA	310	NA
Subjects included	239	NA	288	NA	283	NA	290	NA
Subjects excluded	71	NA	22	NA	27	NA	20	NA
Subjects initially seropositive	**56**	**NA**	**5**	**NA**	**11**	**NA**	**2**	**NA**

8.1.7.1.6 Demographics:

Subjects in each group were comparable in terms of age, race, gender, and with regards to prior therapies or medications (see Table 8.1.5).

Table 8.1.5. Demographics of the Study Population

	ProQuad plus Placebo followed by ProQuad N = 323	MMRII + VARIVAX N=157
Gender	**n (%)**	**n (%)**
Male	150 (46.4)	70 (44.6)
Female	173 (53.6)	87 (55.4)
Age (months)		
Mean	14.1	14.0
SD	1.9	1.7
Median	14.0	14.0
Range	12-22	12-19
Race/Ethnicity		
African American	41(12.7)	21(13.4)
Asian Pacific	30(9.3)	9(5.7)
Caucasian	211(65.3)	107(68.2)
Hispanic	30(9.3)	16(10.2)
Other	11(3.4)	4(2.5)
Specific Prior Therapy		
None	226 (70.0)	110 (70.1)
One or More	97 (30.0)	47 (29.9)

8.1.7.2 Efficacy endpoints: Immunogenicity

8.1.7.2.1 Primary Endpoint, Immunogenicity:

The primary endpoint for efficacy was the immune response to varicella vaccine as measured by the varicella gpELISA in individuals who were either seronegative prior to vaccination or who had a gpELISA titer < 1.25gpELISA units prior to vaccination. Immune responses in children given one or two doses of ProQuad were compared to the immune response to one dose of VARIVAX in children immunized with MMRII and VARIVAX concurrently. The population analyzed included those children who met the pre-specified criteria in the protocol, i.e., had a baseline titer drawn and had the post vaccination serum sample drawn within a specified time period after immunization. After dose 1, 378/480 (78.8%) children were seronegative and evaluable and after dose 2, 367/480 (76.5%) children were evaluable. For the cohort with gpELISA titers < 1.25 gpELISA units pre-vaccine, 435/480 (91%) were evaluable after dose 1 while 423/480 (88.1%) were evaluable after dose 2. The statistical criteria required that the immune response rate for varicella in the ProQuad group be not more than 15% lower than the response rate in the control group, i.e., the 95% confidence interval for the difference in proportions (treatment –control) will not include a decrease of 15 percentage points or more. In the initially seronegative population, the varicella seroresponse rate was 91.2% (95% confidence interval 87.0%-94.4%) while 92.2% (95% confidence interval 86.1%-96.2%) responded after immunization with MMRII + VARIVAX.

Based on this analysis, varicella sero-responses were found to be similar in children after one or two doses of ProQuad versus those immunized with MMRII + VARIVAX. Response rates and estimated differences are listed below in Table 8.1.6.

Table 8.1.6 Comparison of Varicella gpELISA response rates in children immunized with ProQuad (Group A) vs. children immunized with MMRII + VARIVAX.

	Group A N=323			**Group B N=157**					
	ProQuad +Placebo Followed by ProQuad			**MMRII + VARIVAX**			**Estimated Difference (95%CI) A-B**	**One Sided P Value**	**Conclusion**
Population	**Material**	**N**	**Response (95%CI)**	**Material**	**N**	**Response ((95%CI)**			
Initially seronegative subjects	ProQuad 1 dose	250	**91.2% (87.0-94.4)**	MMRII + VARIVAX	128	**92.2% (86.1-96.2)**	**-0.9 (-6.5-5.7)**	<0.001	Similar
	ProQuad 2 doses	239	**99.2% 97.0-99.9)**				**7.0 (3.2-13.1)**	<0.001	Similar
Subjects with baseline titer <1.25gpELISA units	ProQuad 1 dose	290	**91.1% (87.1-94.1)**	MMRII + VARIVAX	145	**92.4% (86.8-96.2)**	**-1.3 (-6.5-4.8)**	<0.001	Similar
	ProQuad 2 doses	278	**98.9% (96.9-99.8)**				**6.5 (2.9-12.1)**	<0.001	Similar

8.1.7.3 Primary Endpoint, Safety:

The primary endpoint for safety stated that there would be no serious vaccine related adverse reactions in the ProQuad group. The primary endpoint for safety was satisfied. **There were no serious vaccine related adverse experiences in either vaccine group A after the first or the second dose of ProQuad or in vaccine group B immunized with MMRII + VARIVAX.** There were no deaths in this study. There were 2 serious adverse reactions reported but they were not related to vaccination. One SAE occurred in a 15month old white, female 36 days after her second dose of ProQuad when she developed fever, rash, and vomiting treated in the hospital for 2 days with IV fluids and medications including antibiotics. This event was attributed to a viral illness and not to vaccination. Another 13month old female was hospitalized with severe rotavirus gastroenteritis and dehydration that required intravenous fluids. This event was not attributed to prior vaccination.

8.1.7.4 Secondary Endpoint, Immunogenicity:

Secondary immunogenicity endpoints included the immune response to measles, mumps, and rubella. The study was designed to see if administration of 1 dose of ProQuad would elicit similar seroconversion rates 6 weeks after vaccination when compared to 1 dose of currently licensed MMRII given concomitantly with VARIVAX. The study was designed so that the vaccines would be considered to have similar immunogenicity if the seroresponse rates for ProQuad were not more than 10% lower than the seroresponse rate for MMRII for each vaccine antigen. The expected seroresponse rates after MMRII was 95% for each vaccine antigen.

The seroresponse rate to measles was 96.0% and 100% after 1 dose of ProQuad and MMRII respectively, with an estimated difference of –4.0%. The immune responses were declared similar ($p < 0.001$).

The seroresponse rate to mumps was 99.0% and 98.7%, after 1 dose of ProQuad and MMRII, respectively, with an estimated difference of 0.3%. The immune responses were declared similar ($p < 0.001$).

The seroresponse rates to rubella were 95.8% and 91.8% after 1 dose of ProQuad and MMRII, respectively with an estimated difference of 4.1 %. The immune responses were declared similar ($p<0.001$).

The comparison of the seroresponse rates, estimated differences and p-values are listed in Table 8.1.7 below.

Table 8.1.7 Comparison of measles, mumps and rubella seroreponses following immunization with ProQuad and Placebo (Group A) versus MMRII and VARIVAX (Group B)

Assay	**Group A N=323**		**Group B N=157**		**Estimated Difference A-B (95%CI)**	**p=Value**	**Conclusion**
	ProQuad + Placebo followed by ProQuad		**MMRII + VARIVAX**				
	N	**Estimated Response Rate**	**N**	**Estimated Response Rate**			
Measles	302	96.0%	145	100%	-4.0 (-6.8-1.4)	<0.001	Similar
Mumps	295	99.0%	150	98.7%	0.3 (-1.9-3.8)	<0.001	Similar
Rubella	304	95.8% (>10IU/mL: 95.1% 92.0, 97.2%)	153	91.8% (>10IU/mL 92.8% 87.5, 96.4%)	4.1 (-0.6-10.0)	<0.001	Similar

8.1.7.4.1 Secondary Endpoint, Immunogenicity of a Second Dose of ProQuad:

The immune response to a second dose of ProQuad was evaluated as a secondary immunogenicity endpoint to see if this response was superior to the immune response 6 weeks after a single dose of ProQuad. This analysis was restricted to children with varicella antibody titers obtained at baseline and after both dose 1 and dose 2. In subjects who were initially seronegative, 91.6% developed a gpELISA titer $\geq$5 after one dose while 99.4% achieved this titer after 2 doses of ProQuad. In subjects with baseline varicella antibody titers < 1.25 gpELISA units, 91.2% responded after dose 1 and 99.2% responded after dose 2. Both analyses indicated that varicella responses after 2 doses of ProQuad were superior to the responses seen after one dose. A summary of the seroresponse rates and this analysis are listed in Table 8.1.8 below.

Table 8.1.8 Comparison of the gpELISA responses to varicella after one or two doses of ProQuad

Population	N	Group A N=323 ProQuad 1 dose %≥5 gpELISA units	Group A2 N=323 ProQuad 2 doses %≥5 gpELISA units	Estimated Difference (95% CI) Group A2-GroupA	One sided p-Value	Conclusion
Initially seronegative subjects	235	91.6%	99.4%	7.8 (4.0-11.7)	<0.001	Superior
Subjects with baseline titers <1.25 gpELISA units	274	91.2%	99.2%	8.0 (4.3-11.7)	<0.001	Superior

8.1.7.4.2 Additional immunogenicity endpoints evaluated included:

8.1.7.4.2.1 Post-dose 1 comparison of the fold rise in GMTs for antibody against each vaccine antigen.

There was a less than two-fold difference in geometric mean antibody titers for measles, mumps, rubella, and varicella when responses were compared after one dose of ProQuad vs. one dose of MMRII + VARIVAX. The comparison of GMTs is listed in Table 8.1.9 below.

Table 8.1.9 Post Dose 1: Fold-rise in GMTs for Measles, Mumps, Rubella and Varicella Antibody

Assay	Group A N=323 ProQuad + Placebo followed by ProQuad		Group B N=157 MMRII + VARIVAX		Fold difference Group A/ Group B (95%CI)
	N	GMT ELISA UNITS (IU/mL)	N	GMT ELISA UNITS (IU/mL)	
Measles	302	284.7 (2777.1mIU/mL)	145	201.0 (1961.3mIU/mL)	1.4 (1.2,1.7)
Mumps	295	94.5	150	68.1	1.4 (1.1, 1.7)
Rubella	304	106.2 (83 IU/mL)	153	101.9 (79.6IU/mL)	1.0 (0.9, 1.3)
Varicella	250	13.0	128	13.3	1.0 (0.8, 1.2)
Varicella: Subjects with baseline titer <1.25 gpELISA units	290	12.7	145	13.0	1.0 (0.8, 1.1)

8.1.7.4.2.2 Fold-rise in GMTs from post dose 1 to post dose 2.

Six weeks after a second dose of ProQuad, there was a 1.3 fold increase in measles antibody, a 2.5 fold increase in mumps antibody, and a 1.3 fold increase in rubella antibody. Interestingly, there was a 45.4 fold rise in varicella antibody following ProQuad dose two. GMTs and the comparison of antibody titers for each vaccine antigen after one and two doses of ProQuad are listed in Table 8.1.10 below.

Table 8.1.10 Summary of Observed Fold Rise from Post Dose 1 to Post Dose 2 in Subjects who Received 2 Doses of ProQuad (Per Protocol Analysis)

Assay	**Group A1 N=323**		**Group A2 N=323**	**Fold difference Group A1/ Group A2 (95%CI)**
	ProQuad After 1 Dose		**After 2 Doses**	
	N	**GMT**	**GMT**	
Measles	284	281.9	370.7	1.3
Mumps	278	93.7	230.7	2.5
Rubella	286	107.4	137.9	1.3
Varicella: seronegative at baseline	235	12.9	586.6	45.4
Varicella: Subjects with baseline titer <1.25 gpELISA units	274	12.6	609.5	48.5

8.1.7.4.2.3 Reverse cumulative distribution of post vaccination antibody titers were compared for each vaccine antigen. This comparison did not reveal any new findings regarding the immune responses to ProQuad vs. immune responses to MMRII + VARIVAX (data not shown).

8.1.7.4.2.4 An analysis of all subjects with serology was consistent with the results of the per protocol analysis (data not shown).

8.1.7.4.2.5 The immunogenicity summary for initially sero-positive subjects is given in Table 8.1.11 below.

Table 8.1.11 Immune responses in subjects seropositive at baseline:

Assay	**Group A N=323**					**Group B N=157**				
	ProQuad +Placebo Followed by ProQuad				**% with ≥ 4-fold rise**	**MMRII + VARIVAX**				**% with ≥ 4-fold rise**
	Pre		**Post**			**Pre**		**Post**		
Measles	5	23.4	5	535.9	80.0%	8	14.7	8	332.8	87.5%
Mumps	11	7.1	11	97.6	100%	3	11.8	3	33.2	33.3%
Rubella	2	40.8	2	174.5	50%	0	NA	0	NA	NA
Varicella	60	1.0	57	11.7	84.2%	25	1.0	25	11.9	88%

8.1.7.5 Safety endpoints.

8.1.7.6 Summary of Clinical Adverse Experiences (AEs):

76.0% of subjects reported an AE after their first dose of ProQuad while 63.2% reported an adverse reaction after the second dose. 71.8% of subjects immunized with MMRII + VARIVAX reported AEs. A summary of clinical adverse experiences reported in Study 009 is listed in Table 8.1.12.

Table 8.1.12 Summary of clinical adverse reactions reported following immunization with Proquad + placebo or MMRII + VARIVAX

	ProQuad + Placebo Followed by ProQuad N=323				**MMRII +VARIVAX N-157**	
	Post Dose 1		Post Dose 2		Post Dose 1	
	N	%	n	%	N	%
Number of subjects	323		310		157	
Number with follow-up	321		307		156	
Number (%) of subjects						
With no AE	77	(24.0)	113	(36.8)	44	(28.2)
With 1 or more AE	244	(76.0)	194	(63.2)	112	(71.8)
With Injection Site Reaction	34	(10.6)	15	(4.9)	15	(9.6)
With Systemic Reaction	241	(75.1)	189	(61.6)	108	(69.2)
With Vaccine Related Systemic AE	153	(47.7)	67	(21.8)	65	(41.7)
With Serious AE	0	(0)	1	(0.3)	1	(0.6)
With Serious Vaccine Related AE	0	(0)	0	(0)	0	
Who Died	0	(0)	0	(0)	0	(0)
Discontinued due to AE	1	(0.3)	0	(0)	0	(0)

8.1.7.7 Subjects immunized with ProQuad + placebo or with MMRII + VARIVAX were followed for 42 days after each immunization. Clinical follow-up was obtained on 321 of 323 (99.3%) ProQuad recipients after dose 1 and 307 of 310 (99.0%) immunized with a second dose. Follow-up was obtained on 156 of 157 (99.4%) children immunized with MMRII + VARIVAX.

8.1.7.8 Serious Vaccine Related Adverse Reactions:

The primary endpoint for safety was any observation of vaccine-related serious adverse reactions. No serious vaccine-related adverse reactions were expected in either the ProQuad or MMRII + VARIVAX group. A serious adverse reaction was defined as any adverse experience that resulted in death, was life threatening, results in persistent or significant disability, resulted in hospitalization or prolonged an existing hospitalization, caused a congenital anomaly or birth defect or any other medical event that could be judged serious as well as any cancer or overdose.

There were no deaths in this study. Two serious adverse reactions were reported but they were not related to vaccination. Therefore, the primary endpoint for safety for this study was met, as **no serious vaccine related**

adverse reactions were observed following ProQuad vaccination giving 95% confidence that the true rate for a serious vaccine related AE was less than or equal to 0.9%.

Of the two serious AEs reported in this study, one occurred in a ProQuad recipient and one in a child immunized with MMR + VARIVAX.

Case 1: A 15month old child with fever, vomiting and dehydration was admitted to the hospital 4 days after her second dose of ProQuad for IV hydration, antibiotics, and observation. Bacterial cultures were negative and she was discharged the next day with a diagnosis of viral illness.

Case 2: A 13month old child developed fever and acute rotavirus gastroenteritis with dehydration requiring hospitalization 21 days after her first dose of MMRII and VARIVAX.

Two other children had febrile seizures 9 days after ProQuad Dose 1 and one day after MMRII + VARIVAX. These seizures lasted one minute each and were judged not serious by the investigators who evaluated them. These events were not thought to be due to vaccination because of the timing or because there were other underlying infections (e.g., otitis media) that contributed to the illness.

8.1.7.9 **Injection Site Reactions:**

As expected, injection site reactions after ProQuad occurred slightly less frequently than injection site reactions after MMRII and VARIVAX combined with the exception of local swelling at the injection site. Injection site reactions were reported from 7.5% of children after ProQuad immunization and the most common complaints were erythema, pain, tenderness, and soreness at the injection site while 11.5% reported injection site reactions after concomitant administration of MMRII and VARIVAX. These data are summarized in Table 8.1.13 below.

Table 8.1.13 Summary of injection site reactions following immunization with ProQuad + Placebo versus MMRII + VARIVAX

Dose and Number of Subjects Vaccinated	ProQuad + Placebo Followed by ProQuad									MMRII + VARIVAX								
	Dose 1 323			Placebo			Dose 2 310			MMRII 157			VARIVAX 157			Total 157		
	N	%	VR	N	%	VR	n	%	VR	n	%	VR	N	%	VR	n	%	VR
Subjects Without Follow-up	2			2			3			1			1			1		
Subjects with Follow-up	321			321			307			156			156			156		
N/ % with one or more injection site AEs	24	**7.5**		19	**5.9**		15	**4.9**		5	**3.2**		13	**8.3**		18	**11.5**	18
Ecchymosis	3	**0.9**	3	5	**1.6**	5	1	**0.3**	1	1	**0.6**	1	5	**3.2**	5	6	**3.8**	6
Erythema	9	**2.8**	9	8	**2.5**	8	7	**2.3**	7	1	**0.6**	1	8	**5.1**	8	9	**5.8**	9
Lump	0	**0**		1	**0.3**	1	0	**0**		0	**0**		0	**0**		0	**0**	
Pain/tenderness/ Soreness	9	**2.8**	9	7	**2.2**	7	9	**2.9**	9	3	**1.9**	3	3	**1.9**	3	6	**3.8**	6
Pruritus	0	**0**		0	**0**		0	**0**		1	**0.6**	1	1	**0.6**	1	2	**1.3**	2
Rash	5	**1.6**	5	1	**0.3**	1	0	**0**		0	**0**		0	**0**		0	**0**	
Swelling	6	**1.9**	6	2	**0.6**	2	3	**1.0**	3	0	**0**		2	**1.3**	2	2	**1.3**	2

8.1.7.10 Systemic Adverse Reactions:

Systemic adverse reactions were not significantly increased in children immunized with ProQuad when compared to reports after concomitant immunization with MMRII and VARIVAX. Systemic AEs that occurred at a frequency of 1% or greater in either group are summarized in Table 8.1.14 below. AEs that were reported two times or more frequently in the ProQuad group were anorexia, sneezing, contact dermatitis, eczema, measles-like rash, and otitis media. There were also increases in nasal congestion, cough, rhinitis, wheezing, and upper respiratory tract infections reported after ProQuad Dose 2 when compared to the number reported after MMRI and VARIVAX.

Table 8.1.14 Summary of systemic adverse reactions following immunization with ProQuad + placebo versus MMRII + VARIVAX

	ProQuad + Placebo followed by ProQuad						MMRII + VARIVAX		
	Dose 1			Dose 2					
	N	%	VR	n	%	VR	n	%	VR
Number of Subjects	323			310			157		
Without follow-up	2			3			1		
With follow-up	321			307			156		
With one or more AE	241	**75.1**		189	**61.6**		108	**69.2**	
With no AE	80	**24.9**		118	**38.4**		48	**30.8**	
Body as Whole	135	**42.1**	102	79	**25.7**	33	60	**38.5**	37
Cardiovascular	1	**0.3**	0	2	**0.7**	0	2	**1.3**	0
Digestive	58	**18.1**	24	30	**9.8**	11	31	**19.9**	11
Metabolic/Nutritional/Immune	3	**0.9**	0	1	**0.3**	0	2	**1.3**	0
Nervous System/Psychiatric	46	**14.3**	36	28	**9.1**	11	21	**13.5**	17
Respiratory	78	**24.3**	30	119	**38.8**	38	30	**19.2**	9
Skin	101	**31.5**	42	35	**11.4**	9	46	**29.5**	22
Special Senses	34	**10.6**	6	19	**6.2**	2	8	**5.1**	2

8.1.7.11 **Fever:**

Although the incidence of fever ≥102 F was greater in the ProQuad group, the proportion with fever was not significantly increased when compared to the proportion with fever in MMRII + VARIVAX recipients. Fevers were of short duration and lasted mean of 1.7 days after ProQuad (median of 1 day, range of 1-7 days) and 1.5 days after MMRII + VARIVAX (median 1 day and range 1-7 days). There were more reports of high fever (≥104 F) days 5-12 after immunization in the ProQuad group (5.3%) than in the MMRII + VARIVAX group (1.9%) but there was not a statistically significant difference. See Table 8.1.15 for a summary of fevers reported in each group.

Table 8.1.15 Summary of the proportion with fever following immunization with ProQuad + placebo versus MMRII + VARIVAX

	ProQuad + Placebo followed by ProQuad				MMRII + VARIVAX	
	Dose 1		Dose 2			
	N	%	n	%	n	%
Number of Subjects	323		310		157	
Without follow-up	5		6		2	
With follow-up	318		304		155	
Maximum Temperature ≥102 F	126	**39.6**	71	**23.4**	54	**34.8**

8.1.7.12 Measles Like Rashes:

Measles like rashes reported 0-42 days after immunization occurred at a significantly higher rate after ProQuad (5.9%) than after MMRII + VARIVAX immunization (1.3%) with a risk difference of 4.6% (95% CI, 0.9-8.0) and p = 0.021. Measles like rashes 5 to 12 days after immunization also occurred significantly more frequently in the ProQuad group (5.3%) than in the MMRII + VARIVAX group (1.3%) with a p-value of 0.036. The majority of measles like rashes in the ProQuad + placebo group occurred 5-12 days after immunization. Also, 30% (6 of 20) reported fever ≥102 F coincident with rash.

8.1.7.13 Varicella Rashes:

Varicella like rashes were reported more frequently after ProQuad immunization (7/321, 2.2%) than after MMRII + VARIVAX (3/156, 1.9%) but this increase was not significant (p = 0.854).

8.1.8 Comments & Conclusions (Study 009):

8.1.8.1 The seroresponse rates to each component of the vaccine in ProQuad formulated to contain a higher potency of varicella virus were similar to immunogenicity seen after MMRII + VARIVAX.

8.1.8.2 The varicella seroresponse rates after two doses of ProQuad formulated to contain a higher potency of varicella virus were superior to immunogenicity seen after one dose of MMRII + VARIVAX.

8.1.8.3 No serious vaccine-related AEs were seen in this clinical trial. In addition, the overall incidence of adverse reactions seen after ProQuad was similar to that seen after MMRII + VARIVAX with a few exceptions.

8.1.8.4 There was a significant increase in the rate of measles like rashes reported after ProQuad immunization when compared to MMRII + VARIVAX given at separate sites. This increased incidence in measles-like rash occurred predominantly on day 5-12 after immunization and coincided with the onset of high fevers. In addition, there was a trend for higher measles antibody titers in children with measles-like rash and fever suggesting that there was an increase in measles vaccine virus replication associated with the use of ProQuad.

8.1.8.5 Increasing the potency of the varicella component in ProQuad overcame problems with virus interference encountered with vaccines formulated to contain lower varicella doses.

8.2 Trial #011:

A Dose Selection Study in Healthy Children Comparing Measles, Mumps, Rubella, and Varicella (ProQuad) Vaccine to MMRII Given Concomitantly with Process Upgrade Varicella Vaccine (PUVV) in Separate Injections

8.2.1 Objective/Rationale:

Study 011 was designed as a dose ranging study to test ProQuad using three dose levels of varicella in order to select a formulation that would give varicella immune responses equivalent to that seen after MMRII + VARIVAX immunization. It was also designed to show that the immunogenicity of the measles, mumps, and rubella components were similar to that seen after MMRII + VARIVAX immunization and to demonstrate that ProQuad was generally safe and well tolerated.

8.2.2 Design Overview:

Study 011 was a partially double-blinded, multi-center, randomized study at 18 study sites in healthy children who received ProQuad formulated to contain one of three formulations of varicella (Low, Medium or High Dose) or MMRII + VARIVAX on Day 0. ProQuad recipients also received a second dose of the same material on Day 90. Parents or legal guardians provided informed consent and subjects were randomized and vaccinated on Study Day 0 and then followed for 42 days for adverse reactions. ProQuad recipients returned on Day 90 for a second dose of the same vaccine formulation and were followed for an additional 42 days after receipt of the second dose. The study was conducted as an open label study because participants and personnel knew whether or not they would receive ProQuad or MMRII + PUVV. The study was double-blinded only with regard to the varicella formulation administered. The person assigning the allocation number, reconstituting the vaccine, and drawing the vaccine into the syringe was not blinded to vaccine assignment but did not know the ProQuad varicella dose level of the vaccine administered. Syringes were labeled with the subject's allocation number and initials prior to delivery to the blinded study person in the clinic for administration. Parents, guardians, children, study personnel administering the vaccine and performing follow-up for adverse events, were blinded to vaccine formulation until the subject had completed the study. The sponsor personnel performing the laboratory testing were blinded to group assignment, dose formulation, and dose number. The IRB at each site reviewed and approved the clinical protocol and approved the Informed Consent Form used to enroll subjects in this study. Planned enrollment was for 1520 children starting on **April 8, 1999;** the study ended on **April 3, 2000**. Subjects who provided serum samples were offered revaccination with any component of the vaccine to which they did not respond. Serum samples were obtained prior to each dose of vaccine and 6 weeks after vaccination. An overview of the study design is provided in table 8.2.1 below:

Table 8.2.1 Overview of the Study 011 Design

Time	Group 1, 2, and 3 (ProQuad + Placebo)	Group 4 (MMRII + PUVV)
Day 0	History/consent/eligibility Obtain pre-vaccine serum sample. Administer vaccine and placebo. Provide vaccination report cards.	History/consent/eligibility Obtain pre-vaccine serum sample. Administer vaccines. Provide vaccination report cards.
Day 0-42	Parents and guardians perform follow-up for Adverse Reactions	Parents and guardians perform follow-up for Adverse Reactions
Day 42	Obtain post vaccination serum sample. Collect and review vaccination report cards Collect information on exposure to measles, mumps rubella or varicella	Obtain post vaccination serum sample. Collect and review vaccination report cards Collect information on exposure to measles, mumps rubella or varicella
>Day 42 < Day 90	Inform subjects of need to return at Day 90 for second dose of ProQuad	-
Day 90	Administer second dose of ProQuad (formulation same as dose #1) and distribute VRC.	-
Days 90-132	Perform follow-up for AEs	-
Day 132	Obtain post vaccination #2 serum sample Collect and review vaccination report cards Collect information on exposure to measles, mumps rubella and varicella.	-

8.2.2.1 **Randomization:**

Subjects were randomly assigned to one of 4 treatment groups 1:1:1:1 according to a computer-generated allocation schedule provided by the Merck statistician. Each study center was given a list of unique allocation numbers and children were randomized in blocks of 8. An un-blinded study nurse assigned allocation numbers. This person also provided the vaccine for injection. Allocation numbers were not re-assigned for any reason. This nurse did not have any other direct contact with the study subject. ProQuad was provided in identical vials labeled with an allocation number, so the person re-constituting the vaccine was blinded to varicella vaccine dose. Likewise, the allocation schedule used at each site did not specify the vaccine formulation.

MMRII and VARIVAX recipients were randomized but not blinded to vaccine administered.

Subjects who signed an Informed Consent but were not randomized submitted a non-randomized CRF to report demographic data and reason for exclusion but no other data was submitted for these subjects.

8.2.2.2 **Interim analyses:**

Interim analyses were planned after 25% of the subjects received 1 injection of ProQuad and after the 6 week serum sample had been drawn and again 6 weeks after 25% of the subject received dose 2. Interim

analyses of measles, mumps, and rubella serology were for administrative purposes in order to make programmatic decisions.

8.2.2.3 **Study Population:**

The vaccines were evaluated in healthy children, 12-23 months of age who met the following criteria:

8.2.2.3.1 **Inclusion criteria**:

- Good health
- 12-23 months of age
- Negative history for varicella, shingles, measles, mumps and rubella

8.2.2.3.2 **Exclusion criteria**:

- Previous receipt of measles, mumps rubella, or varicella vaccine either alone or in any combination.
- Immune impairment or deficiency, neoplastic disease, depressed immunity from steroid or other therapy
- History of anaphylactic reaction to neomycin
- History of anaphylactic or other immediate allergic reactions subsequent to egg ingestion.
- Any exposure to measles, mumps, rubella, varicella, or shingles in the 4 weeks prior to each vaccination involving:
    - Continuous household contact
    - Playmate contact > 1 hour indoors
    - Hospital contact in the same room or prolonged face-to-face contact
    - Contact with a newborn whose mother had chickenpox 5 days or less prior to delivery or within 48 hours of delivery.
- Vaccination with an inactivated vaccine within 14 days prior to receipt of each dose of vaccine or scheduled within 42 days thereafter.
- Vaccination with a live virus vaccine within 30 days of a dose of the study vaccine or scheduled within 42 days thereafter.
- Immune globulin or any blood products administered 3 months prior to or within 2 months after each vaccination.
- Any contraindications to either MMRI or VARIVAX as stated in the package circulars.
- Any condition that in the opinion of the investigator might interfere with the evaluation of the study objectives.
- It was recommended that subjects not receive salicylates during the 6 weeks after vaccination because of aspirin use in children with varicella infection has been associated with Reye's syndrome.

8.2.2.3.3 Subjects were discontinued from the study if they developed an anaphylactic reaction after vaccine administration or if they developed varicella, measles, mumps or rubella prior to the administration of the study vaccine. Subjects who received other vaccines or

blood products before serologic follow-up samples were obtained were not necessarily discontinued from the study but their serology data may have been excluded from the group analyses.

8.2.3 Products used:

Products used in this protocol were manufactured by Merck. All clinical materials were supplied in 0.7mL single-dose vials. Vaccines were lyophilized. ProQuad vials were labeled with individual allocation numbers. Vaccines were re-supplied as needed throughout the study on a site-by-site basis. Doses were administered on Day 0, the day of entry into the study. Vaccine lot numbers and potencies are summarized in Table 8.2.2 below.

Table 8.2.2 Vaccine Lot Numbers and Potency

Vaccine	Lot Number	Fill Number	Bulk Lot Number	Potency/ 0.5mL dose	Vol. ML	Route
ProQuad Low Dose	1565W/e474	------	---------- -------- -------- --------	3.84 $log_{10}TCID_{50}$ 4.98 $log_{10}TCID_{50}$ 3.68 $log_{10}TCID_{50}$ 3.48$log_{10}PFU$	0.5	Subcutaneous
ProQuad Medium Dose	1567W/E476	-----	**-------** ---------- ---------- ----------	3.88 $log_{10}TCID_{50}$ 4.88 $log_{10}TCID_{50}$ 3.72 $log_{10}TCID_{50}$ 3.97$log_{10}PFU$	0.5	Subcutaneous
ProQuad High Dose	1568W/E477	------	---------- ---------- --------- ----------	3.94 $log_{10}TCID_{50}$ 4.77 $log_{10}TCID_{50}$ 3.65 $log_{10}TCID_{50}$ 4.25$log_{10}PFU$	0.5	Subcutaneous
PUVV	1558W/E467	------	---------	3.96 $og_{10}PFU$	0.5	Subcutaneous
MMRII	1569W/E478	-----	-------- ------- --------	3.7 $log_{10}TCID_{50}$ 5.0 $log_{10}TCID_{50}$ 3.7 $log_{10}TCID_{50}$	0.5	Subcutaneous
Diluent	1068H	NA	NA	NA	NA	NA

** Diluent: sterile water for injection.
N/A: not applicable.

8.2.4 Study Objectives:

8.2.4.1 Primary Hypothesis:

At least one dose level and regimen of ProQuad will have a similar immune response to varicella that is less than 10 percentage points different as measured by the percent of subjects with gpELISA titers ≥5 units at 6 weeks after immunization and compared to the immune response seen after with MMRII + PUVV given concomitantly at separate sites.

8.2.4.2 Secondary hypothesis (1):

At least one dose level and regimen of ProQuad will elicit similar seroresponse rates to measles, mumps, and rubella at 6 weeks after completion of the regimen as a single dose of MMRII + PUVV given concomitantly at separate sites.

8.2.4.3 Secondary hypothesis (2):

At least one dose level and regimen of ProQuad will elicit similar GMTs of measles, mumps, and rubella antibody at 6 weeks after completion of the regimen as a single dose of MMRII + PUVV given concomitantly at separate sites.

8.2.4.4 Secondary hypothesis (3):

There will be no vaccine related serious adverse reaction in any of the treatment groups in this study during the 42day follow-up period.

The first objective was to select a dose level of varicella that has a similar immune response to varicella in the control group immunized with MMRI + PUVV

The second objective was to demonstrate that the immunogenicity for measles, mumps, and rubella was similar to that seen after MMRII + PUVV immunization.

The third objective was to show that ProQuad was generally safe and well tolerated.

8.2.4.5 Study Endpoints:

Immunogenicity endpoints were measured using immunological assays that specifically measured IgG antibody responses to each vaccine virus. **Safety endpoints** were assessed using the Vaccination Report Card that was completed by each subject's parent or legal guardian.

8.2.4.5.1 **Detection of Measles IgG Antibody (ELISA):**

The measles ELISA used measles antigen purchased from ---------
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-------------------------------- The limit of detection of this assay was determined to be 2.13 measles antibody units. The quantifiable range for serum tested at a dilution of 1:160 was 2.13 to 136.15 measles antibody units. The assay precision was 23%. Pre-vaccination samples were tested at a dilution of 1:160 and were considered to be seronegative if they were below the OD cut-off of 2.13 antibody units per ml. Post vaccination samples were tested at dilutions of 1:160 and 1:1600 and were considered to be positive if they had ≥21.3 ELISA antibody units (equivalent to 207.8mIU measles antibody/mL). At a serum dilution of 1:1600 the quantifiable range of the measles ELISA was 21.3 –1360

ELISA antibody units. To convert to mIU the ELISA titer was divided by 0.1025.

8.2.4.5.2 **Detection of Mumps IgG Antibody (ELISA):**

Mumps virus antigen used for this assay was produced at MRL. The mumps antigen was ---------- The quantity of anti-mumps IgG was determined by comparing the response in the test sample to the standard curve. The cut-off was determined by running 10 replicates of the negative control serum. The assay cut-off was equivalent to the mean O.D. +0.15 for the 10 assays on the negative control serum where 0.15 was 3 S.D. of the mean of a panel of know mumps negative sera. Samples with ODs less than or equal to the cut-off were serostatus negative and assigned a titer of < 2.0 AB units. Samples with OD values greater than the cut-off were quantified using the standard curve. The quantifiable range was 2.0 to 40 mumps antibody units/mL. Sera whose titers exceeded this range were re-analyzed at greater dilutions until an endpoint titer was obtained. The negative control for the assay was a pool of human sera known to be mumps negative. The low positive control was a pool of human sera while the high positive was also a pool of human sera. A single mumps positive serum was used to generate the standard curve. The standard curve data were fit using a quadratic polynomial. The LOD was 1.75 Ab units and the quantifiable range of the assays was 1.25 to 40 mumps Ab units/mL. Samples with low titers of mumps antibody measured 1.85 fold lower at the lowest dilution tested while medium and high titers pools showed no evidence of lack of dilutability. The precision of the assay was 14%.

8.2.4.5.3 Detection of Rubella IgG (ELISA):

Inactivated rubella antigen purchased from ---------- The cut-off for the assay was determined by determining the mean OD value for 10 known rubella negative control sera plus 5 times the S.D. of the negative controls. Samples with OD values less than the cut-off were considered to be seronegative and were assigned a value of 10 AB units. The quantity of rubella antibody in positive samples was determined relative to the standard curve. The negative control for this assay was a single human serum known to be negative for rubella antibody. The low positive and high positive controls were the WHO International Standard diluted to 40 and 160 mIU/mL, respectively. The WHO reference serum was also used to generate the standard curve. Standard curve data were fit using a quadratic polynomial. The LOD was 0.91 rubella antibody

units/mL. The quantifiable range of the assay was 1-32 antibody units /mL. There was no evidence of significant dilution bias and the overall assay variability was 22.4%. A pre-vaccination sample was considered to be seronegative if it was below the OD cut-off and a post vaccination sample was considered to be seropositive if it contained ≥12.8 ELISA antibody units (=10 IU/mL). Antibody titers expressed in ELISA units were divided by 1.28 to obtain Rubella International Units (IU).

8.2.4.5.4 Detection of Varicella IgG (gp ELISA antibody):

The purpose of the glycoprotein enzyme-linked immunosorbent assay (gpELISA) was to detect IgG antibody to varicella-zoster virus (VZV) before and after vaccination with VZV-containing vaccine(s). This method detects antibodies to VZV glycoproteins (gp), which have been lectin affinity-purified from MRC-5 cells infected with the KMcC strain of VZV. The assay and the purification of the VZV gp from VZV-infected cells have been described ----------

Serum sample titers determined by gpELISA correlate with neutralizing antibody titers (Krah, DL, Cho I, Schofield T, et al. Comparison of gpELISA and neutralizing antibody responses to Oka/Merck live varicella vaccine in children and adults. Vaccine 1997 15(1):61-64.) and with protective efficacy (White CJ, Kuter BJ, Ngai A, et al. Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. Pediatr Infect Dis J 1992 11(1):19-23.).

Results for the assay are reported as concentration of antibody in gpELISA units/mL. The negative control used for this assay was an individual human serum at a dilution of 1:50, found to be negative for anti-VZV. The high positive marker was a VZV-antibody-positive serum, diluted 1:15,000, which gave a response in the assay at the upper end of the standard curve. The low positive marker was a VZV-antibody-positive serum diluted 1:50,000, which gave a response in the assay at the lower end of the standard curve. A VZV-antibody-positive individual human serum was used to generate a standard curve (range of 0.625 to 20 gpELISA units/mL).

Prior to June-2001, the standard curve was approximated using a quadratic function fit to the 0.625 to 20 gpELISA units/mL concentration range of the standard. Since June-2001, the

standard curve has been approximated using the four-parameter weighted logistic regression function. A statistical analysis comparing the two fit procedures showed that the quadratic and logistic processing methods yield similar titers (generally within 3%) when interpolating from the 0.625 to 20 gpELISA units/mL region of the standard curve.

During the validation, the limit of detection (LOD) was mathematically determined to be 0.3 gpELISA units/mL. However, because no standard concentrations below 0.625 gpELISA units/mL are run in the assay, the LOD is reported as <0.625 gpELISA units/mL. The quantifiable range of the assay is 0.625 to 20 gpELISA units/mL. Dilutability is defined as the attribute of a standard curve assay whereby it is demonstrated that a test sample can be diluted through a series, yielding equivalent titers across that series. The assay is dilutable for samples tested in the 1:500 to 1:40,000 dilution range. The precision of the assay for a sample titer was 11%. There was no statistical evidence of increased variability in test sample results due to different analysts performing the assay.

8.2.4.6 Changes in the Conduct of the Study:

8.2.4.6.1 CBER asked Merck to evaluate the measles serology using ----mIU/mL as a sero-protective cut-off. However, the LOD for this assay was ----mIU/mL which was equivalent to ----- ELISA antibody units so ----------mIU/mL was used in lieu of ---mIU/mL as the cut-off.

8.2.4.6.2 CBER asked Merck to evaluate rubella serology using 10IU/mL as a sero-protective cut-off. This corresponds to 12.8 rubella ELISA units and is above the LOD for the MRL assay.

8.2.4.6.3 The planned analysis of missing data was not conducted using multiple imputations for the evaluation of differential loss to follow-up because the loss was lower than anticipated.

8.2.4.6.4 At CBER's request, the primary varicella immunogenicity analysis was performed on subjects with a baseline antibody titer < 1.25 gpELISA units instead of on subjects with baseline titers < 5gpELISA units.

8.2.4.6.5 A procedure to identify the minimally acceptable dose was added to the original analysis.

8.2.4.6.6 The Cochran-Armitage Linear Trend test was used to examine whether or not there was a linear trend in specific AEs with increasing varicella dose. The specific AEs evaluated were: erythema/pain/tenderness 0-4 days after dose 1 or dose 2; injection site rashes 0-42 days after immunization with dose 1 or dose 2; measles-like,

rubella-like or varicella-like rashes 0-42 days after immunization and fever ≥102 F within 0-42 days after immunization after dose 1 or dose 2.

8.2.5 Surveillance

8.2.5.1 MRL conducted its own Quality Assurance and Quality Control Program and surveillance included on-site monitoring of investigators sites, on site and in-house review of clinical data and resultant databases, review of the clinical study reports and summary documents.

8.2.5.2 No formal interim analysis was performed. Pre-vaccination varicella antibody titers were monitored at approximately 25, 50 and 75% enrollment in order to insure that sufficient numbers of susceptibles (> 80% for each group) were enrolled for the primary analysis. If varicella seropositivity rates rose above 35% then the power of the study would drop below 80% and additional subjects would have been recruited into the study.

8.2.5.3 No formal surveillance for cases of measles, mumps, rubella and varicella in the community was performed. Parents and guardians were required to submit exposure surveys and children with a history of exposure after immunization and prior to the post vaccination serum sample were excluded from the immunogenicity analyses.

8.2.5.4 Follow-up visits for safety assessments and serology were as follows:

Parents filled out the Vaccination Report Cards for 42 days after each vaccination. They were required to note local and systemic AEs and record temperatures for 42 days after immunization. They were to contact study personnel immediately if any serious AEs were noted. Study personnel evaluated all children with rash immediately. Varicella-like lesions were cultured and tested by --- for varicella genome after additional informed consent was obtained from the parent/guardian.

Blinded study personnel provided follow-up and collected information regarding the adverse reactions.

8.2.6 Statistical considerations:

8.2.6.1 The primary purpose of the study was to select a varicella dose/vaccine formulation and ProQuad regimen (1 or 2 doses) that would elicit an immune response to varicella that was similar to that seen after as concomitant MMRII and PUVV immunization. The primary endpoint for evaluating the immunogenicity hypothesis was the percent of subjects whose varicella antibody titer was ≥5gpELISA units at 6 weeks after a dose of vaccine. The study population for the primary analysis was the per protocol population with varicella antibody titers < 1.25 gpELISA units at baseline.

Six one-sided, non-inferiority tests were performed to show that varicella antibody titers were similar to the responses seen after MMRII + PUVV immunization. Similarity was defined as less than 10-percentage-point decrease in seroconversion rates. The significance level was adjusted for multiplicity at the one-sided α=0.025 level. The analysis was stratified by study center. Rejection of the null hypothesis (H_0; $p_A \geq p_B$-0.10 vs. H_A: $p_A > p_B$-0.10 where p_A is the proportion with varicella antibody after ProQuad immunization and p_B is the proportion with varicella antibody after MMRII + PUVV immunization) led to the conclusion that the varicella response rates were similar in the two groups.

In order to control the overall significance level at the one-sided alpha= 0.025 level, and allow for multiple comparisons, a modified Bonferroni approach with a stepped Hochberg adjustment was used.

Three hundred and eighty subjects were enrolled in each group with the expectation that 5% would be excluded from the primary analysis because baseline varicella antibody titers were > 5 gpELISA units and an additional 10% of subjects would be lost to follow-up after each vaccination. The expected varicella response rate in the control group was 85%. Assuming that there would be at least 323 evaluable subjects in each of the ProQuad as well as the control groups, the study would have 94% power to rule out a difference of 10 percentage points or more after 1 injection. Assuming that there would be at least 285 evaluable subjects after dose 2, the study would have 92.1% power to rule out a difference of 10 percentage points or more. Power calculations were based on an alpha level of 0.025 (one-sided) for each comparison without multiplicity adjustment.

Because CBER recommended a change to the protocol to evaluate subjects with baseline varicella titers < 1.25 gpELISA units instead of < 5 gpELISA units as originally planned, there were only 307 evaluable per protocol subjects after dose 1 and the power of the study dropped from 94.0 to 92.1%. In contrast, the drop out rate after dose 1 was lower than anticipated and the power to detect a difference in the varicella responses after 2 doses of ProQuad was essentially unchanged in spite of the change to the protocol.

8.2.6.2 The first of the three secondary immunogenicity analyses for measles, mumps, and rubella responses rates consisted of 6 one-sided, non-inferiority tests of the null hypothesis H_0; $p_A \leq p_B$-0.10 vs. H_A: $p_A > p_B$-0.10 where p_A is the proportion with antibody after ProQuad immunization and p_B is the proportion with antibody after MMRII + PUVV immunization to demonstrate that the post-vaccination responses after one dose of ProQuad were similar to the responses after one dose of MMRII given with PUVV. An equivalence margin of 10% was allowed because this was a pilot study. Rejection of the null hypothesis allowed one to come to the conclusion that the immune responses to the vaccines were similar.

The primary immunogenicity analysis for measles, mumps and rubella antibody was based on the comparison of immune responses in the per-protocol population of subjects who were initially seronegative to the respective vaccine antigen.

The proportion of subjects with post-vaccination measles, mumps, and rubella antibody was adjusted for study center.

8.2.6.3 The second secondary hypothesis evaluated the similarity between ProQuad and MMRII +PUVV groups with regards to GMTs to measles, mumps, rubella, and varicella using ANOVA to compare the log titers to test the null hypothesis H_0: $GMT_A/GMT_B \leq 0.5$ (that is, less than a 2-fold decrease) against the alternative hypothesis H_0: $GMT_A/GMT_B > 0.5$ where GMT_A is the response 6 weeks after immunization with ProQuad and GMT_B is the response 6 weeks after immunization with MMRII + PUVV. The significance level for each test was controlled to ensure that the overall Type I error rate did not exceed 0.025 (one-sided test). Two-sided 95% confidence intervals for the ratio of GMTs were also provided.

8.2.6.4 The third of the secondary endpoints was an analysis for safety. The incidence of vaccine related serious adverse experiences were compared. Two additional comparisons were made of the safety profiles for ProQuad vs. MMRII + PUVV. First, for reactions that were prompted in the VRC, the proportion of subjects experiencing each AE were estimated and the 95% CI for the risk differences between treatment groups and two-sided p values presented. This includes AEs such as injection site reactions, elevated temperature, varicella-like rash, measles-like rash, rubella-like rash, and mumps-like symptoms. In addition, the risk differences between groups and the corresponding 95% confidence intervals for the risk differences were compared for adverse reactions occurring in at least 1% of subjects in any treatment group Days 0-42 after immunization.

8.2.6.5 An additional aim of this study was to identify a clinically acceptable minimum dose for the varicella component of ProQuad. The minimum clinically acceptable varicella dose was chosen to ensure that at least 76% of initially seronegative children would have post-vaccination titers of ≥5 gpELISA units. This approach to identify the minimum dose for varicella was chosen because VARIVAX was licensed in the US based on the ability to elicit ≥5 gpELISA units of varicella antibody post-vaccination, a titer that has been correlated with protection against infection. Also, in the pivotal efficacy studies in support of VARIVAX licensure, the seroresponse rate was 76%.

Therefore, the varicella dose with the lower limit of the 95% CI for the seroresponse rate equal to or greater than 76% was chosen as the minimal clinically acceptable dose for ProQuad. By choosing the minimum dose in this way, one can be 97.5% confident that at least 76% of varicella naïve children immunized

with this vaccine will develop a specific antibody response that is ≥5 gpELISA units.

8.2.6.6 Handling Drop-Outs or Missing Data:

8.2.6.6.1 An additional analysis was performed on all subjects with any valid serology and this analysis was compared to the per protocol analysis.

8.2.6.6.2 Because the drop-out rate after dose 1 was less than anticipated, all subjects who were included in the post-dose 1 analysis but who were not available for the post-dose 2 evaluation were assumed to have varicella antibody titers of < 5 gpELISA units post dose 2 and the analysis of varicella responses was repeated on this dataset.

8.2.7 Results

8.2.7.1 Populations enrolled/analyzed

8.2.7.1.1 Multi-center Study:

This study was conducted at 18 sites. The sites and principal investigators are listed in Table 8.2.3 below. Enrollment at each study site ranged from 12 to 339 and is shown in Table 8.2.4. In cases where the site did not enroll at least 10 evaluable subjects in each treatment group, this small site was combined with the largest site in the same geographic region to form a new “combined study center”. Study sites were combined until each had at least 10 evaluable subjects in each vaccine group.

Table 8.2.3 Listing of Principal Investigators and Study Sites

Combined Study Center Number	Original Study Center Number	Investigator	Location
1	011001	Reisinger, Keith	Pittsburgh, PA
	011007	Greenberg, David	
2	011004	Wheeler, J, Gary	Little Rock, AK
	011018	Stewart, Tracy	
3	011005	Moore, William	Dallas, TX
	011008	Guerrero, Juan	Austin, TX
4	011006	Allen, Brian	Onalaska, WI
	011009	Sullivan, Bradley	Marshfield, WI
	011015	Karasov, Robert	Minneapolis, MN
5	011003	Marshall, Gary	Louisville , KY
	011011	Anderson, Edwin	St. Louis, MO
6	011002	Black, Steven	Oakland, CA
	011014	Milnes, Philip	Wenatchee, WA
	011016	Bettis, Robert	Edmonds, WA
7	011010	Watson, Barbara	Philadelphia, PA
8	011012	Marchant, Colin	Boston, MA
9	011013	Meissner, Cody	Boston, MA
10	011017	Bernstein, Hank	Boston, MA

Table 8.2.4 Distribution of Study Participants by Group at Each Site:

STUDY NUMBER	PI	PROQUAD LOW DOSE	PROQUAD MED DOSE	PROQUAD HIGH DOSE	MMRII + PUVV	UNKNOWN	DILUENT	TOTAL
		N=387	N=393	N=381	N=390	N=7	N=1	
011001	REISINGER	37	40	39	38	0	0	154
011002	BLACK	85	86	84	84	0	0	339
011003	MARSHALL	14	14	14	14	0	0	56
011004	WHEELER	10	11	8	13	7	0	
011005	MOORE	6	6	5	5	0	0	22
011006	ALLEN	10	9	9	11	0	0	39
011007	GREENBERG	11	13	12	14	0	0	50
011008	GUERRERO	25	26	26	28	0	1	106
011009	SULLIVAN	10	10	10	10	0	0	40
011011	WATSON	22	20	20	20	0	0	
011012	ANDERSON	3	3	3	3	0	0	12
011013	MARCHANT	52	52	52	51	0	0	207
011014	MEISSNER	29	28	30	27	0	0	114
011015	MILNES	8	8	8	8	0	0	32
011016	KARASOV	5	6	5	5	0	0	21
011017	BETTIS	4	5	4	4	0	0	17
011018	BERNSTEIN	19	20	18	18	0	0	
011019	STEWART	37	36	34	37	0	0	144

8.2.7.1.2 1559 subjects were enrolled and 1395 completed the study. 156 subjects discontinued the study including 2 who did not receive any vaccine. The identity of the vaccine material administered was not known for 7 additional subjects and 1 other subject received diluent only. Subject accounting by vaccine group is listed in Table 8.2.5 below:

Table 8.2.5 Enrollment and Study Dropouts by Vaccine Group

	ProQuad Low Dose		**ProQuad Medium Dose**		**ProQuad High Dose**		**MMRII + PUVV**		**Unknown**		**Diluent Only**		**Total**	
	N = 387		N = 393		N = 381		N = 390		N = 7		N= 1		1559	
	n	%	n	%	n	%	N	%	N	%	n	%	N	%
Male	201		212		189		229		6		0		837	
Female	186		181		192		161		1		1		722	
Vaccinated at														
Dose 1	387	100	393	100	381	100	390	100	7	100	1	100	1559	100
Dose 2	360	93.0	365	92.9	360	94.5	0	0	3	42.9	1	100	1089	69.9
Completed	336	86.8	343	87.3	346	90.8	370	94.9	0	0	0	0	1395	89.5
Discontinued	51	13.2	50	12.7	35	9.2	20	5.1	7	100	1	100	164	10.5
Clinical AE	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Deviation from Protocol	2	0.5	3	0.8	0	0	1	0.3	7	100	1	0	14	0.9
Refusal to participate	13	3.4	16	4.1	12	3.1	3	0.8	0	0	0	0	44	2.8
Lost to follow-up	19	4.9	12	3.1	12	3.1	7	1.8	0	0	0	0	50	3.2
Discontinued due to AE	1	0.3	2	0.5	3	0.8	0	0	0	0	0	0	6	0.4
Missed bleed	13	3.4	10	2.5	6	1.6	7	1.8	0	0	0	0	36	2.3
Incomplete safety follow-up	3	0.8	7	1.8	2	0.5	2	0.5	0	0	0	0	14	0.9

8.2.7.1.3 Protocol deviations that resulted in data being excluded from the primary immunogenicity analysis are describe below:

8.2.7.1.3.1 **Reasons for exclusion after dose 1 included**:

8.2.7.1.3.2 ProQuad Low Dose: one subject received MMRII +PUVV and another developed measles.

8.2.7.1.3.3 ProQuad Medium Dose: One subject was exposed to varicella; one subject (AN 01284) received MMRII + PUVV, in addition to the study vaccine; one subject was randomized to the Low Dose group but received the Medium Dose at both visit 1 and visit 2; one subject was not vaccinated.

8.2.7.1.3.4 ProQuad High Dose: one subject received the vaccine IM and not sc; and one subject received OPV 31 days after the study vaccine.

8.2.7.1.3.5 MMRII + PUVV: one subject was younger than 12 months; one subject received an injection to boost his white blood cell count; one subject was randomized to the ProQuad High Dose

group but instead received MMRII + PUVV; one subject was not vaccinated.

8.2.7.1.3.6 Some protocol deviations occurred after the Post Dose 1 blood sample but before the second vaccination:

8.2.7.1.3.7 One subject was supposed to receive ProQuad High Dose but received MMRII +PUVV instead.

8.2.7.1.3.8 One subject in the ProQuad Middle Dose group received immune serum globulin after vaccination and was discontinued from the study.

8.2.7.1.3.9 Protocol deviations that caused subject to be excluded from the Post Dose 2 immunogenicity analyses included:

8.2.7.1.3.10 ProQuad Low Dose: one subject developed measles; 1 subject received the incorrect treatment; one subject received a non-study live virus vaccine 11 days prior and 39 days after the second vaccination; another subject also received a non-study live virus vaccine on Day 0 of the second vaccination.

8.2.7.1.3.11 ProQuad Middle Dose: one subject was exposed to varicella two days prior to the second dose; one subject received the incorrect treatment; one subject was randomized to the Low Dose group but received Medium Dose ProQuad at Visit 1 and Visit 2. 3 subjects received non-study live virus vaccines during the prohibited period.

8.2.7.1.3.12 ProQuad High Dose: one subject received the vaccine IM instead of SC.

8.2.7.1.3.13 All subjects were included in the safety analysis if follow-up data were available.

8.2.7.1.4 No subjects were prematurely un-blinded as to their dose level during the 42 days of follow-up after administration of dose 1 or dose 2. Subjects receiving MMRII + PUVV were not blinded.

8.2.7.1.5 The primary analysis of immunogenicity was based on the per-protocol population and only subjects who were initially seronegative for the vaccine antigen were evaluated. Serostatus for each vaccine antigen at baseline for each group is listed in Table 8.2.6 below:

Table 8.2.6 Serostatus for Each Vaccine Antigen at Baseline, (Pre-Vaccination)

	Exclusion for Corresponding Vaccine Components/Dose 1															
	Varicella				**Measles**				**Mumps**				**Rubella**			
	PQ Low	**PQ Med**	**PQ High**	**MMR PUVV**	**PQ Low**	**PQ Med**	**PQ High**	**MMR PUVV**	**PQ Low**	**PQ Med**	**PQ High**	**MMR PUVV**	**PQ Low**	**PQ Med**	**PQ High**	**MMR PUVV**
Subjects vaccinated at visit 1	387	393	381	390	387	393	381	390	387	393	381	390	387	393	381	390
Subjects included	310*	313*	307*	320*	324	342	323	350	334	347	331	351	335	347	333	357
Subjects excluded	77	80	74	70	63	51	58	40	53	46	50	39	52	46	48	33
Subject initially seronegative	**269***	**262***	**262***	**261***	**364**	**375**	**354**	**375**	**373**	**379**	**364**	**378**	**374**	**380**	**365**	**382**
Subjects initially seropositive	**32**	**45**	**36**	**44**	**17**	**12**	**19**	**13**	**8**	**8**	**9**	**10**	**7**	**7**	**8**	**6**
	Exclusion for Corresponding Vaccine Components/Dose 2															
Subjects vaccinated at visit 2	360	365	360	NA	360	365	360	NA	360	365	360	NA	360	365	360	NA
Subjects included	300	303	309	NA	313	328	326	NA	322	331	335	NA	319	333	335	NA
Subjects excluded	60	62	51	NA	47	37	34	NA	38	34	25	NA	41	32	25	NA
Subjects initially seropositive**	**30**	**38**	**35**	**NA *****	**16**	**11**	**19**	**NA**	**6**	**8**	**9**	**NA**	**5**	**7**	**7**	**NA**

*Subjects included in the immunogenicity analyses for antibody responses to varicella met the per protocol criteria and had baseline antibody titers that were < 1.25gpELISA units/mL but may have been > the LOD for the assay. Subjects who were seronegative for varicella antibody at baseline had antibody levels that were below the LOD in the gpELISA. **Subjects were initially seropositive prior to dose #1. ***NA: not applicable because these subjects were not given a second dose of vaccine.

8.2.7.1.6 Demographics:

Subjects in each group were comparable in terms of age, race, gender, and with regards to prior therapies or medications (see Table 8.2.7).

Table 8.2.7 Demographics of the Study Population

	PROQUAD LOW DOSE	**PROQUAD MED DOSE**	**PROQUAD HIGH DOSE**	**MMRII + PUVV**	**UNKNOWN**	**DILUENT**	**TOTAL**
	N=387	**N=393**	**N=381**	**N=390**	**N=7**	**N=1**	**1559**
Gender							
Male	201	212	189	229	6	0	837
Female	186	181	192	161	1	1	722
Age (months)							
Mean	12.9	12.9	12.9	13.0	13.4	12.0	12.9
SD	1.5	1.5	1.5	1.7	1.6		1.5
Median	12.0	12.0	12.0	12.0	13.0	12.0	12.0
Range	12-22	12-23	12-21	11-23	12-16	12	11-23
Race/Ethnicity							
African American	65	55	64	54	2	0	240
Asian Pacific	19	11	19	13	0	0	62
Caucasian	241	267	251	262	5	0	1026
Hispanic	34	44	22	25	0	0	125
Native American	1	1	1	0	0	0	3
Other	27	15	24	36	0	1	103
Specific Prior Therapy							
None	262	269	250	247	6	0	1034
One or More	125	124	131	143	1	1	525

8.2.7.2 Efficacy endpoints: Immunogenicity

8.2.7.2.1 **Primary Endpoint for Efficacy:**

The primary endpoint for efficacy was the immune response to varicella vaccine as measured by gpELISA antibody in individuals who had a gpELISA titer < 1.25gpELISA units prior to vaccination and gpELISA ≥5 after immunization with ProQuad.

According to the closed testing procedure, the antibody responses after the second dose of ProQuad High Dose were first compared to the responses in the control group. Immune responses were declared similar (p-value < 0.001) so responses after two doses of ProQuad Medium Dose were compared to the control. Immune responses for this comparison were found to be similar (p-value < 0.001) so in the next comparison the immune responses after one injection of ProQuad High Dose were compared to the control. The immune responses in this comparison were found to be similar with a p-value of 0.014. In the next set of comparisons the immune responses after two injections of ProQuad Low Dose and one injection of ProQuad Medium Dose were compared to the control. Immune responses after two injections of ProQuad Low Dose were similar to the antibody responses seen after one dose of MMRII + PUVV in the control group (p-value < 0.001) but the immune responses after one injection of ProQuad Medium Dose were not found to be similar to the control group (p-value = 0.082) and no further comparisons were made.

The comparisons indicated that each of the two injection regimens (using Low, Medium, or High Dose vaccine) and a single injection of ProQuad High Dose induced varicella antibody responses that were statistically similar (< 10 percentage points different) to the varicella responses seen in the control group immunized with MMRII + PUVV. However, antibody responses after one injection of either Low Dose ProQuad or Medium Dose ProQuad were not similar to those seen in the control group.

Seroresponse rates and varicella GMTs after one or two doses of ProQuad at each dose level as well as varicella responses in the control vaccinated group are listed in the Table 8.2.8 below. The comparisons of varicella antibody responses in each group are listed in Table 8.2.9 below.

Table 8.2.8 Seroresponse Rates and Varicella GMTs after One or Two Doses of ProQuad or after One Dose of MMRII + PUVV given concomitantly in the per-protocol Population

Varicella Antibody Responses	**ProQuad Low Dose**				**ProQuad Medium Dose**				**ProQuad High Dose**				**MMR+PUVV**	
	After 1 Injection		After 2 Injections		After 1 Injection		After 2 Injections		After 1 Injection		After 2 Injections		After 1 Injection	
	n	Response (95% CI)	N	Response (95%CI)	N	Response (95%CI)	N	Response (95%CI)	n	Response (95%CI)	n	Response (95%CI)	n	Response (95%CI)
% ≥5 gpELISA units	310	63.9% 198/310 (58.2%-69.2%)	300	99.7% 299/300 (98.2%-100%)	313	80.8% 253/313 (76.0%-85.0%)	303	100% 303/303 (98.8%-100%)	307	88.6% 272/307 (84.5%-91.9%)	309	99% 306/309 (97.2%-99.8%)	320	93.1% 298/320 (89.8%-95.6%)
Post Vaccination GMT	310	5.7 (5.0, 6.5)	300	167.7 (145.6, 193.2)	313	10.5 (9.4, 11.7)	303	381.0 (335.8, 432.4)	307	11.9 (10.8, 13.1)	309	469.4 (405.5, 543.4)	320	16.5 (15.1, 18.1)

Table 8.2.9 Comparison of Varicella gpELISA Response Rates after One or Two Doses of ProQuad vs. Immune Response Rates in Children Immunized with MMRII + PUVV

Comparison of 1 and 2 Injections of ProQuad to MMRII + PUVV						Estimated Differences ProQuad-MMRII +PUVV (95%CI)	p=Value	Conclusion
ProQuad				MMRII +PUVV				
Treatment Group	Number of Injections	N	Estimated Response	N	Estimated Response			
ProQuad Low	1	310	63.9%	**320**	**93.2%**	-29.3 (-35.4,-23.3)	NA	Not similar
ProQuad Medium		313	80.7%			-12.4(-17.8,-7.3)	0.824	Not similar
ProQuad High		**307**	**88.6%**			**-4.6(-9.4,-0.1)**	**0.014**	**Similar**
ProQuad Low	2	300	99.7%	320	93.1%	6.6(3.9,10.0)	<0.001	Similar
ProQuad Medium		303	100.0%			6.9(4.3,10.3)	<0.001	Similar
ProQuad High		309	99.0%			5.9(3.0,9.4)	<0.001	Similar

8.2.7.2.2 Secondary Endpoint for Efficacy:

Immune responses to measles, mumps, and rubella were also evaluated and compared to the control MMRII + PUVV immunized group. Seroresponse rates and GMTs post vaccination were compared for the per-protocol population that was initially seronegative.

First, the treatment-by-combined-study-center tests were performed. These analyses did not show any affect of combining treatment centers. Next, the closed testing procedure was followed in the evaluation of the ProQuad response for each vaccine antigen to see if the responses were statistically similar (i.e., < 5 percentage point decrease) to that seen following immunization with MMRII + PUVV.

Seroresponse rates after one dose of ProQuad: In the initially seronegative population, the seroresponse rates for measles, mumps, and rubella were statistically similar (< 5 percentage points different) to those seen in the control group.

The seroresponse rates for **measles** after one dose of ProQuad were 99.1%, 98.8% and 99.4% for the Low, Medium, and High Dose groups, respectively vs. 99.7% in the group immunized with MMRII +PUVV. The immune responses in each group were declared to be similar to the control group.

The seroresponse rates for **mumps** after one dose of ProQuad were 99.7%, 99.1%, and 98.2% for the Low, Medium, and High Dose groups, respectively vs. 99.7% in the group immunized with MMRII +PUVV. The immune responses in each group were declared to be similar to the control group.

The seroresponse rates for **rubella** after one dose of ProQuad were 100%, 98.8%, and 97.9% for the Low Medium and High Dose groups, respectively vs. 98.6% in the group immunized with MMRII +PUVV. The immune responses in each group were declared to be similar to the control group.

These results are summarized in Tables 8.2.10 and 8.2.11 below.

Table 8.2.10 Measles, Mumps and Rubella Antibody Responses Following Immunization with ProQuad vs. MMRII + PUVV

	ProQuad Low Dose				ProQuad Medium Dose				ProQuad High Dose				MMR+PUVV	
	After 1 Injection		After 2 Injections		After 1 Injection		After 2 Injections		After 1 Injection		After 2 Injections		After 1 Injection	
	n	Response (95%CI)	N	Response (95%CI)	n	Response (95%CI)	N	Response (95%CI)	N	Response (95%CI)	n	Response (95%CI)	n	Response (95%CI)
Measles														
% Responding	324	99.1% (97.3%, 99.8%)	313	99.4% (97.7%, 99.9%)	342	98.8% (97.0%, 99.7%)	328	99.7% (98.3%, 100%)	323	99.4% (97.8%, 99.9%)	326	100% (98.9%, 100%)	350	99.7% (98.4%, 100%)
Post Vaccination GMT	324	251.8 (229.9, 275.8)	313	549.7 (484.8, 623.4)	342	309.5 (280.8, 341.3)	328	783.0 (681.8, 899.1)	323	315.4 (286.4, 347.3)	326	747.9 (656.6, 851.7)	350	253.5 (230.5, 278.8)
GMT MIU/mL		2456.7		5363.2		3019.9		7638.6		3076.9		7297.1		2473.0
% ≥255mIU/mL	338	98.5% (96.6, 99.5)	327	99.4% (97.8, 99.9)	352	98.3% (96.3, 99.4)	337	99.7% (98.4, 100)	339	99.1% (97.4, 99.8)	344	99.7% (98.4, 100)	361	98.9% (97.2, 99.7)
Mumps														
% Responding	334	99.7% (98.3%, 100%)	322	100% (98.9%, 100%)	347	99.1% (97.5%, 99.8%)	331	99.7% (98.3%, 100%)	331	98.2% (96.1%, 99.3%)	335	100% (98.9%, 100%)	351	99.7% (98.4%, 100%)
Post Vaccination GMT	334	102.0 (90.0, 115.7)	322	277.7 (252.0, 305.9)	347	106.3 (94.4, 119.8)	331	244.1 (220.3, 270.3)	331	114.7 (101.3, 130.0)	335	286.0 (259.2, 315.7)	351	97.4 (87.5, 108.5)
Rubella														
% Responding	335	100% (98.9%, 100%)	319	100% (98.9%, 100%)	347	98.8% (97.1%, 99.7%)	333	99.7% (98.3%, 100%)	333	97.9% (95.7%, 99.2%)	335	100% (98.9%, 100%)	357	98.6% (96.8%, 99.5%)
Post Vaccination GMT	335	131.4 (119.6, 144.5)	319	263.7 (239.0, 291.0)	347	122.5 (110.7, 135.5)	333	230.7 (207.8, 256.0)	333	115.5 (104.9, 127.2)	335	254.2 (230.5, 280.3)	357	128.5 (116.5, 141.7)
GMT IU/mL		102.7		206.0		95.7		180.2		90.2		198.6		100.4
% ≥10IU/mL	339	100% (98.9, 112.3)	322	100% (98.9, 100)	350	98.9% (97.1, 99.7)	336	99.7% (98.4, 100)	337	97.9% (95.8, 99.2)	339	100% (98.9, 100)	361	98.6% (96.8, 99.5)

Table 8.2.11 Comparison of measles, mumps and rubella seroresponse rates following immunization with ProQuad versus MMRII + PUVV.

Comparison of **MEASLES** responses after 1 and 2 Injections of ProQuad to responses after MMRII + PUVV						Estimated Differences ProQuad-MMRII +PUVV (95%CI)	p=Value	Conclusion
ProQuad				MMRII +PUVV				
Treatment Group	Number of Injections	N	Estimated Response Rate	N	Estimated Response			
ProQuad Low	1	324	99.1%	350	99.7%	-0.6 (-2.4,0.9)	<0.001	Similar
ProQuad Medium		342	98.8%			-0.9 (-2.8,0.6)	<0.001	Similar
ProQuad High		323	99.4%			-0.3 (-2.0,1.1)	<0.001	Similar
ProQuad Low	2	313	99.4%	350	99.7%	-0.3 (-2.0,1.1)	<0.001	Similar
ProQuad Medium		328	99.7%			-0.0 (-1.5,1.4)	<0.001	Similar
ProQuad High		326	100.0			0.3 (-0.9,1.7)	<0.001	Similar
Comparison of **MUMPS** responses after 1 and 2 Injections of ProQuad to responses after MMRII + PUVV						Estimated Differences ProQuad-MMRII +PUVV (95%CI)	p=Value	Conclusion
ProQuad				MMRII +PUVV				
Treatment Group	Number of Injections	N	Estimated Response Rate	N	Estimated Response			
ProQuad Low	1	334	99.7%	351	99.7%	-0.0 (-1.4,1.3)	<0.001	Similar
ProQuad Medium		347	99.1%			-0.6 (-2.3,0.8)	<0.001	Similar
ProQuad High		331	98.1%			-1.6 (-3.8, -0.1)	<0.001	Similar
ProQuad Low	2	322	100.0%	351	99.7%	0.3 (-1.0, 1.6)	<0.001	Similar
ProQuad Medium		331	99.7%			-0.0 (-1.5, 1.3)	<0.001	Similar
ProQuad High		335	100.0%			0.3 (-0.9, 1.6)	<0.001	Similar
Comparison of **RUBELLA** responses after 1 and 2 Injections of ProQuad to responses after MMRII + PUVV						Estimated Differences ProQuad-MMRII +PUVV (95%CI)	p=Value	Conclusion
ProQuad				MMRII +PUVV				
Treatment Group	Number of Injections	N	Estimated Response Rate	N	Estimated Response			
ProQuad Low	1	335	100.0%	357	98.6%	1.4 (0.1, 3.3)	<0.001	Similar
ProQuad Medium		347	98.9%			0.3 (-1.6, 2.3)	<0.001	Similar
ProQuad High		333	97.9%			-0.6 (-3.0, 1.5)	<0.001	Similar
ProQuad Low	2	319	100.0%	357	98.6%	1.4 (0.1, 3.3)	<0.001	Similar
ProQuad Medium		333	99.7%			1.1 (-0.4, 3.0)	<0.001	Similar
ProQuad High		335	100.0%			1.4 (0.1, 3.3)	<0.001	Similar

8.2.7.2.3 **GMTs after one dose of ProQuad**:

The second of the secondary endpoints for immunogenicity stated that at least one dose level and regimen of ProQuad would elicit similar GMTs for varicella, measles, mumps, and rubella antibody 6 weeks after completion of the regimen as seen after one dose of MMRII + PUVV given concomitantly at separate sites. GMTs were considered to be similar if there was < a 2-fold decrease when compared to the control.

Varicella: The analyses listed in Table 8.2.12 below indicate that varicella GMTs following 1 dose of Medium Dose ProQuad or High Dose ProQuad were similar to those seen after immunization with MMRII + PUVV. Varicella GMTs after Medium or High Dose ProQuad were 0.63 and 0.72 fold different, respectively, from the GMT seen after MMRII +PUVV immunization and met the

statistical criteria for similarity and were less than two-fold different from the varicella GMT in the control group.

ProQuad Low Dose did not elicit a varicella antibody response that was similar to that seen after immunization with MMRII + PUVV. GMTs following Low Dose ProQuad were 0.35 that seen after MMRII + PUVV immunization which is more than two fold different from the GMT in the control group; the varicella GMTs for the ProQuad Low Dose groups were declared not similar to the immune responses seen in the control vaccinated group.

Measles: Measles GMTs after one dose of ProQuad were 2456.6mIU/mL, 3019.5mIU/mL, and 3077.1 mIU/mL in the Low, Medium, and High Dose groups respectively vs. 2473.2 mIU/mL in the group immunized with MMRII + PUVV. The measles GMTs for each ProQuad formulation were declared to be similar to the control group. Measles GMTs after one dose of ProQuad were 1.0, 1.2, and 1.2 fold different from the control group in the Low, Medium, and High Dose Proquad groups, respectively.

Mumps: Mumps GMTs after one dose of ProQuad were 102.0, 106.3 and 114.7 ELISA units/mL for the Low, Medium, and High Dose groups, respectively, vs. 97.4 ELISA units/mL in the group immunized with MMRII +PUVV. The mumps GMTs for each ProQuad group were declared to be similar to the control group. Mumps GMTs after one dose were 1.0, 1.1 and 1.2 fold different from the control group in the Low, Medium, and High Dose ProQuad groups, respectively.

Rubella: Rubella GMTs after one dose of ProQuad were 102.6, 95.7, and 90.2 IU/mL for the Low, Medium, and High Dose groups, respectively, vs. 100.4 IU/mL in the group immunized with MMRII +PUVV. The rubella GMTs for each ProQuad group were declared to be similar to the control group. Rubella GMTs after one dose were 1.0, 0.9, and 0.9 fold different from the control group in the Low, Medium, and High Dose ProQuad groups, respectively.

The comparisons of GMTS for varicella, measles, mumps and rubella antibody are listed in Table 8.2.12 below.

Table 8.2.12 Comparison of varicella, measles, mumps and rubella GMTs following immunization with ProQuad versus concomitant immunization with MMRII + PUVV.

Comparison of **VARICELLA GMTs** after 1 and 2 Injections of ProQuad to GMTs after MMRII + PUVV						Estimated Fold Differences ProQuad/MMRII +PUVV (95%CI)	One-Sided p=Value	Conclusion
ProQuad				MMRII +PUVV				
Treatment Group	Number of Injections	N	Estimated GMT ELISA Units	N	Estimated GMT			
ProQuad Low	1	310	5.72	320	16.56	0.35 (0.30, 0.40)	1.000	Not Similar
ProQuad Medium		313	10.50			0.63 (0.54, 0.74)	0.001	Similar
ProQuad High		307	11.95			0.72 (0.62, 0.84)	<0.001	Similar
ProQuad Low	2	300	167.73	320	16.54	10.14 (8.49, 12.11)	<0.001	Similar
ProQuad Medium		303	377.71			22.83 (19.13, 27.25)	<0.001	Similar
ProQuad High		309	465.67			28.15 (23.60, 33.57)	<0.001	Similar
Comparison of **MEASLES GMTs** after 1 and 2 Injections of ProQuad to responses after MMRII + PUVV						Estimated Fold Differences ProQuad/MMRII +PUVV (95%CI)	One-Sided p=Value	Conclusion
ProQuad				MMRII +PUVV				
Treatment Group	Number of Injections	N	Estimated GMT ELISA Units	N	Estimated GMT			
ProQuad Low	1	324	251.4	350	253.4	1.0 (0.9, 1.1)	<0.001	Similar
ProQuad Medium		342	309.2			1.2 (1.1, 1.4)	<0.001	Similar
ProQuad High		323	314.9			1.2 (1.2, 1.4)	<0.001	Similar
ProQuad Low	2	313	549.8	350	253.5	2.2 (1.8, 2.6)	<0.001	Similar
ProQuad Medium		328	781.6			3.1 (2.6, 3.6)	<0.001	Similar
ProQuad High		326	744.8			2.9 (2.5, 3.5)	<0.001	Similar
Comparison of **MUMPS** GMTs after 1 and 2 Injections of ProQuad to GMTs after MMRII + PUVV						Estimated Fold Differences ProQuad-MMRII +PUVV (95%CI)	One-Sided p=Value	Conclusion
ProQuad				MMRII +PUVV				
Treatment Group	Number of Injections	N	Estimated GMT ELISA Units	N	Estimated Response			
ProQuad Low	1	334	101.5	351	98.2	1.0 (0.9, 1.2)	<0.001	Similar
ProQuad Medium		347	106.3			1.1(0.9, 1.3)	<0.001	Similar
ProQuad High		331	114.9			1.2 (1.0, 1.4)	<0.001	Similar
ProQuad Low	2	322	276.0	351	98.5	2.8 (2.4, 3.2)	<0.001	Similar
ProQuad Medium		331	243.6			2.5 (2.2, 2.8)	<0.001	Similar
ProQuad High		335	285.3			2.9 (2.5, 3.3)	<0.001	Similar
Comparison of **RUBELLA** GMTs after 1 and 2 Injections of ProQuad to GMTs after MMRII + PUVV						Estimated Fold Differences ProQuad-MMRII +PUVV (95%CI)	One-Sided p=Value	Conclusion
ProQuad				MMRII +PUVV				
Treatment Group	Number of Injections	N	Estimated Response ELISA Units	N	Estimated Response			
ProQuad Low	1	335	130.5	357	129.1	1.0 (0.9,1.2)	<0.001	Similar
ProQuad Medium		347	122.1			0.9 (0.8, 1.1)	<0.001	Similar
ProQuad High		333	115.9			0.9 (0.8, 1.0)	<0.001	Similar
ProQuad Low	2	319	262.8	357	129.2	2.0 (1.8, 2.3)	<0.001	Similar
ProQuad Medium		333	232.1			1.8 (1.6, 2.1)	<0.001	Similar
ProQuad High		335	255.0			2.0 (1.7, 2.3)	<0.001	Similar

8.2.7.2.4 Seroresponse rates and GMTS after a second dose of ProQuad:

Immune responses (seroresponse rates and GMTs) after a second dose of ProQuad at the same varicella dose level as the first injection given 6 weeks after vaccination were compared to immune responses seen after immunization with MMRII +PUVV. These analyses were restricted to children who were seronegative for each antigen at baseline and had serum available for analysis after both dose 1 and dose 2. These data are also summarized in Table 8.2.12

Following a second dose of ProQuad, **varicella** seroresponse rates were 99.7%, 100%, and 99.0% in the Low, Medium, and High dose groups respectively compared with 93.1% after one dose of MMRII + PUVV. GMTs were 167.7, 381.0, and 469.4 gpELISA units/mL after two doses of Low, Medium or High Dose ProQuad, respectively vs. 16.5 gpELISA units/mL in the group immunized with one dose of MMRII + PUVV. Seroresponse rates and GMTs after two doses of ProQuad were statistically similar to the varicella responses seen after one dose of MMRII + PUVV. Six weeks after immunization varicella GMTs were 10.14, 22.83 and 28.15 fold higher in children immunized with a second dose of Low, Medium, or High Dose ProQuad, respectively when compared to varicella responses after one dose of MMRII + PUVV.

Following a second dose of ProQuad, **measles** seroresponse rates were 99.4%, 99.7%, and 100.0% in the Low, Medium, and High dose groups, respectively, compared with 99.7% after one dose of MMRII + PUVV. GMTs were 5363.2, 7638.6, and 7297.1 mIU/mL after two doses of Low, Medium or High Dose ProQuad, respectively, vs. 2473.0 mIU/mL in the group immunized with one dose of MMRII + PUVV. Seroresponse rates and GMTs after two doses of ProQuad were statistically similar to measles responses seen after one dose of MMRII + PUVV. The results also indicated that ProQuad Medium and High Dose elicited significantly higher measles GMTs compared to the control group. Six weeks after immunization, measles GMTs were 2.2, 3.1 and 2.9 fold higher in children immunized with a second dose of Low, Medium, or High Dose ProQuad, respectively when compared to measles responses after one dose of MMRII + PUVV.

Following a second dose of ProQuad, **mumps** seroresponse rates were 100%, 99.7%, and 100.0% in the Low, Medium, and High dose groups respectively compared with 99.7% after one dose of MMRII + PUVV. GMTs were 277.7, 244.1, and 286.0 ELISA units/mL after two doses of Low, Medium, or High Dose ProQuad, respectively vs. 97.4 ELISA units/mL in the group immunized with one dose of MMRII + PUVV. Seroresponse rates and GMTs after two doses of ProQuad were statistically similar to mumps responses seen after one dose of MMRII + PUVV. Six weeks after immunization mumps GMTs were 2.8, 2.5, and 2.9 fold higher in children immunized with a second dose of Low, Medium or High Dose ProQuad, respectively when compared to mumps responses after one dose of MMRII + PUVV.

Following a second dose of ProQuad, **rubella** seroresponse rates were 100%, 99.7%, and 100.0% in the Low, Medium, and High dose groups respectively compared with 98.6% after one dose of MMRII + PUVV. GMTs were 206.0, 180.2 and 198.6 IU/mL after two doses of Low, Medium, or High Dose ProQuad, respectively, vs. 100.4 IU/mL in the group immunized with one dose of MMRII + PUVV. Rubella seroresponse rates and GMTs after two doses of ProQuad were statistically similar to rubella responses seen after one dose of MMRII + PUVV. Six weeks after immunization rubella GMTs were 2.0, 1.8, and 2.0 fold higher in children immunized with a second dose of Low, Medium, or High Dose ProQuad, respectively when compared to rubella responses after one dose of MMRII + PUVV.

8.2.7.2.5 Additional immunogenicity endpoints that were evaluated included:

8.2.7.2.5.1 Comparisons of reverse cumulative distribution of post-vaccination antibody titers were consistent with the per protocol analysis provided above.

8.2.7.2.5.2 An analysis of all subjects with serology was consistent with the results of the per protocol analysis.

8.2.7.2.5.3 A summary of the immune responses after dose 1 and after dose 2 for seropositive subjects was provided and this showed high seroresponse rates after 2 doses of ProQuad.

8.2.7.2.6 Selection of a Minimum Clinically Acceptable Dose of Varicella in ProQuad.

VARIVAX was licensed based on the ability to elicit post vaccination antibody of ≥5 gpELISA units /mL of antibody in 76% of the initially seronegative subjects after one injection. Therefore, a logistic regression model was used to predict the response rates and 95% confidence intervals for each of the 3 varicella doses tested in Study 011 in order to determine the formulation that could be expected to induce a protective varicella immune response in at least 76% of initially seronegative children. Based on this model the lowest dose whose lower bound of the 95% confidence interval was above 76% was $3.84 \log_{10}$ PFU.

8.2.7.3 Safety endpoints:

Subjects were followed for 42 days for adverse reactions after each dose of vaccine.

8.2.7.4 Summary of Clinical Adverse Experiences:

The proportion of subjects reporting after the first injection was >96% in each vaccine group and the proportions reporting at least one AE in each group were similar with 77.8%, 76.2%, and 79.3% reporting at least one AE in the ProQuad Low, Medium, and High dose groups, respectively, and 79.5% with at least one AE in the MMRII + PUVV group. There was good compliance with reporting after a second dose of ProQuad with greater than 98% of the subjects immunized providing data for the 42 days period following ProQuad Dose 2. After a second dose of ProQuad 74.5%, 72.0%, and 72.6% reported at least one AE in the Low, Medium, and High Dose groups, respectively.

Table 8.2.13 and Table 8.2.14 summarize injections site reactions, systemic reactions and serious adverse reactions by group after Dose 1 and Dose 2, respectively.

Table 8.2.13 Summary of clinical adverse reactions reported following immunization with ProQuad Dose 1 or concomitant immunization with MMRII + PUVV.

Injection #1	**ProQuad Low Dose N=387**		**ProQuad Medium Dose N=393**		**ProQuad High Dose N=381**		**MMRII + PUVV N=390**	
	n	%	N	%	N	%	N	%
Number of subjects	387		393		381		390	
Number with follow-up	378	97.7	387	98.5	377	99.0	381	96.7
Number (%) of subjects								
With no AE	84	22.2	92	23.8	78	20.7	78	20.5
With 1 or more AE	294	77.8	295	76.2	299	79.3	303	79.5
With Injection Site Reaction	115	30.4	104	26.9	100	26.5	126	33.1
With Systemic Reaction	265	70.1	272	70.3	276	73.2	278	73.0
Vaccine Related Systemic AE	100	**26.5**	118	**30.5**	135	**35.8**	108	**28.3**
With Serious AE	2	0.5	3	0.8	1	0.3	1	0.3
With Serious Vaccine Related AE	0	0	1	0.3	0	0	0	0
Who Died	0	0	0	0	0	0	0	0
Discontinued due to AE	1	0.3	2	0.5	2	0.5	0	0

Table 8.2.14 Summary of clinical adverse reactions reported following immunization with ProQuad Dose 2

Injection #2	ProQuad Low Dose N=360		ProQuad Medium Dose N=365		ProQuad High Dose N=360	
	N	%	N	%	n	%
Number of subjects	360		365		360	
Number with follow-up	353	98.1	361	98.9	358	99.4
Number (%) of subjects						
With no AE	90	25.5	101	28.0	98	27.4
With 1 or more AE	263	74.5	260	72.0	260	72.6
With Injection Site Reaction	79	22.4	79	21.9	70	19.6
With Systemic Reaction	246	69.7	239	66.2	239	66.8
Vaccine Related Systemic AE	60	17.0	54	15.0	55	15.4
With Serious AE	1	0.3	0	0	2	0.6
With Serious Vaccine Related AE	0	0	0	0	0	0
Who Died	0	0	0	0	0	0
Discontinued due to AE	0	0	0	0	0	0

8.2.7.5 **Safety outcomes:**

Following the first dose of ProQuad the incidence of clinical adverse reactions in ProQuad Low, Medium, and High dose groups were comparable to or lower than the incidence in the control group.

Following the second dose of ProQuad, the incidence of clinical adverse reactions in the Low, Medium, and High dose groups were similar to or less than the incidence seen in the control group after 1 dose.

8.2.7.6 Serious Vaccine Related Adverse Reactions:

There were no deaths in this study.

Following Dose 1 there were seven serious adverse reactions reported: two subjects in the ProQuad Low Dose group, three subjects in the ProQuad Medium Dose group, 1 in the ProQuad High Dose group and 1 in the MMRII + PUVV group. However, only one of these subjects from the ProQuad Medium Dose group had a vaccine-related serious adverse reaction (described below).

Subject 01500 had 3 febrile seizures while hospitalized for fever and lethargy 7 days after his first injection of ProQuad Medium Dose. He experienced another febrile seizure after discharge from the hospital. Fever and seizures were thought to be possibly due to vaccination and no other cause was identified. This was the only serious adverse reaction that was considered to be vaccine-related in children immunized with ProQuad. The risk difference for serious, vaccine-related adverse reactions in the ProQuad Medium Dose group (1/387, 0.3%) compared to the control group (0/381) showed that there was not a significantly increased risk (0.3, confidence intervals of -0.7, 1.5).

Following Dose 2 of ProQuad, one subject immunized with ProQuad Low Dose, none immunized with ProQuad Medium Dose and 2 immunized

with ProQuad High Dose had serious adverse reactions although none were considered to be vaccine-related.

8.2.7.7 Injection Site Reactions:

Injection site reactions that occurred in ≥ 5% of subjects were erythema, pain/tenderness/soreness, and swelling and were seen in subjects after both the first and second injection of ProQuad as well as in children immunized with MMRII and PUVV. These data are summarized in Tables 8.2.15, 8.2.16, 8.2.17, 8.2.18, 8.2.19, 8.2.20, 8.2.21, and 8.2.22 listed below.

Following ProQuad Dose 1: Injection site reactions Days 0-4 after ProQuad were not significantly increased when compared to reactions at the MMRII or PUVV injection sites with the exception of injection site rashes that occurred more frequently after Low Dose and High Dose ProQuad (p= 0.032) relative to the same at the MMRII injection site. Injection site rashes were not significantly increased relative to the incidence of reports at the PUVV injection site. See Table 8.2.15 and 8.2.16, and 8.2.17. Similar results were seen when the analysis was limited to the quadruple negative population (see table 8.2.18).

Following ProQuad Dose 2: As expected, there were no significant increases in injection site reactions Days 0-4 after Dose 2 of ProQuad at any dose when compared to the frequency after the first injection at the MMRII injection site or at the PUVV injection site. See table 8.2.19, 8.2.20, and 8.2.21. Similar results were seen when the analysis was limited to the population that was quadruple seronegative at baseline (before the first dose of vaccine, see Table 8.2.22).

Table 8.2.15 Summary of injection site reactions following immunization with ProQuad Dose 1 versus MMRII + PUVV

Injection #1 Day 0-42	**ProQuad**						**MMRII +PUVV**			
	Low Dose N=387		Medium Dose N=393		High Dose N=381		MMRII N=390		PUVV N=390	
	n	%	N	%	n	%	n	%	n	%
Subjects with Follow-up	378		387		381		381		381	
N (%) with one or more injection site AEs	115	30.4	104	26.9	100	26.5	108	28.3	111	29.1
Ecchymosis	4	1.1	8	2.1	4	1.1	5	1.3	8	2.1
Erythema	48	12.7	43	11.1	32	8.5	56	14.7	48	12.6
Pain/tenderness/ Soreness	84	22.2	70	18.1	78	20.7	90	23.6	85	22.3
Rash	7	**1.9**	5	1.3	7	**1.9**	1	0.3	7	1.8
Swelling	29	7.7	23	5.9	30	8.0	36	9.4	32	8.4

Table 8.2.16 Comparison of injection site reactions following ProQuad Dose 1 with reactions reported after immunization with MMRII + PUVV at the MMRII injection site.

Injection #1		Comparison	ProQuad Group A			MMRII Site Group B			Risk Difference† (Group A-Group B) Percentage Point 95% CI) †	p-Value†
		Group A vs. Group B (Injection Site)	N	N	%	N	N	%		
Day 0 to 4	Erythema	ProQuad™ (Low Dose) vs. M-M-R™ II	47	378	(12.4)	55	381	(14.4)	-2.0 (-6.9, 2.9)	0.419
		ProQuad™ (Middle Dose) vs. M-M-R™ II	42	387	(10.9)				-3.6 (-8.4, 1.1)	0.135
		ProQuad™ (High Dose) vs. M-M-R™ II	29	377	(7.7)				-6.7 (-11.3, -2.3)	0.003
	Pain/tenderness/ Soreness	ProQuad™ (Low Dose) vs. M-M-R™ II	84	378	(22.2)	90	381	(23.6)	-1.4 (-7.4, 4.6)	0.647
		ProQuad™ (Middle Dose) vs. M-M-R™ II	70	387	(18.1)				-5.5 (-11.3, 0.2)	0.059
		ProQuad™ (High Dose) vs. M-M-R™ II	78	377	(20.7)				-2.9 (-8.9, 3.0)	0.331
	Swelling	ProQuad™ (Low Dose) vs. M-M-R™ II	29	378	(7.7)	36	381	(9.4)	-1.8 (-5.9, 2.3)	0.382
		ProQuad™ (Middle Dose) vs. M-M-R™ II	23	387	(5.9)				-3.5 (-7.4, 0.3)	0.068
		ProQuad™ (High Dose) vs. M-M-R™ II	29	377	(7.7)				-1.8 (-5.8, 2.3)	0.388
Days 0 to 42	Ecchymosis	ProQuad™ (Low Dose) vs. M-M-R™ II	4	378	(1.1)	5	381	(1.3)	-0.3 (-2.1, 1.5)	NA
		ProQuad™ (Middle Dose) vs. M-M-R™ II	8	387	(2.1)				0.8 (-1.2, 2.9)	
		ProQuad™ (High Dose) vs. M-M-R™ II	4	377	(1.1)				-0.3 (-2.1, 1.5)	
	Erythema	ProQuad™ (Low Dose) vs. M-M-R™ II	48	378	(12.7)	56	381	(14.7)	-2.0 (-6.9, 2.9)	NA
		ProQuad™ (Middle Dose) vs. M-M-R™ II	43	387	(11.1)				-3.6 (-8.4, 1.2)	
		ProQuad™ (High Dose) vs. M-M-R™ II	32	377	(8.5)				-6.2 (-10.9, -1.7)	
	Pain/tenderness/ Soreness	ProQuad™ (Low Dose) vs. M-M-R™ II	84	378	(22.2)	90	381	(23.6)	-1.4 (-7.4, 4.6)	NA
		ProQuad™ (Middle Dose) vs. M-M-R™ II	70	387	(18.1)				-5.5 (-11.3, 0.2)	
		ProQuad™ (High Dose) vs. M-M-R™ II	78	377	(20.7)				-2.9 (-8.9, 3.0)	
	Rash	**ProQuad™ (Low Dose) vs. M-M-R™ II**	**7**	**378**	**(1.9)**	**1**	**381**	**(0.3)**	**1.6 (0.2, 3.5)**	**0.032**
		ProQuad™ (Middle Dose) vs. M-M-R™ II	5	387	(1.3)				1.0 (-0.3, 2.8)	0.105
		ProQuad™ (High Dose) vs. M-M-R™ II	**7**	**377**	**(1.9)**				**1.6 (0.2, 3.6)**	**0.032**

Table 8.2.17 Comparison of injection site reactions following immunization with ProQuad Dose 1 Days 0 to 4 or Days 0-42 versus MMRII + PUVV at the PUVV injection site

Injection #1		Comparison	Group A			PUVV Site Group B			Risk Difference † (Group A-Group B) Percentage Point (95% CI) †	p-Value †
		Group A vs. Group B (Injection Site)	n	N	%	n	N	%		
Day 0 to 4	Erythema	ProQuad™ (Low Dose) vs. PUVV	47	378	(12.4)	44	381	(11.5)	0.9 (-3.8, 5.6)	0.708
		ProQuad™ (Middle Dose) vs. PUVV	42	387	(10.9)				-0.7 (-5.2, 3.8)	0.760
		ProQuad™ (High Dose) vs. PUVV	29	377	(7.7)				-3.9 (-8.2, 0.4)	0.072
	Pain/tenderness/ soreness	ProQuad™ (Low Dose) vs. PUVV	84	378	(22.2)	85	381	(22.3)	-0.1 (-6.0, 5.9)	0.977
		ProQuad™ (Middle Dose) vs. PUVV	70	387	(18.1)				-4.2 (-9.9, 1.5)	0.145
		ProQuad™ (High Dose) vs. PUVV	78	377	(20.7)				-1.6 (-7.5, 4.3)	0.588
	Swelling	ProQuad™ (Low Dose) vs. PUVV	29	378	(7.7)	29	381	(7.6)	0.1 (-3.8, 3.9)	0.975
		ProQuad™ (Middle Dose) vs. PUVV	23	387	(5.9)				-1.7 (-5.4, 1.9)	0.358
		ProQuad™ (High Dose) vs. PUVV	29	377	(7.7)				0.1 (-3.8, 4.0)	0.967
Days 0 to 42	Ecchymosis	ProQuad™ (Low Dose) vs. PUVV	4	378	(1.1)	8	381	(2.1)	-1.0 (-3.2, 0.9)	NA
		ProQuad™ (Middle Dose) vs. PUVV	8	387	(2.1)				-0.0 (-2.3, 2.2)	
		ProQuad™ (High Dose) vs. PUVV	4	377	(1.1)				-1.0 (-3.2, 0.9)	
	Erythema	ProQuad™ (Low Dose) vs. PUVV	48	378	(12.7)	48	381	(12.6)	0.1 (-4.7, 4.9)	NA
		ProQuad™ (Middle Dose) vs. PUVV	43	387	(11.1)				-1.5 (-6.1, 3.1)	
		ProQuad™ (High Dose) vs. PUVV	32	377	(8.5)				-4.1 (-8.6, 0.3)	
	Pain/tenderness/ soreness	ProQuad™ (Low Dose) vs. PUVV	84	378	(22.2)	85	381	(22.3)	-0.1 (-6.0, 5.9)	NA
		ProQuad™ (Middle Dose) vs. PUVV	70	387	(18.1)				-4.2 (-9.9, 1.5)	
		ProQuad™ (High Dose) vs. PUVV	78	377	(20.7)				-1.6 (-7.5, 4.3)	
	Rash	ProQuad™ (Low Dose) vs. PUVV	7	378	(1.9)	7	381	(1.8)	0.0 (-2.1, 2.2)	0.988
		ProQuad™ (Middle Dose) vs. PUVV	5	387	(1.3)				-0.5 (-2.6, 1.4)	0.543

Table 8.2.18 Injection site reactions following immunization with ProQuad Dose 1 or MMRII + PUVV in the quadruple negative population

Injection #1 Quadruple Negatives	ProQuad						MMRII +PUVV			
	Low Dose N=326		Medium Dose N=329		High Dose N=315		MMRII N=322		PUVV N=322	
	n	%	N	%	n	%	n	%	n	%
Subjects with Follow-up	318		323		311		314		314	
N(%) with one or more injection site AEs	93	29.2	85	26.3	81	26.0	89	28.3	92	29.3
Ecchymosis										
Erythema	38	11.9	34	10.5	25	8.0	48	15.3	42	13.4
Pain/tenderness/ Soreness	69	21.7	55	17.0	63	20.3	75	23.9	71	22.6
Rash	6	1.9	5	1.5	6	1.9	1	0.3	7	2.2
Swelling	24	7.5	20	6.2	25	8.0	28	8.9	25	8.0

Table 8.2.19 Summary of injection site reactions following immunization with ProQuad Dose 2

Injection #2	ProQuad					
	Low Dose N=360		Medium Dose N=365		High Dose N=360	
	N	%	n	%	n	%
Subjects with Follow-up	353		361		358	
N and % with one or more injection site AEs	79	22.4	79	21.9	70	19.6
Ecchymosis	4	1.1	4	1.1	2	0.6
Erythema	36	10.2	46	12.7	38	10.6
Pain/tenderness/ Soreness	57	16.1	52	14.4	51	14.2
Rash	2	0.6	1	0.3	2	0.6
Swelling	31	8.8	22	6.1	21	5.9

Table 8.2.20 Comparison of injection site reactions following ProQuad Dose 2 with reactions reported after 1 immunization with MMRII + PUVV at the MMRII injection site

ProQuad Injection #2		Comparison	Group A			MMRII Site Group B			Risk Difference† (Group A-Group B) Percentage Point (95% CI)†	p-Value†
			N	N	%	n	N	%		
Day 0 to 4	Erythema	ProQuad™ (Low Dose) vs. M-M-R™ II	36	353	(10.2)				-4.2 (-9.0, 0.6)	0.082
		ProQuad™ (Middle Dose) vs. M-M-R™ II	46	361	(12.7)	55	381	(14.4)	-1.7 (-6.7, 3.3)	0.502
		ProQuad™ (High Dose) vs. M-M-R™ II	37	358	(10.3)				-4.1 (-8.9, 0.7)	0.092
	Pain/Tenderness/ Soreness	ProQuad™ (Low Dose) vs. M-M-R™ II	57	353	(16.1)				-7.5 (-13.2, -1.7)	0.012
		ProQuad™ (Middle Dose) vs. M-M-R™ II	52	361	(14.4)	90	381	(23.6)	-9.2 (-14.8, -3.6)	0.001
		ProQuad™ (High Dose) vs. M-M-R™ II	51	358	(14.2)				-9.4 (-15.0, -3.8)	0.001
	Swelling	ProQuad™ (Low Dose) vs. M-M-R™ II	31	353	(8.8)				-0.7 (-4.9, 3.6)	0.754
		ProQuad™ (Middle Dose) vs. M-M-R™ II	22	361	(6.1)	36	381	(9.4)	-3.4 (-7.3, 0.5)	0.089
		ProQuad™ (High Dose) vs. M-M-R™ II	21	358	(5.9)				-3.6 (-7.5, 0.3)	0.068
Days 0 to 42	Ecchymosis	ProQuad™ (Low Dose) vs. M-M-R™ II	4	353	(1.1)				-0.2 (-2.1, 1.7)	
		ProQuad™ (Middle Dose) vs. M-M-R™ II	4	361	(1.1)	5	381	(1.3)	-0.2 (-2.1, 1.7)	NA
		ProQuad™ (High Dose) vs. M-M-R™ II	2	358	(0.6)				-0.8 (-2.6, 0.8)	
	Erythema	ProQuad™ (Low Dose) vs. M-M-R™ II	36	353	(10.2)				-4.5 (-9.3, 0.3)	
		ProQuad™ (Middle Dose) vs. M-M-R™ II	46	361	(12.7)	56	381	(14.7)	-2.0 (-6.9, 3.1)	NA
		ProQuad™ (High Dose) vs. M-M-R™ II	38	358	(10.6)				-4.1 (-8.9, 0.7)	
	Pain/Tenderness/ Soreness	ProQuad™ (Low Dose) vs. M-M-R™ II	57	353	(16.1)				-7.5 (-13.2, -1.7)	
		ProQuad™ (Middle Dose) vs. M-M-R™ II	52	361	(14.4)	90	381	(23.6)	-9.2 (-14.8, -3.6)	NA
		ProQuad™ (High Dose) vs. M-M-R™ II	51	358	(14.2)				-9.4 (-15.0, -3.8)	
	Rash	ProQuad™ (Low Dose) vs. M-M-R™ II	2	353	(0.6)				0.3 (-1.0, 1.8)	0.519
		ProQuad™ (Middle Dose) vs. M-M-R™ II	1	361	(0.3)	1	381	(0.3)	0.0 (-1.2, 1.3)	0.970
		ProQuad™ (High Dose) vs. M-M-R™ II	2	358	(0.6)				0.3 (-1.0, 1.8)	0.527

Table 8.2.21 Comparison of injection site reactions following immunization with ProQuad Dose 2 Days 0 to 4 or Days 0-42 versus 1 dose of MMRII + PUVV at the PUVV injection site

ProQuad Injection #2		Comparison	Group A			PUVV Site Group B			Risk Difference† (Group A-Group B) Percentage Point (95% CI) †	p-Value †
			N	N	%	n	N	%		
Day 0 to 4	Erythema	ProQuad™ (Low Dose) vs. PUVV	36	353	(10.2)	44	381	(11.5)	-1.4 (-5.9, 3.2)	0.558
		ProQuad™ (Middle Dose) vs. PUVV	46	361	(12.7)				1.2 (-3.5, 6.0)	0.619
		ProQuad™ (High Dose) vs. PUVV	37	358	(10.3)				-1.2 (-5.8, 3.4)	0.598
	Pain/tenderness/ /soreness	ProQuad™ (Low Dose) vs. PUVV	57	353	(16.1)	85	381	(22.3)	-6.2 (-11.8, -0.4)	0.035
		ProQuad™ (Middle Dose) vs. PUVV	52	361	(14.4)				-7.9 (-13.5, -2.3)	0.006
		ProQuad™ (High Dose) vs. PUVV	51	358	(14.2)				-8.1 (-13.6, -2.5)	0.005
	Swelling	ProQuad™ (Low Dose) vs. PUVV	31	353	(8.8)	29	381	(7.6)	1.2 (-2.8, 5.3)	0.563
		ProQuad™ (Middle Dose) vs. PUVV	22	361	(6.1)				-1.5 (-5.3, 2.2)	0.414
		ProQuad™ (High Dose) vs. PUVV	21	358	(5.9)				-1.7 (-5.5, 2.0)	0.345
Days 0 to 42	Ecchymosis	ProQuad™ (Low Dose) vs. PUVV	4	353	(1.1)	8	381	(2.1)	-1.0 (-3.1, 1.0)	NA
		ProQuad™ (Middle Dose) vs. PUVV	4	361	(1.1)				-1.0 (-3.1, 1.0)	
		ProQuad™ (High Dose) vs. PUVV	2	358	(0.6)				-1.5 (-3.6, 0.2)	
	Erythema	ProQuad™ (Low Dose) vs. PUVV	36	353	(10.2)	48	381	(12.6)	-2.4 (-7.0, 2.3)	NA
		ProQuad™ (Middle Dose) vs. PUVV	46	361	(12.7)				0.1 (-4.7, 5.0)	
		ProQuad™ (High Dose) vs. PUVV	38	358	(10.6)				-2.0 (-6.7, 2.7)	
	Pain/tenderness /soreness	ProQuad™ (Low Dose) vs. PUVV	57	353	(16.1)	85	381	(22.3)	-6.2 (-11.8, -0.4)	NA
		ProQuad™ (Middle Dose) vs. PUVV	52	361	(14.4)				-7.9 (-13.5, -2.3)	
		ProQuad™ (High Dose) vs. PUVV	51	358	(14.2)				-8.1 (-13.6, -2.5)	
	Rash	ProQuad™ (Low Dose) vs. PUVV	2	353	(0.6)	7	381	(1.8)	-1.3 (-3.3, 0.4)	0.118
		ProQuad™ (Middle Dose) vs. PUVV	1	361	(0.3)				-1.6 (-3.5, -0.1)	0.040
		ProQuad™ (High Dose) vs. PUVV	2	358	(0.6)				-1.3 (-3.3, 0.4)	0.113

Table 8.2.22. Injection site reactions following immunization with ProQuad Dose 2 **in the quadruple negative population**.

Injection #2 Quadruple Seronegative	ProQuad					
	Low Dose N=304		Medium Dose N=309		High Dose N=298	
	N	%	n	%	n	%
Subjects with Follow-up	297		305		296	
N(%) with one or more injection site AEs	68	22.9	67	22.0	57	19.3
Ecchymosis	NR	NR	NR	NR	NR	NR
Erythema	29	9.8	38	12.5	30	10.1
Pain/tenderness/ Soreness	49	16.5	45	14.8	43	14.5
Rash	2	0.7	1	0.3	1	0.3
Swelling	27	9.1	19	6.2	20	6.8

8.2.7.8 Systemic Adverse Reactions:

Following Dose 1: The rates of systemic clinical adverse reactions after the first dose of ProQuad were similar to the rates reported after immunization with MMRII +PUVV. Rates of AEs summarized by body system are listed in Table 23 below. Overall, there were 159 individual AEs compared following Dose 1 (data not shown). Although there was a 4.9% increase in AEs for body system complaints after immunization with ProQuad High Dose, there was no increase in any one specific AE that could account for the difference. Similarly, nervous system and psychiatric AEs were reported 4.3% more frequently in children immunized with ProQuad Low Dose when compared to the control group and this was due to an increase in reports of irritability after immunization. The risk differences for all other AEs in the ProQuad groups were < 1.6% different for all other comparisons. These data are summarized in Table 8.2.23.

Following Dose 2: 129 AEs were compared after ProQuad Dose 2 to the incidence of the same AE following one injection of MMRII + PUVV in the control group. For the most part, ProQuad Dose 2 was less reactogenic than MMRII + PUVV with the following exceptions: Adverse reactions related to the respiratory system were reported more frequently in children immunized with ProQuad Low, Medium, and High Dose with increases of 7.1%, 10.6% and 10.1%, respectively when compared to the control group. This was due to increases in the frequency of reporting of respiratory congestion, cough and upper respiratory tract infection. Otitis media was also reported 4.0%, 2.3% and 5.7% more frequently after ProQuad Low, Medium, and High dose immunization than MMRII + PUVV. These data are summarized in Table 8.2.24.

Table 8.2.23 Summary of systemic adverse reactions following immunization with ProQuad Dose 1 versus MMRII + PUVV

ProQuad Injection #1	ProQuad						MMR+PUVV	
	Low Dose N=387		Medium Dose N=393		High Dose N=381		N=390	
	n	%	n	%	n	%	n	%
Number of Subjects								
Without follow-up	9		6		4		9	
With follow-up	378		387		377		381	
With one or more AE	265	70.1	272	70.3	276	73.2	278	73.0
With no AE	113	29.9	115	29.7	101	26.8	103	27.0
Body as Whole	136	36.0	143	37.0	161	42.7	144	37.8
Hematologic and Lymphatic	2	0.5	2	0.5	7	1.9	6	1.6
Digestive	67	17.7	51	13.2	66	17.5	63	16.5
Metabolic/Nutritional/Immune	5	1.3	8	2.1	4	1.1	5	1.3
Nervous System/Psychiatric	65	17.2	54	14.0	54	14.3	49	12.9
Respiratory	111	29.4	127	32.8	131	34.7	137	36.0
Skin	89	23.5	99	25.6	95	25.2	95	24.9
Special Senses	57	15.1	56	14.5	53	14.1	63	16.5

Table 8.2.24 Summary of systemic adverse reactions following immunization with ProQuad Dose 2

ProQuad Injection #2	ProQuad					
	Low Dose N=360		Medium Dose N=365		High Dose N=360	
	n	%	n	%	n	%
Number of Subjects						
Without follow-up	7		4		2	
With follow-up	353		361		358	
With one or more AE	246	69.7	239	66.2	239	66.8
With no AE	107	30.3	122	33.8	119	33.2
Body as Whole	98	27.8	107	29.6	111	31.0
Musculoskeletal	2	0.6	1	0.3	5	1.4
Digestive	43	12.2	54	15.0	41	11.5
Metabolic/Nutritional/Immune	2	0.6	4	1.1	7	2.0
Nervous System/Psychiatric	32	9.1	32	8.9	33	9.2
Respiratory	152	43.1	168	46.5	165	46.1
Skin	44	12.5	49	13.6	40	11.2
Special Senses	74	21.0	57	15.8	74	20.7

8.2.7.9 Fever:

Following Dose 1: The proportion with fever ≥102 F in ProQuad Low, Medium and High Dose groups was similar to the proportion with fever in the MMRII + PUVV group. (See Tables 8.2.25 and 8.2.29). The results were similar when the analysis was limited to the quadruple negative population (see Table 8.2.26).

Following Dose 2: The proportion with fever ≥102 F in ProQuad Low, Medium, and High Dose groups was less than the proportion with fever in the MMRII + PUVV group. This was expected because ProQuad vaccinees had already been primed by prior vaccination and most were

immune (See Tables 8.2.27 and 8.2.29.) The results were similar when the analysis was limited to the population that was quadruple negative at baseline. (See Table 8.2.28)

The proportion with fevers ≥104 F after ProQuad immunization at any dose level was not significantly increased when compared to the proportion with fever ≥104 F after MMRII + PUVV.

Table 8.2.25 Summary of the proportion of subjects with fever following immunization with ProQuad Dose 1 versus MMRII + PUVV

ProQuad Injection #1	**ProQuad**						**MMRII + PUVV**	
	Low Dose N=397		Medium Dose N=393		High Dose N=381		N=390	
	N	%	N	%	N	%	n	%
Number of Subjects								
Without follow-up	9		13		8		11	
With follow-up	378		380		373		379	
Maximum Temperature ≥102 F	116	30.7	129	33.9	145	38.9	136	35.9

Table 8.2.26 Summary of the proportion of subjects with fever following immunization with ProQuad Dose 1 versus MMRII + PUVV in the quadruple negative population

Injection #1 in Quadruple Seronegatives	**ProQuad**						**MMRII + PUVV**	
	Low Dose N=326		Medium Dose N=329		High Dose N=381		N=381	
	N	%	N	%	N	%	n	%
Number of Subjects								
Without follow-up	9		12		72		69	
With follow-up	317		317		309		312	
Maximum Temperature ≥102 F	97	30.6	116	36.6	124	40.1	115	36.9

Table 8.2.27. Summary of the proportion of subjects with fever following immunization with ProQuad Dose 2

ProQuad Injection #2	**ProQuad**					
	Low Dose N=360		Medium Dose N=393		High Dose N=390	
	N	%	N	%	N	%
Number of Subjects						
Without follow-up	13		28		30	
With follow-up	347		365		360	
Maximum Temperature ≥102 F	81	23.3	98	27.4	100	28.2

Table 8.2.28 Summary of the proportion of subjects with fever following immunization with ProQuad Dose 2 in the quadruple negative population

Injection #2 in Quadruple Seronegatives	ProQuad					
	Low Dose N=304		Medium Dose N=309		High Dose N=298	
	n	%	n	%	n	%
Number of Subjects						
Without follow-up	12		7		4	
With follow-up	292		302		294	
Maximum Temperature ≥102 F	71	24.3	84	27.8	79	26.9

Table 8.2.29 Comparison of the proportion of subjects with fever following immunization between ProQuad Dose 1 or ProQuad Dose 2 (Group A) versus MMRII + PUVV (Group B).

Comparison		Group A			Group B			Risk Difference † (Group A-Group B) Percentage Points (95% Confidence Interval)†	p-Value
		n	N	%	n	N	%		
Dose 1	ProQuad™ (Low Dose) vs. M-M-R™ II + PUVV	116	378	(30.7)	136	379	(35.9)	-5.2 (-11.9, 1.5)	0.130
	ProQuad™ (Middle Dose) vs. M-M-R™ II + PUVV	129	380	(33.9)				-1.9 (-8.7, 4.9)	0.576
	ProQuad™ (High Dose) vs. M-M-R™ II + PUVV	145	373	(38.9)				3.0 (-3.9, 9.9)	0.397
Dose 2	ProQuad™ (Low Dose) vs. M-M-R™ II + PUVV	81	347	(23.3)				-12.5(-19.1, -5.9)	<0.001
	ProQuad™ (Middle Dose) vs. M-M-R™ II + PUVV	98	358	(27.4)				-8.5 (-15.2, -1.8)	0.013
	ProQuad™ (High Dose) vs. M-M-R™ II + PUVV	100	355	(28.2)				-7.7 (-14.4, -1.0)	0.025

8.2.7.10 Measles-Like Rashes:

Reporting for measles-like rashes was not significantly increased in any of the ProQuad vaccine groups compared with reporting of post-vaccination measles-like rashes for children in the control group. The majority of measles-like rashes started between Days 5-12 and the comparison of the rate of measles-like rashes for each ProQuad group was similar to the rate of rashes in the group immunized with MMRII + PUVV (See Table 8.2.30) The mean duration of rashes that occurred between days 5-12 was 5.1, 4.1, and 3.7 days in the ProQuad Low, Medium, and High Dose groups, respectively vs. 7.2 days following immunization with MMRII + PUVV (See Table 8.2.31) Rashes in all groups were generally considered to be mild (See Table 8.2.32).

Table 8.2.30 Measles-like rashes: comparison of rates after ProQuad Dose 1

Post-vaccination Day Range	Comparison ProQuad™ vs. MMRII + PUVV	ProQuad Group A			MMRII+ PUVV Group B			Risk Difference (Group A - Group B) (Percentage Points) (95% Confidence Interval)†	p-Value
		n	N	(%)	n	N	(%)		
Days 0 to 42	Low Dose	12	378	3.2	13	381	3.4	0.2 (-3.0, 2.5)	0.855
	Middle Dose	17	387	4.4				1.0 (-1.9, 3.9)	0.483
	High Dose	17	377	4.5				1.1 (-1.8, 4.1)	0.439
Days 5 to 12	Low Dose	10	378	2.6	11	381	2.9	-0.2 (-2.8, 2.3)	0.839
	Middle Dose	14	387	3.6				0.7 (-1.9, 3.4)	0.569
	High Dose	13	377	3.4				0.6 (-2.1, 3.3)	0.659

Table 8.2.31 Measles-like rashes: comparison of duration after ProQuad Dose 1

Day Range		ProQuad™ (Low Dose)	ProQuad™ (Middle Dose)	ProQuad™ (High Dose)	M-M-R™ II+PUVV
(Post-vaccination 1)	Parameter	(N=387)	(N=393)	(N=381)	(N=390)
Days 0 to 42	N	12	17	17	13
	Mean Median	4.6 5	4.2 4	4.2 4	7.0 5
	(Min, Max)	(2,8)	(2, 8)	(1, 10)	(2, 28)
Days 5 to 12	N	10	14	13	11
	Mean Median	5.1 5	4.1 4	3.7 4	7.2 4
	(Min, Max)	(2,8)	(2,7)	(1,7)	(2,28)

Table 8.2.32 Measles-like rashes: comparison of severity after ProQuad Dose 1

		ProQuad™ (Low Dose)	ProQuad™ (Middle Dose)	ProQuad™ (High Dose)	M-M-R™ II+PUVV
Day Range (Post-vaccination 1)	Reported Severity†	(N=387) n (%)	(N=393) n (%)	(N=381) n (%)	(N=390) n (%)
Days 0 to 42	Mild	8 (66.7%)	12 (70.6%)	12 (70.6%)	7 (53.8%)
	Moderate	2 (16.7%)	4 (23.5%)	4 (23.5%)	5 (38.5%)
	Severe	2 (16.7%)	1 (5.9%)	1 (5.9%)	1 (7.7%)
Days 5 to 12	Mild	6 (60.0%)	10 (71.4%)	10 (76.9%)	6 (54.5%)
	Moderate	2 (20.0%)	3 (21.4%)	2 (15.4%)	5 (45.5%)
	Severe	2 (20.0%)	1 (7.1%)	1 (7.7%)	0 (0.0%)

8.2.7.11 Varicella-like Rashes:

After Dose 1, 39 children had varicella-like rashes including 31 children immunized with ProQuad (8, Low Dose, 12 Medium Dose, and 11 High Dose) while 8 subjects in the control group reported varicella-like rashes. Ten samples were collected for testing for varicella genome by ---; one sample from the High Dose group gave a negative result and one sample from the control group was positive for vaccine virus and the remaining 8 samples were inadequate for testing. After vaccination #2, 5 subjects had varicella like rashes; only one sample for viral testing was obtained from a child in the Medium Dose group and this sample was inadequate for testing.

Varicella-like rashes were not significantly increased after dose one or dose 2 when compared to the rate of varicella like rashes in the control group immunized with one dose of MMRII + PUVV. (See Table 8.2.33)

Table 8.2.33 Comparison of measles-like, rubella-like and varicella rashes after ProQuad Dose 1 or ProQuad Dose 2 versus MMRII + PUVV

Term	Comparison Group A vs. Group B	Group A			Group B			Risk Difference + (Group A-Group B) Percentage Points (95% Confidence Interval)†	p-Value†
		n	N	%	n	N	%		
Measles-like Rash	ProQuad™ (Low Dose)-Visit 1 vs. M-M-R™ II + PUVV	12	378	(3.2)	13	381	(3.4)	-0.2 (-3.0,2.5)	0.855
	ProQuad™ (Middle Dose)-Visit 1 vs. M-M-R™ II + PUVV	17	387	(4.4)				1.0 (-1.9,3.9)	0.483
	ProQuad™ (High Dose)-Visit 1 vs. M-M-R™ II + PUVV	17	377	(4.5)				1.1 (-1.8,4.1)	0.439
	ProQuad™ (Low Dose)-Visit 2 vs. M-M-R™ II + PUVV	1	353	(0.3)				-3.1 (-5.5,-1.4)	0.002
	ProQuad™ (Middle Dose)-Visit 2 vs. M-M-R™ II + PUVV	2	361	(0.6)				-2.9 (-5.3,-1.0)	0.006
	ProQuad™ (High Dose)-Visit 2 vs. M-M-R™ II + PUVV	1	358	(0.3)				-3.1 (-5.5,-1.4)	0.002
Rubella-like Rash	ProQuad™ (Low Dose)-Visit 1 vs. M-M-R™ II + PUVV	1	378	(0.3)	1	381	(0.3)	0.0 (-1.2,1.2)	0.996
	ProQuad™ (Middle Dose)-Visit 1 vs. M-M-R™ II + PUVV	2	387	(0.5)				0.3 (-1.0,1.6)	0.572
	ProQuad™ (High Dose)-Visit 1 vs. M-M-R™ II + PUVV	1	377	(0.3)				0.0 (-1.2,1.3)	0.994
	ProQuad™ (Low Dose)-Visit 2 vs. M-M-R™ II + PUVV	0	353	(0.0)				-0.3 (-1.5,0.8)	0.336
	ProQuad™ (Middle Dose)-Visit 2 vs. M-M-R™ II + PUVV	0	361	(0.0)				-0.3 (-1.5,0.8)	0.330
	ProQuad™ (High Dose)-Visit 2 vs. M-M-R™ II + PUVV	0	358	(0.0)				-0.3 (-1.5,0.8)	0.332
Varicella-like Rash	ProQuad™ (Low Dose)-Visit 1 vs. M-M-R™ II + PUVV	8	378	(2.1)	8	381	(2.1)	0.0 (-2.2,2.3)	0.987
	ProQuad™ (Middle Dose)-Visit 1 vs. M-M-R™ II + PUVV	12	387	(3.1)				1.0 (-1.4,3.5)	0.384
	ProQuad™ (High Dose)-Visit 1 vs. M-M-R™ II + PUVV	11	377	(2.9)				0.8 (-1.5,3.3)	0.472
	ProQuad™ (Low Dose)-Visit 2 vs. M-M-R™ II + PUVV	1	353	(0.3)				-1.8 (-3.8,-0.3)	0.026
	ProQuad™ (Middle Dose)-Visit 2 vs. M-M-R™ II + PUVV	2	361	(0.6)				-1.5 (-3.6,0.1)	0.068
	ProQuad™ (High Dose)-Visit 2 vs. M-M-R™ II + PUVV	2	358	(0.6)				-1.5 (-3.6,0.2)	0.070

8.2.8 Comments & Conclusions (Study 011):

8.2.8.1 The immune response to varicella (as measured by the percent of subject with antibody titers ≥ 5 gpELISA units) in subjects with a baseline titer of <1.25 gp ELISA antibody units after ProQuad high dose (4.25 PFU log_{10}/dose) was similar to the immune response after MMRII + PUVV.

8.2.8.2 The immune response to varicella after 2 doses of ProQuad at all 3 dose levels (as measured by the percent of subject with antibody titers ≥ 5 gpELISA units) was similar to the immune response to varicella after one dose of MMRII + PUVV in subject with baseline antibody titers < 1.25 gpELISA units.

8.2.8.3 Immune responses to measles, mumps and rubella after either one or two injection of ProQuad at any varicella dose level were statistically similar to the immune response to the same antigens after one dose of MMRII + PUVV.

8.2.8.4 The minimum clinically acceptable dose of ProQuad at expiry was predicted to be 3.84log_{10} PFU/dose. In this clinical study, the lower bound of 95% CI for the seroresponse rate for varicella antibody in the group immunized with ProQuad containing varicella at a dose of 3.97 log10 PFU/dose was 76% so this was taken as the proven clinically acceptable minimum dose and this was used to set the end expiry titer for the varicella component of ProQuad.

8.2.8.5 One vaccine related serious adverse reaction (febrile seizures) was reported after Medium Dose ProQuad. In this study, there were more complaints of congestion, cough and upper respiratory tract infection in ProQuad immunized children after dose 2 than after MMRII + PUVV immunization. Otherwise, the reactogenicity profile after ProQuad immunization was similar to that seen after MMRII + PUVV.

8.3 Trial # 012:

Multi-center Study: Comparison of the Safety, Tolerability, and Immunogenicity of 3 Consistency Lots of Frozen Measles, Mumps, Rubella, and Varicella Vaccine (ProQuad) in Healthy Children

8.3.1 **Objective/Rationale:**

The primary objectives of the study were (1) To confirm manufacturing consistency of ProQuad by demonstrating that the 3 consistency lots of ProQuad elicited similar immune responses to measles, mumps, rubella, and varicella; (2) To determine whether the 3 consistency lots of ProQuad combined elicited an immune response similar to MMRII and VARIVAX given at separate sites; (3) To demonstrate that each of the three consistency lots of ProQuad elicited an acceptable immune response to measles, mumps, and rubella; (4) to demonstrate that the 3 consistency lots of ProQuad are well tolerated; (5) To evaluate the persistence of antibodies to all 4 vaccine antigens 1 year post vaccination.

8.3.2 **Design Overview:**

Partially blinded, multi-center (40 sites) randomized study in four groups of healthy children who received one of three lots of ProQuad, frozen, containing 4.40-4.73 $\log_{10}$PFU/dose of varicella (Group 1, 2 and 3) or MMRII and VARIVAX (Group 4). Parents or legal guardians provided informed consent and subjects were randomized and vaccinated on Study Day 0 and then followed for 42 days for adverse reactions. The person assigning the allocation number, reconstituting the vaccine, and drawing the vaccine into the syringe was not blinded to group assignment. Syringes were labeled with the subject's allocation number and initials and delivered to the blinded study person in the clinic for administration. Parents, guardians, children, study personnel administering the vaccine and performing follow-up for adverse events as well as all person performing serological assays were blinded to group assignments. The IRB at each site reviewed and approved the clinical protocol and the Informed Consent Form used to enroll subjects in this study. Planned enrollment was for 3400 children with 850 children per group. Enrollment started on **April 12, 2000** and ended on **May 11, 2001**. Informed consent was obtained prior to collection of the pre-vaccination blood sample. Serum samples were sometimes obtained prior to vaccination and were obtained again 6 weeks after vaccination. All subjects returned 1 year after immunization so that a blood sample could be drawn to evaluate antibody persistence and to ask about history of exposure to measles, mumps, rubella, and varicella. Subjects who provided serum samples at 42 days and 1 year after immunization were offered revaccination with any component of the vaccine to which they did not respond.

Due to an increase in measles-like rashes observed in study 009, study subjects with rashes were encouraged to return for evaluation in order to determine if rashes were due to an increase in measles viremia. Whole blood samples were collected at the time of the rash and evaluated by RT-PCR for measles virus genome. In addition, samples were collected at 2 study sites days 10-17 after immunization from children without rash as controls (from ~75-150 recipients of ProQuad and from ~75-150 recipients of MMRII + VARIVAX).

The study was conducted as an open trial with respect to assignment to ProQuad or MMRII + VARIVAX. However, parent/guardian, investigator and clinical study site personnel were blinded as to which lot of ProQuad was administered. MRL

personnel who performed the serology testing were blinded to group assignment however, they knew if they were testing a pre-vaccine or post-vaccine serum sample. The study design is summarized in Table 8.3.1.

Table 8.3.1 Summary of Study 012 Design:

Time	Procedures	
	Groups 1, 2, and 3 (ProQuad™)	Group 4 (M-M-R™ II + VARIVAX™)
Day 0	Reviewed eligibility criteria. Obtained history/consent. Obtained 5- to 10-mL blood sample. Administered 0.5 mL of ProQuad™. Distributed and reviewed instructions for vaccination report card (VRC).	Reviewed eligibility criteria. Obtained history/consent. Obtained 5- to 10-mL blood sample. Administered 0.5 mL each of M-M-R™ II and VARIVAX™ at separate injection sites. Distributed and reviewed instructions for vaccination report card (VRC).
Day 10 to 17	Obtained 3- to 5-mL blood sample (subset of ~75 to 150 subjects only).	Obtained 3- to 5-mL blood sample (subset of ~75 to 150 subjects only).
Day 0 to 42	Performed follow-up for adverse experiences using VRC.	Performed follow-up for adverse experiences using VRC.
Day 42 (-7 days/+14 days)†	Obtained 5- to 10-mL blood sample. Collected VRC and reviewed with parent/guardian. Collected exposure/disease survey information for measles, mumps, rubella, varicella, and/or zoster.	Obtained 5- to 10-mL blood sample. Collected VRC and reviewed with parent/guardian. Collected exposure/disease survey information for measles, mumps, rubella, varicella, and/or zoster.
1-year post-vaccination (-30 days/+6 months)	Obtained 5- to 10-mL blood sample. Collected exposure/disease survey for measles, mumps, rubella, varicella, and/or zoster.	Obtained 5- to 10-mL blood sample. Collected exposure/disease survey for measles, mumps, rubella, varicella, and/or zoster.
ProQuad™ Lot 1 = ProQuad™ containing a varicella dose of 4.40 log10 PFU/dose at Day 0. ProQuad™ Lot 2 = ProQuad™ containing a varicella dose of 4.61 log10 PFU/dose at Day 0. ProQuad™ Lot 3 = ProQuad™ containing a varicella dose of 4.73 log10 PFU/dose at Day 0. † This -7 days/+14 days window surrounding the Day 42 time point was provided to sites to ensure that serological follow-up was obtained close to the date specified in the protocol. A different window (27 to 84 days post-vaccination) was used for statistical purposes. COMPLETION OF THE VACCINATION REPORT CARD WAS REQUIRED FOR A FULL 42 DAYS POSTVACCINATION.		

8.3.2.1 Randomization:

Children were randomized into one of 4 groups in a 1:1:1:1 ratio at each of the 40 study sites on Day 0 at the time of enrollment and immediately after informed consent had been given. Subjects were randomly assigned to treatment groups in blocks of 8 using an allocation table supplied by Merck and randomization was performed separately at each study site. An un-blinded study person assigned allocation numbers sequentially. This person was also responsible for reconstituting vaccine. Allocation numbers were not reassigned for any reason.

Two study sites also provided a subset of control samples for RT-PCR for measles virus genome. In order to have an equal number of controls and study recipients providing blood for RT-PCR analysis, participants at these two study sites were randomized 1:1:1:3.

8.3.2.2 Interim analysis

An interim analysis was performed after 50% of the children had been immunized to review the immune responses to all 4 study antigens and to compare rates of measles-like rashes between ProQuad recipients and

children immunized with MMRII and VARIVAX. No changes were made to the study based in the results.

8.3.2.3 Study Population:

The vaccines were evaluated in healthy children 12-23 months of age who met the following criteria:

8.3.2.3.1 Inclusion criteria:

- Good health
- 12-23 months of age
- Negative history for varicella, shingles, measles, mumps, and rubella

8.3.2.3.2 Exclusion criteria:

- Previous receipt of measles, mumps, rubella, or varicella vaccine either alone or in any combination.
- Immune impairment or deficiency, neoplastic disease, depressed immunity from steroid or other therapy
- History of anaphylactic reaction to neomycin or gelatin or any other component of the vaccine.
- History of anaphylactic or other immediate allergic reactions subsequent to egg ingestion.
- Any exposure to measles, mumps, rubella, varicella or shingles in the 4 weeks prior to each vaccination involving:
    - Continuous household contact
    - Playmate contact > 1 hour indoors
    - Hospital contact in the same room or prolonged face-to-face contact
    - Contact with a newborn whose mother had chickenpox 5 days or less prior to delivery or within 48 hours of delivery.
- Vaccination with an inactivated vaccine within 14 days prior to receipt of each dose of vaccine or scheduled within 42 days thereafter.
- Vaccination with a live virus vaccine within 30 days of a dose of the study vaccine or scheduled within 42 days thereafter.
- Immune globulin or any blood products administered 3 months prior to or within 2 months after each vaccination.
- Any contraindications to either MMRII or VARIVAX as stated in the package circulars.
- Any condition that in the opinion of the investigator might interfere with the evaluation of the study objectives.
- It was recommended that subjects not receive salicylates during the 6 weeks after vaccination because aspirin use in children with varicella infection has been associated with Reye's syndrome.

8.3.2.3.3 Subjects were discontinued from the study if they developed an anaphylactic reaction after vaccine administration or if they developed varicella, measles, mumps or rubella prior to the administration of the study vaccine. Subjects who received other vaccines or blood products before serologic follow-up samples were obtained were not necessarily discontinued from

8.3.3 Products used

Products used in this protocol were manufactured by Merck. All clinical materials were supplied in 0.7mL single-dose vials. Study vaccines were re-supplied as needed throughout the study on a site-by-site basis. Doses were administered on Day 0, the day of entry into the study. Potencies of the vaccines used were calibrated to a house standard and were accurate at the time of release.

ProQuad vials were identical and identified by a 3-part, double-blinded label. Each vial had an allocation number that corresponded with a number on the allocation schedule. MMRII and VARIVAX vials were used under open-label conditions and the site could use any sample from their stored stock of vaccine. Storage temperatures were monitored and vaccines re-supplied as needed during the study. Vaccine lot numbers and potency are summarized in Table 8.3.2 below.

Table 8.3.2 Vaccine Lot Numbers and Potency

Vaccine	Lot Number	Fill Number	Bulk Number(s)	Potency/ 0.5 mL dose
ProQuad™	1592/WG698	----	---- ---- ---- -------	4.10 ($log_{10}TCID_{50}$) 4.97 ($log_{10}TCID_{50}$) 4.22 ($log10TCID_{50}$) 4.40 $log_{10}PFU$
	1593/WG699	-------	---------- ---------- ---------- ----------	3.97 ($log_{10T}CID_{50}$) 4.65 ($log_{10}TCID_{50}$) 4.25 ($log_{10T}CID_{50}$) 4.61 log_{10} PFU
	1594/WG700	----	---------- ---------- ---------- ----------	3.88 ($log_{10}TCID_{50}$) 4.50 ($log_{10}TCID_{50}$) 4.22 ($log_{10}TCID_{50}$) 4.73 log_{10} PFU
M-M-R™ II	1676J	----	--------- -------- --------	3.8 ($log_{10}TCID_{50}$) 5.0 ($log_{10}TCID_{50}$) 3.7 ($log_{10}TCID_{50}$)
VARIVAX™	1681J 1686J 0443K	---- ---- ----	------ ------- --------	3.6 log_{10} PFU 3.6 $log_{10}PFU$ 3.5 $log_{10}PFU$
Diluent	1585J 0864H 1941J 0361K	NA NA NA NA	NA NA NA NA	NA NA NA NA
NA = Not applicable.				

*PGS is phosphate, glutamate, sorbitol stabilizer. It is reconstituted using sterile diluent.
** Diluent: sterile water for injection.

8.3.4 Study Objectives:

8.3.4.1 Primary Hypothesis, Immunogenicity:

The three consistency lots of ProQuad will elicit similar immune responses to measles, mumps, rubella, and varicella 6 weeks post vaccination.

The primary endpoints for measles, mumps, and rubella were the respective response rates and GMTs in initially seronegative subjects. The primary endpoints for varicella were the percent of subjects with varicella antibody titers ≥5 gpELISA units in subjects with baseline varicella antibody titers < 1.25 gpELISA units, and GMTs.

The statistical criterion for non-inferiority required that the 3 lots did not differ by more than δ=5 percentage points for measles, mumps and rubella and not more than δ=10 percentage points for varicella, i.e., the two sided 90% confidence interval for the difference in response rates between any pairs of lots was entirely within (-δ%, δ%) for each antigen.

The statistical criterion for consistency with respect to GMTs requires that the two-sided 90% confidence interval for the ratio between any pair of the three lots be entirely within (0.67-1.5) for each antigen.

Immune responses to each of the three lots were found to be consistent according to these criteria, and the data for the three lots were pooled and the following hypothesis addressed:

8.3.4.2 Secondary Hypothesis (1):

The administration of 1 dose of ProQuad will elicit similar seroconversion rates to measles, mumps, and rubella at 6 weeks post-vaccination as the concomitant administration of 1 dose of the currently licensed VARIVAX™ and MMRII™.

(The primary endpoint for measles, mumps, and rubella are the respective response rates and GMTs in initially seronegative subjects. The primary endpoint for varicella was the percent of subject that achieved a varicella antibody titer ≥ 5gpELISA units in subjects whose baseline titer was < 1.25 gpELISA units.

The statistical criteria required that the seroconversion rates for measles, mumps, and rubella were no more than δ=5 percentage points lower and the seroconversion rate for varicella was no more than δ=10 percentage points lower than the corresponding rates in the control group, i.e., the 90% confidence interval for the difference in seroconversion rates [treatment vs. control] will not include a decrease of δ percentage points or more.

The statistical criterion for similarity with respect to GMTs: GMTs would be considered similar if the lower bound of the two-sided 90% confidence interval on the ratio of the GMT ProQuad/GMT control was > 0.67 for each antigen, i.e., less than a 1.5 fold decrease.

8.3.4.3 Secondary hypothesis (2):

In addition to establishing consistency and non-inferiority, the study must also demonstrate an acceptable immune response to each vaccine antigen:

The statistical criterion for an acceptable immune response to measles, mumps, and rubella required that the lower bound of the two-sided 95% confidence interval for each respective response rate be entirely above 90%. The statistical criterion for an acceptable immune response to varicella required that the lower bound of the two-sided 95% confidence interval on the percent of subjects that achieved a titer ≥5gpELISA units be entirely above 76%.

8.3.4.4 Secondary hypothesis (3):

There will be no vaccine related serious adverse reactions in any of the groups receiving ProQuad during the 42-day follow-up period. If no serious AEs are observed in the 2550 subjects enrolled, then the 95% two-sided confidence interval for the true rate is 0.0% to 0.14%.

8.3.4.4.1 Study objectives:

8.3.4.4.1.1 Demonstrate that the three consistency lots of ProQuad will elicit similar immune responses to measles, mumps, rubella, and varicella.
8.3.4.4.1.2 Determine if immune responses to ProQuad are similar to the immune responses elicited by MMRII + VARIVAX given concomitantly but at separate injection sites.
8.3.4.4.1.3 Demonstrate that each of the three lots of ProQuad elicits an acceptable immune response to each vaccine antigen.
8.3.4.4.1.4 Evaluate the persistence of antibody to each of the 4 vaccine antigens 1 year after vaccination.

8.3.4.5 <u>Study Endpoints</u>:

Immunogenicity endpoints were measured using immunological assays that specifically measured IgG antibody responses to each vaccine virus. **Safety endpoints** were assessed using the Vaccination Report Card that was completed by each subject's parent or legal guardian.

8.3.4.5.1 **Detection of Measles IgG Antibody (ELISA):**

The measles ELISA used measles antigen purchased from --------- -- -- -- -- ---------------------- The limit of detection of this assay was determined to be 120mIU/mL for samples tested at 1:1000 dilution. The quantifiable range for samples diluted 1:1000 was 120-7680mIU/mL equivalent to 12.3 to 787 measles antibody units. The assay precision was 23%. Samples were considered to be seronegative if they were below the OD cut-off and samples were considered to be seropositive if they had ≥12.3 ELISA antibody units (equivalent to 120mIU measles antibody/mL).

8.3.4.5.2 **Detection of Mumps IgG Antibody (ELISA):**

Mumps virus (Jeryl-Lynn, wild type) antigen used for this assay was produced at MRL. The mumps antigen was ---------------------- -- -- -- -------------------- The quantity of anti-mumps IgG was determined by comparing the response in the test sample to the standard curve. The cut-off was determined by running 72 mumps negative control sera. The assay cut-off was equivalent to < 10.0 AB units. Samples with OD values greater than the cut-off were quantified using the standard curve. The quantifiable range was 0.5 to 64 mumps Ab units/mL. Sera whose titers exceeded this range were re-analyzed at greater dilutions until an endpoint titer was obtained. The negative control for the assay was a pool of human sera known to be mumps antibody negative. The low positive control was a pool of human sera while the high positive was also

a pool of human sera. A single mumps positive serum was used to generate the standard curve. Standard curve data were fit using a quadratic polynomial. The LOD was < 0.5 Ab units. Samples with low mumps antibody titers measured 1.85 fold lower at the lowest dilution tested while medium and high titers pools showed no evidence of lack of dilutability. The precision of the assay was 18.9-25.3%.

8.3.4.5.3 **Detection of Rubella IgG (ELISA):**

Inactivated rubella antigen purchased from ----------

---------- The cut-off for the assay was determined by determining them mean OD value for 10 known rubella negative control sera plus 5 times the Standard deviation of the negative controls. Samples with OD values less than the cut-off were considered to be seronegative and were assigned a value of 10 AB units. Positive samples were quantified relative to the standard curve. The negative control for this assay was a single human serum known to be negative for rubella antibody. The low positive and high positive controls were the WHO International Standard diluted to 40 and 160 mIU/mL. The WHO reference serum was also used to generate the standard curve. Standard curve data were fit using a quadratic polynomial. The LOD was < 0.005 IU/mL rubella antibody units/mL. The quantifiable range of the assay was0.005-0.32 IU/mL (equivalent to 6.4-409.6 ELISA antibody units). There was some evidence of dilution bias but titers varied < 4 fold and the overall assay variability was 14%. A pre-vaccination sample was considered to be seronegative if it was below the OD cut-off and a post vaccination sample was considered to be seropositive if it contained ≥12.8 ELISA antibody units (=10 IU/mL).

8.3.4.5.4 **Varicella gp ELISA**:

The purpose of the glycoprotein enzyme-linked immunosorbent assay (gpELISA) was to detect IgG antibody to varicella-zoster virus (VZV) before and after vaccination with VZV-containing vaccine(s). This method detects antibodies to VZV glycoproteins (gp), which have been lectin affinity-purified from MRC-5 cells infected with the KMcC strain of VZV. The assay and the purification of the VZV gp from VZV-infected cells have been described ----------
---

Serum sample titers determined by gpELISA correlate with neutralizing antibody titers (Krah, DL, Cho I, Schofield T, et al. Comparison of gpELISA and neutralizing antibody responses to Oka/Merck live varicella vaccine in children and adults. Vaccine 1997 15(1):61-64.) and with protective efficacy (White CJ, Kuter BJ, Ngai A, et al. Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. Pediatr Infect Dis J 1992 11(1):19-23.).

Results for the assay are reported as concentration of antibody in gpELISA units/mL. The negative control used for this assay was an individual human serum at a dilution of 1:50, found to be negative for anti-VZV. The high positive marker was a VZV-antibody-positive serum, diluted 1:15,000, which gave a response in the assay at the upper end of the standard curve. The low positive marker was a VZV-antibody-positive serum diluted 1:50,000, which gave a response in the assay at the lower end of the standard curve. A VZV-antibody-positive individual human serum was used to generate a standard curve (range of 0.625 to 20 gpELISA units/mL).

Prior to June-2001, the standard curve was approximated using a quadratic function fit to the 0.625 to 20 gpELISA units/mL concentration range of the standard. Since June-2001, the standard curve has been approximated using the four-parameter weighted logistic regression function. A statistical analysis comparing the two fit procedures showed that the quadratic and logistic processing methods yield similar titers (generally within 3%) when interpolating from the 0.625 to 20 gpELISA units/mL region of the standard curve.

During the validation, the limit of detection (LOD) was mathematically determined to be 0.3 gpELISA units/mL. However, because no standard concentrations below 0.625 gpELISA units/mL are run in the assay, the LOD is reported as <0.625 gpELISA units/mL. The quantifiable range of the assay is 0.625 to 20 gpELISA units/mL. Dilutability is defined as the attribute of a standard curve assay whereby it is demonstrated that a test sample can be diluted through a series, yielding equivalent titers across that series. The assay is dilutable for samples tested in the 1:500 to 1:40,000 dilution range. The precision of the assay for a sample titer was 11%. There was no statistical evidence of increased variability in test sample results due to different analysts performing the assay.

8.3.4.6 Changes in the Conduct of the Study:

8.3.4.6.1 CBER asked Merck to evaluate the measles serology using 120mIU/mL as a sero-protective cut-off.

8.3.4.6.2 CBER asked Merck to evaluate rubella serology using 10IU/mL as a sero-protective cut-off.

8.3.4.6.3 A planned analysis of missing data was not conducted because so little data was missing.

8.3.5 Surveillance

8.3.5.1 MRL conducts its own Quality Assurance and Quality Control Program and surveillance included on-site monitoring of investigators, on site and in-house review of clinical data and resultant databases, review of the clinical study reports and summary documents.

8.3.5.2 No formal interim analysis was performed.

8.3.5.3 There was no formal surveillance for cases of measles, mumps, rubella or varicella in the community. Parents and guardians noted any exposure to these diseases or diagnosis of measles, mumps, rubella or varicella in vaccinees.

8.3.5.4 Follow-up visits for safety assessments and serology are summarized in Table 8.3. 3 and were as follows:

Table 8.3.3 Schedule for follow-up visits

Time	Group A (Proquad + Placebo)	Group B (MMRII + Varivax)
Day 0	History/consent/eligibility Obtain pre-vaccine serum sample. Administer vaccine and placebo. Provide vaccination report cards.	History/consent/eligibility Obtain pre-vaccine serum sample. Administer vaccines. Provide vaccination report cards.
Days 7,14, 21	First 10 vaccinees: telephone calls for serious AEs	First 5 vaccinees: telephone calls for serious AEs
Day 0-42	Parents and guardians perform follow-up for Adverse Reactions	Parents and guardians perform follow-up for Adverse Reactions
Day 42	Obtain post vaccination serum sample. Collect and review vaccination report cards Collect information on exposure to measles, mumps rubella or varicella	Obtain post vaccination serum sample. Collect and review vaccination report cards Collect information on exposure to measles, mumps rubella or varicella
Day 100	Obtain post vaccination #2 serum sample Collect and review vaccination report cards Collect information on exposure to measles, mumps rubella and varicella.	

Parents completed the Vaccination Report Cards for 42 days after vaccination. They were required to note local and systemic AEs and record temperatures for 42 days after immunization. They were to contact study personnel immediately if any serious AEs were noted. Study personnel immediately saw all children with rashes who were brought in for evaluation. Blood samples were obtained from children with measles-like rashes for testing by RT-PCR. Varicella-like lesions were cultured and tested by --- after informed consent was obtained from the parent/guardian.

Local reactions: included pain soreness, tenderness, redness, swelling, rash (like chickenpox) and other local reactions. Severity and duration were also noted.

Temperatures: fever was defined as a body temperature ≥102 F oral or equivalent (101 F, axillary or 103 F, rectal) or reported as "warm to touch". For each fever, the investigator recorded maximum intensity, seriousness, action taken and relationship to vaccine.

Serious AEs: an AE that resulted in death or that was immediately life threatening, resulted in permanent or substantial disability, prolonged hospitalization or required medical or surgical intervention to prevent it from becoming serious.

Rashes: for any measles-like rash, a whole blood sample was obtained for RT-PCR for measles genome and acute and convalescent blood for testing. Photographs were also taken. Additional informed consent was requested and obtained before obtaining blood or taking pictures.

Blinded study personnel provided follow-up and collected information regarding the adverse reactions.

8.3.6 Statistical considerations:

Data from subjects were evaluated on a per protocol basis. Subjects included in the immunogenicity analysis were seronegative (or had varicella antibody titers < 1.25 gpELISA units) at baseline and provided serum samples within a pre-specified time frame and included only subjects providing both pre-vaccination and post-vaccination samples. The analysis of response rates tests a non-zero difference in proportions based on a method proposed by Farrington and Manning. The analysis of GMTs used an ANOVA model with the natural log of the individual titers as the dependent variable. The analysis of acceptable immune response used two-sided 95% confidence intervals on the response rate for each antigen.

The analysis of response rates was adjusted for study center and the analysis of GMTs adjusted for study center and treatment-by-center interaction.

A secondary immunogenicity analysis evaluated responses in all subjects with serology regardless of initial serostatus.

This was a multi-center study conducted at 40 study sites. Enrollment at each site ranged from 1-1511. Centers with < 10 evaluable subjects were pooled with other sites from the same geographic region until there were at least 40 evaluable subjects. This process produced 20 pooled centers

8.3.6.1 Lot-to-lot Consistency:

The primary purpose of the study was to demonstrate similarity in the immune responses among three lots of ProQuad for each vaccine antigen. A consistent antibody response for each antigen required similar seroresponse rates for each pair wise comparison as well as similar GMTS for each pair wise comparison 6 weeks after immunization.

For **measles**, the vaccine response rate was the percent of subjects with post-vaccination antibody titers ≥120 mIU/mL by ELISA. For **mumps** the vaccine response rate was the percent of subjects that achieved post-vaccination antibody titers ≥10 ELISA units. For **rubella**, the vaccine response rate was the percent of subjects that achieved a post-vaccination antibody titer of 12.8 ELISA antibody units or 10 IU/mL. For **varicella** the vaccine response rate was the percent of subjects that achieved a post-vaccination antibody titer of ≥5gpELISA units/mL. The primary immunogenicity analysis was based on subjects initially seronegative prior to vaccination (baseline measles antibody titer < 120mIU/mL, baseline mumps antibody titer < 10 ELISA units/mL, baseline rubella antibody titer < 10IU/mL and baseline varicella antibody titer < 1.25 gpELISA units/mL).

For the comparison of response rates: For each antigen, 2, one-sided tests were conducted at α = 0.05 level for each pair of lots to show equivalence. A difference in response rates greater than 5% for measles, mumps or rubella or 10% for varicella corresponded to the 90% two-sided confidence interval for each pair of lots and would lead to rejection of the null hypothesis. If response rates were entirely contained within the 90% two-sided confidence interval, then similarity was declared for that pair of lots. If similarity was declared for each of the three pair wise comparisons within an antigen, then it was concluded that the three lots were consistently immunogenic.

850 subjects were enrolled assuming 85% of those would be evaluable (5% seropositive at baseline and 10% lost to follow-up). The study had 97.8% power to establish consistency in response rates for measles, mumps, and rubella with < 5 percentage points difference and α=0.05 level and 99.9% power for demonstrating consistency in the varicella response rate with < 10 percentage points difference in a test conducted at α=0.05 level. If the response to each antigen was considered to be independent, then the overall power of the study to show consistency was 93.5%.

For the comparison of GMTS: For each antigen, two, one-sided tests were conducted at α=0.05 for each pair of lots to show equivalence with a δ= 1.5 fold for all 4 antigens. The null hypothesis was rejected if there was a greater than 1.5 fold difference in GMTs which corresponded to the 90% two-sided confidence interval. If the GMTs were less than 1.5 fold different, then similarity was declared. If each of the three pair wise comparisons were found to be similar, then it was concluded that there was a consistent immune response to that antigen among the three lots of ProQuad.

Using the same assumptions as noted above, this study had >99.9% power to establish consistency in the GMTs for measles, mumps, rubella, and varicella and assuming that the immune response to each antigen was independent, the overall power of the study to show consistency as was still >99.9%.

Consistency of ProQuad lots required rejection of all 6 null hypotheses for response rates and all 6 null hypotheses for GMTs for each antigen. No multiplicity adjustments were made.

8.3.6.2 Similarity of ProQuad Immune Responses to Responses Seen after Vaccination with MMRII and VARIVAX:

Once consistent antibody responses for measles, mumps, rubella, and varicella were demonstrated, the results for the three ProQuad lots were combined and compared to the control group that was immunized with MMRII and VARIVAX at separate sites to demonstrate a similar antibody response to each vaccine antigen. Vaccine response rates and GMTs were compared using 8, one-sided, non-inferiority tests. For each antigen a one-sided test was conducted at the α=0.05 level (< 5 percentage points for measles, mumps, and rubella and < 10 percentage points for varicella response rates and <1.5 fold difference in GMTs for each vaccine antigen). Rejection of the 8, one-sided, null hypotheses led to the conclusion that the immune responses to ProQuad were similar to the immune responses to MMRII + VARIVAX. No multiplicity adjustments were made.

The following assumptions were made in estimating the power of the study: 2550 subjects were enrolled and of those, 85% were evaluable (~2166) while 850 subjects received MMRII + VARIVAX with 85% of the subjects evaluable (~722). Response rates for measles, mumps, rubella and varicella were assumed to be 95, 95, 95 and 90%, respectively and the study had 99.95% power for each comparison of measles, mumps and rubella responses and >99.9% power for the varicella comparison.

For establishing similarity of GMTs, an additional assumption was made: the standard deviation of the natural log of the GMT for each antigen was 1.2. The study had 99.9% power to demonstrate that GMTs for each vaccine antigen were similar between groups.

8.3.6.3 Acceptability of Immune Responses for Each Antigen In ProQuad:

The third hypothesis addressed whether the immune responses to each vaccine antigen were acceptable, i.e., at least 90% for measles, mumps and rubella and at least 76% for varicella. Acceptability was determined on a lot-by-lot and antigen-by- antigen basis using 12, one-sided, one-sample binomial tests conducted at the one-sided, α=0.025 significance level. Success required rejection of all three hypotheses for each antigen and no multiplicity adjustments were made.

The same assumptions were made regarding samples size (850 enrolled per lot) and drop-out rates (~15% leaving 722 evaluable subjects per lot). Testing against the 90% lower bound and assuming response rates of 95% for measles, mumps, and rubella, the study had 99.97% power to establish acceptable immune response for each lot and each antigen. Likewise, when the immune response to varicella was tested against the 76% lower bound, with an expected response rate of 90%, the study had 99.9% power to establish an acceptable immune response.

If consistency, similarity, and acceptability hypotheses are assumed to be independent, the overall power of the primary immunogenicity hypotheses was 92.3%

8.3.6.4 Primary Endpoint for Safety:

The primary endpoint for safety was the incidence of serious vaccine related adverse reactions in subjects immunized with ProQuad.

With 850 subjects per group, the study had 97.5% power to detect a 5percentage point increase in incidence rates from 5 to 10%. When comparing ProQuad to MMRII + VARIVAX, this study had 99.8% power to detect a 5 percentage point increase in an event from 5 to 10%.

8.3.6.5 Secondary Endpoint for Safety:

The secondary endpoint for safety consisted of comparing the safety profile for ProQuad vs. the safety profile for MMRII + VARIVAX.

8.3.6.5.1 Risk differences between treatment groups and two – sided p values were presented for adverse reactions listed on the Vaccination Report Card including injection site reactions, injection site rashes, mumps-like symptoms, rubella-like rashes, and varicella-like rashes.

8.3.6.5.2 For adverse reactions not listed on the Vaccination Report Card, for any AE occurring at an incidence ≥1% in either group, the risk difference and corresponding 95% confidence intervals were presented.

8.3.7 Results:

8.3.7.1 Populations enrolled/analyzed

8.3.7.1.1 The study was conducted at 40 study sites in the United States and Canada. The Principal Investigators, study sites and number of children enrolled at each site are listed in Table 8.3.4.

Table 8.3.4 Listing of Study Sites, Primary Investigators, and Number of Children Enrolled at each Site

Study Number	Investigator	Location	Total Subjects Enrolled (N=3928)
012001	Dennehy, Penelope	Providence, RI	48
012003	Rudoy, Raul	Honolulu, HI	27
012004	Sawyer, Mark	La Jolla, CA	2
012005	Milnes, Philip	Wenatchee, WA	19
012006	Walter, Emmanuel	Durham, NC	111
012007	Werzberger, Alan	Monroe, NY	152
012008	Meissner, H. Cody	Boston, MA	74
012009	Bernstein, Hank	Boston, MA	57
012010	Block, Stan	Bardstown, KY	54
012011	Chatterjee, Archana	Omaha, NE	83
012012	Black, Steven	Oakland, CA	1511
012014	Marchant, Colin D.	Boston, MA	206
012015	Sullivan, Bradley J.	Marshfield, WI	85
012016	Henderson, Frederick	Chapel Hill, NC	244
012018	Bromberg, Kenneth	Brooklyn, NY	45
012019	Pollara, Bernard	Tampa, FL	25
012020	Williams, Karen	Baton Rouge, LA	70
012021	Nachman, Sharon	Stony Brook, NY	25
012023	Ford, Robert V.	Winston-Salem, NC	21
012025	Allen, Brian	Lacrosse, WI	39
012026	Lieberman, Jay M.	Long Beach, CA	247
012027	Rees, William C.	Burke, VA	30
012028	Butler, John L.	Birmingham, AL	52
012029	Halperin, Scott	Halifax, Nova Scotia	130
012030	Law, Barbara J.	Winnipeg, Manitoba	42
012031	Russell, Margaret	Calgary, Alberta	20
012032	Lepow, Martha L.	Albany, NY	1
012033	Yogev, Ram	Chicago, IL	25
012034	Danhauer, David E.	Owensboro, KY	41
012035	Alvey, Justin C.	Layton, UT	47
012036	Hornick, Richard	Orlando, FL	15
012037	Schneider, David L.	New Orleans, LA	8
012038	Shapiro, Steven A.	Norristown, PA	7
012039	Brayden, Robert	Denver, CO	23
012040	Senders, Shelly D.	Cleveland, OH	51
012041	Dionne, Marc	D'estimauville, Quebec	64
012042	Lebel, M	Montreal, Quebec	100
012043	Andrews, Wilson P.	Marietta, GA	24
012044	Rennels, Margaret B.	Baltimore, MD	1

8.3.7.1.2 Subject enrollment and drop-outs:

3928 subjects were enrolled with 3758 (96%) completing the study. Reasons for dropouts were similar in each group and are listed in Tables 5a and 5b below. 8 subjects were randomized but never vaccinated. One subject randomized to receive MMRII + VARIVAX inadvertently received MMRII + ProQuad. 170 subjects dropped out with 123 of 2915 subjects (4.2%) in the ProQuad group and 47 of 1012 (4.6%) in the MMRII + VARIVAX group. No subject discontinued due to a clinical adverse reaction. These data are summarized in Tables 8.3.5a 9 (for ProQuad Lots 1, 2 and 3) and 8.3.5b (for MMRII and PUVV)

Table 8.3.5a Enrollment and Study Dropouts by Group:

	ProQuad Lot 1		**ProQuad Lot 2**		**ProQuad Lot 3**		**ProQuad Total**	
	N	(%)	N	(%)	N	(%)	N	(%)
Entered	985†		968†		962†		2915†	
Male	507	(12 to 23)	520	(12-20)	520	(11-23)	1547	(11-23)
Female	478	(12 to 20)	448	(11- 23)	442	(12-20)	1368	(11-23)
Vaccinated	985	(100)	968	(100)	962	(100)	2915	(100)
Completed	950	(96.4)	924	(95.5)	918	(95.4)	2792	(95.8)
Discontinued:	35	(3.6)	44	(4.5)	44	(4.6)	123	(4.2)
AE	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Protocol deviation	3	(0.3)	2	(0.2)	4	(0.4)	9	(0.3)
Refused participation	6	(0.6)	11	(1.1)	11	(1.1)	28	(1.0)
Lost to follow up	16	(1.6)	19	(2.0)	17	(1.8)	52	(1.8)
Missed bleed	7	(0.7)	7	(0.7)	8	(0.8)	22	(0.8)
Safety data incomplete	3	(0.3)	5	(0.5)	4	(0.4)	12	(0.4)
Other	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

Table 8.3.5b Enrollment and Study Dropouts by Group (cont.)

	M-M-R™ II + VARIVAX™ (N=1012)		**M-M-R™ II + ProQuad™ (N=1)**		**Total (N=3928)**	
	N	(%)	N	(%)	N	(%)
Entered:	1012†		1		3928†	
Male	544	(12 to 22)	1	(12 to 12)	2092	(11 to 23)
Female	468	(11 to 22)	0		1836	(11 to 23)
Vaccinated	1012	(100)	1	(100)	3928	(100)
Completed:	965	(95.4)	1	(100)	3758	(95.7)
Discontinued:	47	(4.6)	0	(0.0)	170	(4.3)
AE	0	(0.0)	0	(0.0)	0	(0.0)
Protocol deviation	3	(0.3)	0	(0.0)	12	(0.3)
Refused participation	10	(1.0)	0	(0.0)	38	(1.0)
Lost to follow up	20	(2.0)	0	(0.0)	72	(1.8)
Missed bleed	10	(1.0)	0	(0.0)	32	(0.8)
Safety data incomplete	4	(0.4)	0	(0.0)	16	(0.4)
Other	0	(0.0)	0	(0.0)	0	(0.0)

8.3.7.1.3 Protocol Deviations

Protocol deviations that resulted in data being excluded from the primary immunogenicity analysis included: blood sample outside of the acceptable day range, subject lost to follow-up, subject refused further participation, blood sample was difficult to obtain, baseline sample was missing, subject was given the incorrect allocation number or the incorrect clinical material, subject was outside of the specified age limit, subject received prior vaccination with MMRII or VARIVAX or subject received MMRII or VARIVAX during follow-up but prior to the post-vaccination blood draw, or subject received a live vaccine during follow-up.

8.3.7.1.4 No subjects were prematurely un-blinded during the 42 days of follow-up after vaccine administration.

8.3.7.1.5 Serostatus at baseline:

The primary analysis of immunogenicity was based on the per protocol population and only subjects who were initially seronegative for the vaccine antigen were evaluated. Serostatus for each vaccine antigen at baseline is listed in Table 8.3.6 below:

Table 8.3.6 Serostatus at Baseline

	ProQuad™				M-M-R™ II + VARIVAX™
Initial Serostatus:	(Lot 1) (N=985)	(Lot 2) (N=968)	(Lot 3) (N=962)	(Combined Lots)† (N=2915)	(N=1012)
Measles‡					
Negative	863 (87.6%)	831 (85.8%)	835 (86.8%)	2529 (86.8%)	869 (85.9%)
Positive	74 (7.5%)	77 (8.0%)	73 (7.6%)	224 (7.7%)	91(9.0%)
Unknown	48 (4.9%)	60 (6.2%)	54 (5.6%)	162 (5.6%)	52(5.1%)
Subjects analyzed at 6 weeks	802	771	779	2352	811
Mumps§					
Negative	920 (93.4%)	884 (91.3%)	891 (92.6%)	2695 (92.5%)	942 (93.1%)
Positive	16 (1.6%)	25 (2.6%)	18 (1.9%)	59 (2.0%)	18 (1.8%)
Unknown	49 (5.0%)	59 (6.1%)	53 (5.5%)	161 (5.5%)	52 (5.1%)
Subjects analyzed at 6 weeks	856	823	830	2509	872
Rubella_					
Negative	935 (94.9%)	906 (93.6%)	905 (94.1%)	2746 (94.2%)	956 (94.5%)
Positive	3 (0.3%)	3 (0.3%)	4 (0.4%)	10 (0.3%)	4 (0.4%)
Unknown	47 (4.8%)	59 (6.1%)	53 (5.5%)	159 (5.5%)	52 (5.1%)
Subjects analyzed at 6 weeks	861	835	836	2532	866
Varicella¶					
<1.25 gpELISA Units#	851 (86.4%)	809 (83.6%)	836 (86.9%)	2496 (85.6%)	875 (86.5%)
Negative	669 (67.9%)	651 (67.3%)	678 (70.5%)	1998 (68.5%)	721 (71.2%)
Positive but <1.25 gpELISA units	182 (18.5%)	158 (16.3%)	158 (16.4%)	498 (17.1%)	154 (15.2%)
≥1.25 gpELISA units	87 (8.8%)	100 (10.3%)	73 (7.6%)	260 (8.9%)	85 (8.4%)
Unknown	47 (4.8%)	59 (6.1%)	53 (5.5%)	159 (5.5%)	52 (5.1%)
Subjects analyzed at 6 weeks	791	761	779	2331	813

† Combined lots denote the combination of Lots 1, 2, and 3 of ProQuad™. Seronegative to measles corresponds to an antibody
‡ titer <120U/mL. Seropositive to measles corresponds to an antibody titer ≥120 mIU/mL. Seronegative to mumps corresponds
§ to an antibody titer <10 ELISA Ab units. Seropositive to mumps corresponds to an antibody titer ≥10 ELISA Ab units. For most
_ samples, seronegative to rubella corresponds to an antibody titer <12.8 ELISA Ab units (=10 IU/mL) and seropositive to
¶ rubella corresponds to an antibody titer ≥12.8 ELISA Ab units (=10 IU/mL). However, some samples were tested in the new
# format rubella ELISA, and seronegative to rubella in this assay corresponds to an antibody titer <10 IU/mL and seropositive corresponds to an antibody titer ≥10 IU/mL. Varicella serostatus is determined by comparison to a cutoff optical density (OD), which was derived as a function of historical, known negative controls. The category <1.25 gpELISA units is the sum of the categories Negative and Positive but <1.25 gpELISA units.

Other reasons for exclusion from the per protocol analysis Included: age younger than 12 months, immunized prior to the study, missing baseline result, allocation violation due to investigator error, chickenpox, received extra dose of MMRII + VARIVAX during study, received non-study live viral vaccine, missing or not evaluable post-vaccination result, lost to follow-up and refusal to participate further.

In addition, 70 samples were excluded from the rubella analysis because the samples were tested in the new modified format rubella assay that reported titers in rubella IU/mL instead of ELISA AB units/mL.

8.3.7.1.6 Demographics:

Subjects in each group were comparable in terms of age, race, gender, and with regards to prior therapies or medications with 53.3% male, mean age of 12.7 months and median age of 12.0 months. The three major ethnic groups enrolled in the study were Caucasian (64.0%), African-American (13.4%) and Hispanic (12.7%). (See Table 8.3.7)

Table 8.3.7 Demographics of the Study Population

	ProQuad™								M-M-R™ II + VARIVAX™		M-M-R™ II + Pro Quad™		Total	
	(Lot 1) (N=985)		(Lot 2) (N=968)		(Lot 3) (N=962)		Combined Lots (N=2915)		(N=1012)		(N=1)		(N=3928)	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Gender														
Male	507	(51.5)	520	(53.7)	520	(54.1)	1547	(53.1)	544	(53.8)	1	(100)	2092	(53.3)
Female	478	(48.5)	448	(46.3)	442	(45.9)	1368	(46.9)	468	(46.2)	0	(0.0)	1836	(46.7)
Age (Months)														
Mean	12.7		12.8		12.7		12.7		12.8		12.0		12.7	
SD	1.3		1.4		1.3		1.4		1.4				1.4	
Median	12.0		12.0		12.0		12.0		12.0		12.0		12.0	
Range	12 to 23		11 to 23		11 to 23		11 to 23		11 to 22		12 to 12		11 to 23	
Male	12 to 23		12 to 20		11 to 23		11 to 23		12 to 22		12 to 12		11 to 23	
Female	12 to 20		11 to 23		12 to 20		11 to 23		11 to 22		To		11 to 23	
Race/Ethnicity														
African-American	120	(12.2)	122	(12.6)	136	(14.1)	378	(13.0)	150	(14.8)	0	(0.0)	528	(13.4)
Asian/Pacific	49	(5.0)	59	(6.1)	40	(4.2)	148	(5.1)	43	(4.2)	0	(0.0)	191	(4.9)
Caucasian	615	(62.4)	630	(65.1)	622	(64.7)	1867	(64.0)	647	(63.9)	1	(100)	2515	(64.0)
Hispanic	150	(15.2)	113	(11.7)	116	(12.1)	379	(13.0)	119	(11.8)	0	(0.0)	498	(12.7)
Native American	4	(0.4)	0	(0.0)	3	(0.3)	7	(0.2)	2	(0.2)	0	(0.0)	9	(0.2)
Other	47	(4.8)	44	(4.5)	45	(4.7)	136	(4.7)	51	(5.0)	0	(0.0)	187	(4.8)
Prior therapy														
	329	(33.4)	316	(32.6)	307	(31.9)	952	(32.7)	310	(30.6)	1	(100)	1263	(32.2)

8.3.7.2 Efficacy endpoints: Immunogenicity

8.3.7.2.1 The immunogenicity results in the per protocol population are listed in Table 8.3.8 below:

Table 8.3.8 Immune responses following vaccination with ProQuad vs. MMRII and VARIVAX

Vaccine		ProQuad™				M-M-R™ II + VARIVAX™
		Lot 1	Lot 2	Lot 3	Combined	
Component (Assay)	***Parameter***	***Observed Response (95% CI)***	***Observed Response (95% CI)***	***Observed Response (95% CI)***	***Observed Response (95% CI)***	***Observed Response (95% CI)***
Measles (ELISA)	***Response Rate‡***	98.5% (790/802) (97.4%, 99.2%)	97.7% (753/771) (96.3%, 98.6%)	96.7% (753/779) (95.1%, 97.8%)	97.6% (2296/2352) (96.9%, 98.2%)	98.6% (800/811) (97.6%, 99.3%)
	GMT§	3031.6 (2841.6, 3234.3)	2939.3 (2740.0, 3153.2)	2852.2 (2651.0, 3068.7)	2941.0 (2825.7, 3061.1)	2095.6 (1964.2, 2235.7)
Mumps (ELISA)	***Response Rate‡***	96.4% (825/856) (94.9%, 97.5%)	96.7% (796/823) (95.3%, 97.8%)	94.9% (788/830) (93.2%, 96.3%)	96.0% (2409/2509) (95.2%, 96.7%)	97.9% (854/872) (96.8%, 98.8%)
	GMT§	100.5 (94.3, 107.2)	102.3 (96.0, 109.0)	85.6 (79.9, 91.8)	95.9 (92.3, 99.6)	89.7 (84.7, 94.9)
Rubella (ELISA)	***Response Rate‡***	99.0% (852/861) (98.0%, 99.5%)	98.3% (821/835) (97.2%, 99.1%)	99.0% (828/836) (98.1%, 99.6%)	98.8% (2501/2532) (98.3%, 99.2%)	99.2% (859/866) (98.3%, 99.7%)
	GMT§	113.5 (107.5, 119.8)	114.8 (108.2, 121.8)	114.2 (108.1, 120.6)	114.2 (110.5, 117.9)	132.6 (125.7, 140.0)
Varicella (gpELISA)	***Response Rate‡***	91.8% (726/791) (89.6%, 93.6%)	94.3% (718/761) (92.5%, 95.9%)	94.5% (736/779) (92.6%, 96.0%)	93.5% (2180/2331) (92.4%, 94.5%)	95.0% (772/813) (93.2%, 96.4%)
	GMT§	16.0 (14.9, 17.2)	18.4 (17.2, 19.7)	19.7 (18.4, 21.0)	17.9 (17.2, 18.7)	17.6 (16.6, 18.7)

Combined lots denote the combination of Lots 1, 2, and 3 of ProQuad™.
‡ The response rate for measles is the percent of initially seronegative subjects (baseline antibody titer <120 mIU/mL) with post-vaccination antibody titer ≥120 mIU/mL.
The response rate for mumps is the percent of initially seronegative subjects (baseline antibody titer <10 ELISA Ab units) with post-vaccination antibody titer ≥10 ELISA Ab units. The response rate for rubella is the percent of initially seronegative subjects (baseline antibody titer <12.8 ELISA Ab units (=10 IU/mL)) with post-vaccination antibody titer ≥12.8 ELISA Ab units (=10 IU/mL). The response rate for varicella is the percent of subjects with baseline antibody titer <1.25 gpELISA units with post-vaccination antibody titer ≥5 gpELISA units.
§ GMTs and associated confidence intervals are presented in units of mIU/mL for measles, ELISA Ab units for mumps, ELISA Ab units for rubella, and gpELISA units for varicella.
N = Number of subjects vaccinated in each treatment group.
ELISA = Enzyme-linked immunosorbent assay.
gpELISA = Glycoprotein enzyme-linked immunosorbent assay.
CI = Confidence interval.
GMT = Geometric mean titer.

8.3.7.2.2 Evaluation of consistent antibody responses to three lots of ProQuad (Lot-to-lot consistency).

In order to demonstrate that the three lots elicited similar immune responses, the seroresponse rates for measles, mumps, and rubella had to be no more than 5 percentage points different for each pair-wise comparison for each antigen and no more than 10 percentage points different for the comparisons of the varicella responses rates. In addition, the GMTs had to be no more than 1.5 fold different for each pair of lots compared and for all four antigens.

A consistent immune response was established for response rates and GMTS to **measles** and is summarized in Table 8.3.9 below. The 3 pair-wise comparisons of response rates were declared similar because all three confidence intervals were less than ±5 percentage points different and because all three confidence

intervals for the fold difference in GMTs were contained within 0.67-1.5) which corresponded to 6 one sided p values being < 0.05.

Table 8.3.9 Comparison of Measles Antibody Responses to Demonstrate Lot-to-Lot Consistency

Parameter	Comparison Group A vs. Group B	Group A N	Group A n	Group A Response†	Group B N	Group B n	Group B Response†	Estimated Difference†§/Fold Difference†_ (90% CI)†	One-Sided p-Values†‡ for Lower Bound, Upper Bound	Conclusion
Response Rate¶	Lot 1 vs. Lot 2	985	802	98.5%	968	771	97.6%	0.9 (-0.2, 2.2)	<0.001*, <0.001*	Similar
	Lot 1 vs. Lot 3	985	802	98.5%	962	779	96.7%	1.9 (0.6, 3.2)	<0.001*, <0.001*	Similar
	Lot 2 vs. Lot 3	968	771	97.6%	962	779	96.7%	0.9 (-0.5, 2.4)	<0.001*, <0.001*	Similar
GMT#	Lot 1 vs. Lot 2	985	802	3026.3	968	771	2932.5	1.03 (0.95, 1.12)	<0.001*, <0.001*	Similar
	Lot 1 vs. Lot 3	985	802	3026.3	962	779	2848.9	1.06 (0.98, 1.15)	<0.001*, <0.001*	Similar
	Lot 2 vs. Lot 3	968	771	2932.5	962	779	2848.9	1.03 (0.95, 1.12)	<0.001*, <0.001*	Similar

* A p-value ≤0.05 implies that the difference is statistically significantly less than the pre-specified difference of 5 percentage points or 1.5-fold. Within the pair of lots being compared, both p-values being ≤0.05 corresponds to the two-sided 90% CI on the difference or fold difference being entirely contained in (-5.0, 5.0) percentage points (for response rates) or (0.67, 1.50) fold (for GMTs), respectively, and allows for a conclusion of similarity. † Responses, their differences, associated confidence intervals, and p-values are based on a statistical analysis model adjusting for study center (for response rates) and for study center and treatment group by center interaction (for GMTs). ‡ The p-values are for the comparison to the lower bound (-5.0 percentage points for response rates and 0.67-fold for GMTs) and to the upper bound (5.0 percentage points for response rates and 1.5-fold for GMTs).

§ Group A - Group B. _ Group A/Group B. ¶ The response rate for measles is the percent of initially seronegative subjects (baseline measles antibody titer <120 mIU/mL) with post-vaccination measles antibody titer ≥120 mIU/mL. # GMTs are presented in units of mIU/mL. N = Number of subjects vaccinated in each treatment group. n = Number of subjects initially seronegative to measles contributing to the per-protocol analysis. CI = Confidence interval. GMT = Geometric mean titer.

A consistent immune response was established for response rates and GMTS to **mumps** and is summarized in Table 8.3.10 below. The 3 pair-wise comparisons of response rates were declared similar because all three confidence intervals were less than ±5 percentage points different and because all three confidence intervals for the fold difference in GMTs were contained within 0.67-1.5) which corresponded to 6 one sided p values being < 0.05.

Table 8.3.10 Comparison of Mumps Antibody Responses to Demonstrate Lot-to-Lot Consistency

Parameter	Comparison Group A vs. Group B)	Group A N	Group A n	Group A Response†	Group B N	Group B n	Group B Response†	Estimated Difference†§/Fold Difference†_ (90% CI)†	One-Sided p-Values†‡ for Lower Bound, Upper Bound	Conclusion
Response Rate¶	Lot 1 vs. Lot 2	985	856	96.5%	968	823	96.6%	-0.1 (-1.6, 1.4)	<0.001*, <0.001*	Similar
	Lot 1 vs. Lot 3	985	856	96.5%	962	830	94.9%	1.6 (0.0, 3.2)	<0.001*, <0.001*	Similar
	Lot 2 vs. Lot 3	968	823	96.6%	962	830	94.9%	1.7 (0.1, 3.3)	<0.001*, <0.001*	Similar
GMT#	Lot 1 vs. Lot 2	985	856	101.6	968	823	101.7	1.00 (0.93, 1.08)	<0.001*, <0.001*	Similar
	Lot 1 vs. Lot 3	985	856	101.6	962	830	85.6	1.19 (1.10, 1.28)	<0.001*, <0.001*	Similar
	Lot 2 vs. Lot 3	968	823	101.7	962	830	85.6	1.19 (1.10, 1.28)	<0.001*, <0.001*	Similar

* A p-value ≤0.05 implies that the difference is statistically significantly less than the pre-specified difference of 5 percentage points or 1.5-fold. Within the pair of lots being compared, both p-values being ≤0.05 corresponds to the two-sided 90% CI on the difference or fold difference being entirely contained in (-5.0, 5.0) percentage points (for response rates) or (0.67, 1.50) fold (for GMTs), respectively, and allows for a conclusion of similarity. † Responses, their differences, associated confidence intervals, and p-values are based on a statistical analysis model adjusting for study center (for response rates) and for study center and treatment group by center interaction (for GMTs). ‡ The p-values are for the comparison to the lower bound (-5.0 percentage points for response rates and 0.67-fold for GMTs) and to the upper bound (5.0 percentage points for response rates and 1.5-fold for GMTs).

§ Group A - Group B. _ Group A/Group B. ¶ The response rate for mumps is the percent of initially seronegative subjects (baseline mumps antibody titer <10 ELISA Ab units) with post-vaccination mumps antibody titer ≥10 ELISA Ab units. # GMTs are presented in ELISA Ab units. N = Number of subjects vaccinated in each treatment group. n = Number of subjects initially seronegative to mumps contributing to the per-protocol analysis. CI = Confidence interval. GMT = Geometric mean titer.

A consistent immune response was established for response rates and GMTs to **rubella** and is summarized in Table 8.3.11 below. The 3 pair-wise comparisons of response rates were declared similar because all three confidence intervals were less than ±5 percentage points different and because all three confidence intervals for the fold difference in GMTs were contained within 0.67-1.5) which corresponded to 6, one-sided p values being < 0.05.

Table 8.3.11 Comparison of Rubella Antibody Responses to Demonstrate Lot-to–Lot Consistency

Parameter	Comparison Group A vs. Group B)	Group A N	Group A n	Group A Response †	Group B N	Group B n	Group B Response †	Estimated Difference†§ /Fold Difference† (90% CI)†	One-Sided p-Values†‡ for Lower Bound, Upper Bound	Conclusion
Response Rate¶	Lot 1 vs. Lot 2	985	861	98.9%	968	835	98.3%	0.6 (-0.4, 1.6)	<0.001*, <0.001*	Similar
	Lot 1 vs. Lot 3	985	861	98.9%	962	836	99.0%	-0.1 (-1.0, 0.8)	<0.001*, <0.001*	Similar
	Lot 2 vs. Lot 3	968	835	98.3%	962	836	99.0%	-0.7 (-1.8, 0.2)	<0.001*, <0.001*	Similar
GMT#	Lot 1 vs. Lot 2	985	861	113.4	968	835	114.5	0.99 (0.93, 1.06)	<0.001*, <0.001*	Similar
	Lot 1 vs. Lot 3	985	861	113.4	962	836	114.0	0.99 (0.93, 1.06)	<0.001*, <0.001*	Similar
	Lot 2 vs. Lot 3	968	835	114.5	962	836	114.0	1.00 (0.94, 1.07)	<0.001*, <0.001*	Similar

* A p-value ≤0.05 implies that the difference is statistically significantly less than the pre-specified difference of 5 percentage points or 1.5-fold. Within the pair of lots being compared, both p-values being ≤0.05 corresponds to the two-sided 90% CI on the difference or fold difference being entirely contained in (-5.0, 5.0) percentage points (for response rates) or (0.67, 1.50) fold (for GMTs), respectively, and allows for a conclusion of similarity. † Responses, their differences, associated confidence intervals, and p-values are based on a statistical analysis model adjusting for study center (for response rates) and for study center and treatment group-by-center interaction (for GMTs). ‡ The p-values are for the comparison to the lower bound (-5.0 percentage points for response rates and 0.67-fold for GMTs) and to the upper bound (5.0 percentage points for response rates and 1.5-fold for GMTs).

§ Group A - Group B. _ Group A/Group B. ¶ The response rate for rubella is the percent of initially seronegative subjects (baseline rubella antibody titer <12.8 ELISA Ab units (=10 IU/mL)) with post-vaccination rubella antibody titer ≥12.8 ELISA Ab units (=10 IU/mL). # GMTs are presented in ELISA Ab units. N = Number of subjects vaccinated in each treatment group. n = Number of subjects initially seronegative to rubella contributing to the per-protocol analysis. CI = Confidence interval. GMT = Geometric mean titer.

A consistent immune response was established for response rates and GMTs to **varicella** and is summarized in Table 8.3.12 below for subjects with baseline varicella antibody titers < 1.25gpELISA units. The 3 pair-wise comparisons of response rates were declared similar because all three confidence intervals were less than ±5 percentage points different and because all three confidence intervals for the fold difference in GMTs were contained within 0.67-1.5, which corresponded to 6, one-sided p values being < 0.05.

Varicella gpELISA antibody responses rates and GMTs for subjects seronegative at baseline were similar for the three pair-wise comparisons. Likewise, when the data for the varicella immune responses for the three consistency lots were combined and compared to seroresponse rates and GMTs after immunization with MMRII + VARIVAX in the initially seronegative population, no significant differences were found.

Table 8.3.12 Comparison of Varicella Antibody Responses to Demonstrate Lot-to-Lot Consistency

Parameter	Comparison (Group A Versus Group B)	Group A N	Group A N	Group A Estimated Response	Group B N	Group B n	Group B Estimated Response	Estimated Difference†§/Fold Difference† (90% CI)†	One-Sided p-Values†‡ for Lower Bound, Upper Bound	Conclusion
Response Rate¶	Lot 1 versus Lot 2	985	791	91.6%	968	761	94.3%	-2.7 (-4.9, -0.5)	<0.001*, <0.001*	Similar
	Lot 1 versus Lot 3	985	791	91.6%	962	779	94.5%	-2.9 (-5.1, -0.8)	<0.001*, <0.001*	Similar
	Lot 2 versus Lot 3	968	761	94.3%	962	779	94.5%	-0.2 (-2.2, 1.7)	<0.001*, <0.001*	Similar
GMT#	Lot 1 versus Lot 2	985	791	15.9	968	761	18.4	0.86 (0.80, 0.94)	<0.001*, <0.001*	Similar
	Lot 1 versus Lot 3	985	791	15.9	962	779	19.6	0.81 (0.75, 0.88)	<0.001*, <0.001*	Similar
	Lot 2 versus Lot 3	968	761	18.4	962	779	19.6	0.94 (0.87, 1.02)	<0.001*, <0.001*	Similar

* A p-value ≤0.05 implies that the difference is statistically significantly less than the pre-specified difference of 10 percentage points or 1.5-fold. Within the pair of lots being compared, both p-values being ≤0.05 corresponds to the two-sided 90% CI on the difference or fold difference being entirely contained in (-10.0, 10.0) percentage points (for response rates) or (0.67, 1.50) fold (for GMTs), respectively, and allows for a conclusion of similarity. † Responses, their differences, associated confidence intervals, and p-values are based on a statistical analysis model adjusting for study center (for response rates) and for study center and treatment group-by-center interaction (for GMTs). ‡ The p-values are for the comparison to the lower bound (-10.0 percentage points for response rates and 0.67-fold for GMTs) and to the upper bound (10.0 percentage points for response rates and 1.5-fold for GMTs).

§ Group A - Group B. _ Group A/Group B. ¶ The response rate for varicella is the percent of subjects with **baseline varicella antibody titer <1.25 gpELISA units** with post-vaccination varicella antibody titer ≥5 gpELISA units. # GMTs are presented in gpELISA units. N = Number of subjects vaccinated in each treatment group. n = Number of subjects with baseline varicella antibody titer <1.25 gpELISA units contributing to the per-protocol analysis. CI = Confidence interval. GMT = Geometric mean titer.

8.3.7.2.3 Evaluation of Equivalence of Antibody Responses between ProQuad and the Control Group.

Success on the second primary immunogenicity hypothesis required demonstration of a similar antibody response to measles, mumps, rubella and varicella between subjects who received ProQuad and subjects immunized with MMRII + VARIVAX. Because the responses rates to each of the three lots of ProQuad were declared consistent, the data were combined and compared to the immune responses seen after immunization with MMRII + VARIVAX. In order for immune responses to be declared similar, there had to be no more than a 5-percentage point difference in response rates to measles, mumps, and rubella or a 10-percentage point difference between varicella response rates between ProQuad and the control group. For GMTs, there had to be no more than a 1.5-fold difference between ProQuad and the control group.

This study demonstrated that the antibody responses to measles, mumps, rubella, and varicella in ProQuad recipients were equivalent to the immune responses seen in recipients of MMRII + VARIVAX and the data are summarized in Table 8.3.13 below:

Table 8.3.13 Comparison of Antibody Responses after ProQuad vs. MMRII and VARIVAX

Vaccine Component (Assay)	Parameter	ProQuad (Combined Lots†) (N=2915)		MMRII + VARIVAX™ (N=1012)		Estimated Difference‡§/ Fold Difference‡ (90% CI)¶		Similarity Criterion¶	p-Value‡	Conclusion¶
		n	Estimated Response‡	N	Estimated Response					
Measles	Response Rate#	2352	97.6%	811	98.8%	-1.1	(-2.0, -0.2)	LB>-5.0	<0.001*	Similar
(ELISA)	GMT††	2352	2935.0	811	2104.4	1.39	(1.31, 1.49)	LB>0.67	<0.001*	Similar
Mumps	Response Rate#	2509	96.1%	872	97.8%	-1.8	(-2.8, -0.7)	LB>-5.0	<0.001*	Similar
(ELISA)	GMT††	2509	96.0	872	89.3	1.08	(1.01, 1.14)	LB>0.67	<0.001*	Similar
Rubella	Response Rate#	2532	98.8%	866	99.2%	-0.4	(-1.0, 0.3)	LB>-5.0	<0.001*	Similar
(ELISA)	GMT††	2532	113.9	866	133.2	0.86	(0.81, 0.90)	LB>0.67	<0.001*	Similar
Varicella	Response Rate#	2331	93.5%	813	94.9%	-1.4	(-2.8, 0.2)	LB>-10.0	<0.001*	Similar
(gpELISA)	GMT††	2331	17.9	813	17.6	1.02	(0.96, 1.09)	LB>0.67	<0.001*	Similar

8.3.7.2.4 Evaluation of an acceptable antibody response to Measles, Mumps, Rubella and Varicella (Per Protocol Population).

The third primary immunogenicity hypothesis required demonstrating an acceptable immune response to each vaccine antigen. This was defined as a response rate that is at least 90% (meaning that the lower bound of the 95% confidence interval was greater than 90%) for measles, mumps, and rubella responses and a response rate of at least 76% for varicella (meaning that the lower bound of the 95% confidence interval was above 76%) for varicella responses.

Acceptable immune responses to measles, mumps, rubella, and varicella were demonstrated for each lot of ProQuad using 9, one-sided comparisons (3 for each antigen times 3 lots) which corresponds to p values ≤ 0.025. Three additional one-sided comparisons were performed to determine if varicella responses were acceptable and in each case the lower bound of the 95% confidence interval for the response rates were found to be above 76% which corresponded to a one-sided p value ≤ 0.025. Therefore, each of the three consistency lots of ProQuad elicited acceptable immune responses by these criteria and the data are summarized in Table 8.3.14 below:

Table 8.3.14 Immune Responses elicited by each of the 3 ProQuad Lots Tested in Lot-to-Lot Consistency Studies were Acceptable

Vaccine Component (Assay)	Lot of ProQuad™	Parameter	N	n	Observed Response (95% CI)†		Acceptability Criterion	p-Value	Conclusion†
Measles (ELISA)	Lot 1	Response Rate‡	985	802	98.5%	(97.4, 99.2)	LB >0.90	<0.001*	Acceptable
	Lot 2	Response Rate‡	968	771	97.7%	(96.3, 98.6)	LB >0.90	<0.001*	Acceptable
	Lot 3	Response Rate‡	962	779	96.7%	(95.1, 97.8)	LB >0.90	<0.001*	Acceptable
Mumps (ELISA)	Lot 1	Response Rate‡	985	856	96.4%	(94.9, 97.5)	LB >0.90	<0.001*	Acceptable
	Lot 2	Response Rate‡	968	823	96.7%	(95.3, 97.8)	LB >0.90	<0.001*	Acceptable
	Lot 3	Response Rate‡	962	830	94.9%	(93.2, 96.3)	LB >0.90	<0.001*	Acceptable
Rubella (ELISA)	Lot 1	Response Rate‡	985	861	99.0%	(98.0, 99.5)	LB >0.90	<0.001*	Acceptable
	Lot 2	Response Rate‡	968	835	98.3%	(97.2, 99.1)	LB >0.90	<0.001*	Acceptable
	Lot 3	Response Rate‡	962	836	99.0%	(98.1, 99.6)	LB >0.90	<0.001*	Acceptable
Varicella (gpELISA)	Lot 1	Response Rate‡	985	791	91.8%	(89.6, 93.6)	LB >0.76	<0.001*	Acceptable
	Lot 2	Response Rate‡	968	761	94.3%	(92.5, 95.9)	LB >0.76	<0.001*	Acceptable
	Lot 3	Response Rate‡	962	779	94.5%	(92.6, 96.0)	LB >0.76	<0.001*	Acceptable

* A p-value ≤0.025 implies that the parameter is statistically significantly

† greater than the pre-specified acceptability criterion of 90% for measles,

‡ mumps, and rubella, or 76% for varicella. The lower bound of the 95% CI being >90% (for measles, mumps, and rubella) or >76% (for varicella) implies that the parameter is statistically significantly greater than the pre-specified acceptability criterion (90% for measles, mumps, and rubella and 76% for varicella) and allows for a conclusion of acceptability. The response rate for measles is the percent of initially seronegative subjects (baseline antibody titer <120 mIU/mL) with post-vaccination antibody titer ≥120 mIU/mL. The response rate for mumps is the percent of initially seronegative subjects (baseline antibody titer <10 ELISA Ab units) with post-vaccination antibody titer ≥10 ELISA Ab units. The response rate for rubella is the percent of initially seronegative subjects (baseline antibody titer <12.8 ELISA Ab units (=10 IU/mL)) with post-vaccination antibody titer ≥10 IU/mL. The response rate for varicella is the percent of subjects with baseline varicella antibody titer <1.25 gpELISA units with post-vaccination antibody titer ≥5 gpELISA units. N = Number of subjects vaccinated in each treatment group. n = Number of initially seronegative subjects (for measles, mumps, and rubella) or number of subjects with pre-vaccination varicella antibody titer <1.25 gpELISA units contributing to the per-protocol analysis. ELISA = Enzyme-linked immunosorbent assay. gpELISA = Glycoprotein enzyme-linked immunosorbent assay. CI = Confidence interval. LB = Lower bound (of the 95% confidence interval).

8.3.7.2.5 Summary of Varicella responses by baseline varicella serostatus:

Table 8.3.15. Summary of Varicella Antibody Responses by Baseline Serostatus (Per-Protocol Analysis)

Population	Parameter	ProQuad™ (Lot 1) (N=985) Observed Response (95% CI)	ProQuad™ (Lot 2) (N=968) Observed Response (95% CI)	ProQuad™ (Lot 3) (N=962) Observed Response (95% CI)	ProQuad™ (Combined Lots†) (N=2915) Observed Response (95% CI)	M-M-R™ II + VARIVAX™ (N=1012) Observed Response (95% CI)
Initially seronegative	SCR	99.0% (614/620) (97.9%, 99.6%)	99.8% (616/617) (99.1%, 100%)	99.5% (623/626) (98.6%, 99.9%)	99.5% (1853/1863) (99.0%, 99.7%)	99.1% (662/668) (98.1%, 99.7%)
	%≥5 gpELISA units	91.5% (567/620) (89.0%, 93.5%)	94.8% (585/617) (92.8%, 96.4%)	94.7% (593/626) (92.7%, 96.3%)	93.7% (1745/1863) (92.5%, 94.7%)	94.5% (631/668) (92.4%, 96.1%)
	Post Vaccination GMT	15.8 (14.6, 17.2)	19.3 (17.9, 20.7)	20.2 (18.7, 21.8)	18.3 (17.5, 19.2)	17.3 (16.1, 18.6)
Initially seropositive but <1.25 gpELISA units	%≥5 gpELISA units	93.0% (159/171) (88.1%, 96.3%)	92.4% (133/144) (86.7%, 96.1%)	93.5% (143/153) (88.3%, 96.8%)	92.9% (435/468) (90.2%, 95.1%)	97.2% (141/145) (93.1%, 99.2%)
	Post Vaccination GMT	16.5 (14.3, 19.0)	15.2 (13.2, 17.6)	17.5 (15.4, 20.0)	16.4 (15.2, 17.8)	19.1 (16.8, 21.7)
Subjects with baseline antibody titer ≥1.25 and <5 gpELISA units	%≥5 gpELISA units	86.4% (57/66) (75.7%, 93.6%)	77.5% (55/71) (66.0%, 86.5%)	89.7% (52/58) (78.8%, 96.1%)	84.1% (164/195) (78.2%, 88.9%)	95.5% (63/66) (87.3%, 99.1%)
	Post Vaccination GMT	10.8 (8.7, 13.2)	10.4 (8.3, 13.2)	15.5 (12.4, 19.3)	11.8 (10.4, 13.5)	14.9 (11.8, 18.7)
Subjects with baseline antibody titer <5 gpELISA units	%≥5 gpELISA units	91.4% (783/857) (89.3%, 93.2%)	92.9% (773/832) (90.9%, 94.6%)	94.1% (788/837) (92.3%, 95.6%)	92.8% (2344/2526) (91.7%, 93.8%)	95.0% (835/879) (93.3%, 96.3%)
	Post Vaccination GMT	15.5 (14.5, 16.6)	17.5 (16.5, 18.7)	19.3 (18.1, 20.6)	17.4 (16.7, 18.0)	17.4 (16.4, 18.5)

† Combined lots denote the combination of Lots 1, 2, and 3 of ProQuad™. N = number of subjects vaccinated in each treatment group. CI = Confidence interval. SCR = Seroconversion rate. GMT = Geometric mean titer. gpELISA = Glycoprotein-based enzyme-linked immunosorbent assay.

Table 8.3.15 shows varicella antibody responses by baseline serostatus. When subjects with pre-existing anti-VZV gpELISA titers < 1.25 were immunized, 92.9% of those receiving a single dose of ProQuad and 97.2% of those receiving the vaccines separately achieved antibody titers above 5.0. When subjects with pre-existing anti-VZV gpELISA antibody titers between 1.25 and 5 were immunized, 84.1% of those receiving a single dose of ProQuad and 95.5% of those receiving the vaccines separately achieved antibody titers above 5.0. According to Table 64 (not shown), in vaccinees with baseline varicella titers >1.25 gpELISA units receiving a first dose of vaccine, 62.4% of those receiving ProQuad vs.71.4% of those receiving the vaccines separately achieved 4-fold rises in antibody titer. These data suggest that ProQuad may be less immunogenic with respect to the VZV component than MMR + VARIVAX in subjects with residual pre-existing maternal anti-VZV antibodies. However, there are insufficient subjects in these studies to draw a firm conclusion, as the confidence intervals are overlapping. Nonetheless, ProQuad response rates were high, even among those with prior anti-VZV immunity.

8.3.7.2.6 Additional immunogenicity endpoints that were evaluated included:

8.3.7.2.6.1 All subjects with serology:

An analysis of all subjects with serology was consistent with the results of the per protocol analysis described above.

8.3.7.3 Efficacy Endpoints: Safety.

8.3.7.4 Summary of Clinical Adverse Experiences:

Overall, the proportion reporting at least one adverse experience was comparable among treatment groups. After ProQuad, 83.3%, 81.2% and 82.9% of subjects after lots 1, 2, and 3 respectively had at least one AE while 80.5% of those given MMRII + VARIVAX reported at least one AE.

8.3.7.5 Follow-up:

Subjects immunized with ProQuad or MMRII + VARIVAX were followed for 42 days after immunization. Parents and guardians completed the vaccination report cards during this period to capture fevers and any signs of local injection site reactions or systemic complaints. Clinical follow-up was obtained on 969 of 985 ProQuad Lot 1 recipients, 950 of 968 ProQuad Lot 2 recipients, 941 of 962 ProQuad Lot 3 recipients, and 993 of 1012 subjects after MMRII + VARIVAX.

8.3.7.6 Serious Vaccine Related Adverse Reactions:

The primary endpoint for safety was any observation of vaccine-related serious adverse reactions. No serious vaccine-related adverse reactions were expected in either the ProQuad or MMRII + VARIVAX group.

There were no deaths in this study during the 42day follow-up period however, one death occurred 59 days after immunization. This 13 month old male child was not ill but was found apneic and cyanotic after a nap. The child was admitted to the hospital and life support was withdrawn two days later.

36 subjects reported serious adverse reactions to vaccination including six after Lot 1, 8 after lot 2, 11 after lot 3 and 11 after MMRII + VARIVAX. Serious AEs reported during this study included febrile seizures, pneumonia, bronchiolitis, asthma, severe RSV infection, gastroenteritis with or without dehydration, urinary tract infection, accidental drug ingestion, and fractures.

Of the subjects reporting serious adverse reactions, six were considered to be vaccine-related including 5 after ProQuad (0.2%) (2 after Lot 1, 1 after lot 2, 2 after lot 3) and 1(0.1%) after MMRII + VARIVAX. All subjects recovered and no one discontinued the study due to an adverse experience. The serious vaccine related AEs are listed in Table 8.3.16 below.

Table 8.3.16 Serious Vaccine-Related Adverse Events Reported in Study 012

Lot Number	Study Number	Age Months	Adverse Experience	Relative Day of Onset Post dose
1	00156	12	Febrile Seizure	15
1	01222	15	Febrile Seizure	9
2	07555	11	Bronchiolitis	24
3	01972	14	Fever	8
3	02089	12	Febrile Seizure	8
MMR +VARIVAX	01669	16	Febrile Seizure	10

8.3.7.7 Summary of Clinical Adverse Experiences:

Overall, the proportion reporting at least one adverse experience was comparable among treatment groups. After ProQuad, 83.3%, 81.2% and 82.9% of subjects after lots 1, 2, and 3, respectively, had at least one AE while 80.5% of those given MMRII + VARIVAX reported at least one AE. These data are summarized in Table 8.3.17.

Table 8.3.17 Reporting of AEs, post immunization days 0-42

	ProQuad™								**M-M-R™ II + VARIVAX™**		**M-M-R™ II + ProQuad™**	
	Lot 1		**Lot 2**		**Lot 3**		**Total**					
	(N=985)		(N=968)		(N=962)		(N=2915)		(N=1012)		(N=1)	
	n	(%)	N	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Number of subjects	985		968		962		2915		1012		1	
Without follow-up	16		18		21		55		19		0	
With follow-up	969		950		941		2860		993		1	
Number (%) of subjects:												
No AE	162	(16.7)	179	(18.8)	161	(17.1)	502	(17.6)	194	(19.5)	0	(0.0)
1 or more AEs	807	(83.3)	771	(81.2)	780	(82.9)	2358	(82.4)	799	(80.5)	1	(100)
Injection-site AEs	318	(32.8)	330	(34.7)	329	(35.0)	977	(34.2)	379	(38.2)	1	(100)
Systemic AE	760	(78.4)	717	(75.5)	737	(78.3)	2214	(77.4)	728	(73.3)	1 1	(100)
Vaccine-related AE†	519	(53.6)	500	(52.6)	504	(53.6)	1523	(53.3)	518	(52.2)		(100)
Injection-site AEs	317	(32.7)	328	(34.5)	326	(34.6)	971	(34.0)	375	(37.8)	1	(100)
Systemic AEs	312	(32.2)	306	(32.2)	313	(33.3)	931	(32.6)	277	(27.9)	0	(0.0)
Serious AEs	6	(0.6)	8	(0.8)	11	(1.2)	25	(0.9)	11	(1.1)	0	(0.0)
Serious vaccine-related AE	2	(0.2)	1	(0.1)	2	(0.2)	5	(0.2)	1	(0.1)	0	(0.0)
Died	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to AE	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to vaccine-related AE	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a serious AE	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a serious vaccine-related AE	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

8.3.7.8 Injection Site Reactions:

After ProQuad, injection site reactions were reported in 32.8, 34.7 and 35.0% of subjects given Lot 1, 2, and 3, respectively, and in 33.6 after VARIVAX and 33.3% after MMRII. The most common injection site AE after ProQuad was pain/tenderness/soreness in 24.4% while 26.4% reported this at the VARIVAX injection site and 26.8% at the MMRII injection site. For the most part, injection site reactions were considered to be mild. Injection site reactions are summarized in Tables 8.3.18 and 8.3.19 below:

Table 8.3.18 Summary of Injection Site Reactions following ProQuad Vaccination

	ProQuad™											
	(Lot 1) (N=985)			**(Lot 2) (N=968)**			**(Lot 3) (N=962)**			**(Combined Lots) (N=2915)**		
	N	(%)	VR	n	(%)	VR	N	(%)	VR	n	(%)	VR
Number of subjects	985			968			962			2915		
Subjects without follow-up	16			18			21			55		
Subjects with follow-up	969			950			941			2860		
Number (%) with one or more injection-site AE	318	(32.8)		330	(34.7)		329	(35.0)		977	(34.2)	
Discoloration	0	(0.0)		1	(0.1)	1	0	(0.0)		1	(0.0)	1
Dry skin, injection site	0	(0.0)		0	(0.0)		1	(0.1)		1	(0.0)	
Ecchymosis	17	(1.8)	16	14	(1.5)	13	16	(1.7)	15	47	(1.6)	44
Eczema	0	(0.0)		0	(0.0)		0	(0.0)		0	(0.0)	
Erythema	149	(15.4)	149	163	(17.2)	163	163	(17.3)	160	475	(16.6)	472
Hematoma	1	(0.1)	1	0	(0.0)		0	(0.0)		1	(0.0)	1
Hive-like rash	0	(0.0)		0	(0.0)		0	(0.0)		0	(0.0)	
Induration	0	(0.0)		2	(0.2)	2	2	(0.2)	2	4	(0.1)	4
Lump, injection site	1	(0.1)	1	1	(0.1)	1	1	(0.1)	1	3	(0.1)	3
Pain/tenderness/soreness	**221**	**(22.8)**	**220**	246	(25.9)	245	230	(24.4)	229	697	(24.4)	694
Pruritus	0	(0.0)		1	(0.1)	1	0	(0.0)		1	(0.0)	1
Rash	13	(1.3)	12	33	(3.5)	32	35	(3.7)	35	81	(2.8)	79
Rash, nonspec, injection site	**0**	**(0.0)**		**2**	**(0.2)**	**1**	**1**	**(0.1)**		**3**	**(0.1)**	**1**
Reaction, local	0	(0.0)		0	(0.0)		1	(0.1)	1	1	(0.0)	1
Swelling	89	(9.2)	89	86	(9.1)	86	86	(9.1)	85	261	(9.1)	260
Warmth	2	(0.2)	2	1	(0.1)	1	1	(0.1)	1	4	(0.1)	4

Table 8.3.19 Summary of Injection Site Reactions Following MMRII and VARIVAX Vaccination

	M-M-R™ II + VARIVAX™						M-M-R™ II + ProQuad™					
	VARIVAX™ (N=1012)			M-M-R™ II (N=1012)			ProQuad™ (N=1)			M-M-R™ II (N=1)		
	N	(%)	VR	n	(%)	VR	n	(%)	VR	n	(%)	VR
Number of subjects	1012			1012			1			1		
Subjects without follow-up	19			19			0			0		
Subjects with follow-up	993			993			1			1		
Number (%) of subjects with one or more injection-site AEs	334	(33.6)		331	(33.3)		1	(100)		0	(0.0)	
Discoloration	0	(0.0)		0	(0.0)		0	(0.0)		0	(0.0)	
Dry skin, injection site	0	(0.0)		0	(0.0)		0	(0.0)		0	(0.0)	
Ecchymosis	15	(1.5)	14	15	(1.5)	15	0	(0.0)		0	(0.0)	
Eczema	1	(0.1)		0	(0.0)		0	(0.0)		0	(0.0)	
Erythema	136	(13.7)	134	134	(13.5)	133	0	(0.0)		0	(0.0)	
Hematoma	0	(0.0)		1	(0.1)	1	0	(0.0)		0	(0.0)	
Hive-like rash	1	(0.1)	1	1	(0.1)	1	0	(0.0)		0	(0.0)	
Induration	3	(0.3)	3	1	(0.1)	1	0	(0.0)		0	(0.0)	
Lump, injection site	0	(0.0)		0	(0.0)		0	(0.0)		0	(0.0)	
Pain/tenderness/soreness	262	(26.4)	260	266	(26.8)	265	1	(100)	1	0	(0.0)	
Pruritus	1	(0.1)	1	0	(0.0)		0	(0.0)		0	(0.0)	
Rash	17	(1.7)	17	7	(0.7)	6	0	(0.0)		0	(0.0)	
Rash, nonspecific, injection site	**0**	**(0.0)**		**0**	**(0.0)**		0	(0.0)		0	(0.0)	
Reaction, local	0	(0.0)		0	(0.0)		0	(0.0)		0	(0.0)	
Swelling	91	(9.2)	91	80	(8.1)	79	0	(0.0)		0	(0.0)	
Warmth	1	(0.1)	1	1	(0.1)	1	0	(0.0)		0	(0.0)	

Percentages are calculated based on the number of subjects with follow-up after each visit.
Although a subject may have had 2 or more injection-site adverse experiences, the subject is counted only once in the overall total.
VR = Vaccine related. Numbers in this column refer to subjects with injection-site adverse experiences that were determined by the investigator to be possibly, probably, or definitely related to the vaccine.

8.3.7.9 Systemic Adverse Reactions:

Systemic adverse reactions were compared between each lot of ProQuad and the control group and between the combined lots of ProQuad and the control group. The percentages reporting one or more systemic adverse reactions were similar for each lot of ProQuad and the percentages of adverse reactions reported by body system were also similar between lots.

Systemic adverse reactions were reported more frequently after ProQuad than after MMRII and VARIVAX (77.4% vs. 73.3%, respectively) and the increase in reporting was seen for reports for each body system for ProQuad relative to reports for MMRII and VARIVAX. (Tables 8.3.20 and 8.3.21 below).

Table 8.3.20 Comparison of Systemic Adverse Events Following Vaccination with Three Lots of ProQuad

	ProQuad								
	Lot 1			Lot 2			Lot 3		
	N	%	VR	N	%	VR	n	%	VR
Number of Subjects	985			968			962		
Without follow-up	16			18			21		
With follow-up	969			950			941		
With one or more AE	760	78.4		717	75.5		737	78.3	
With no AE	209	21.6		233	24.5		204	21.7	
Body as Whole	415	42.8	220	391	41.2	209	409	43.5	202
Digestive	173	17.9	21	184	19.4	32	175	18.6	31
Metabolic/Nutritional/Immune	10	1.0		8	0.8		14	1.5	
Nervous System/Psychiatric	121	12.5	56	115	12.1	56	136	14.5	75
Respiratory	407	42.0	24	376	39.6	28	391	41.6	23
Skin	309	31.9	90	286	30.1	74	295	31.3	66
Special Senses	191	19.7	4	155	16.3	3	171	18.2	3

Table 8.3.21 Comparison of Systemic Adverse Reactions after ProQuad vs. MMRII and VARIVAX

	ProQuad Total			MMRII + VARIVAX			MMRII + ProQuad		
	N	%	VR	N	%	VR	n	%	VR
Number of Subjects	2915			1012			1		
Without follow-up	55			19			0		
With follow-up	2860			993			1		
With one or more AE	2214	**77.4**		728	**73.3**		1	100.0	
With no AE	646	**22.6**		265	**26.7**		0	0.0	
Body as Whole	1215	**42.5**	631	358	**36.1**	160	1	100	0
Cardiovascular									
Digestive	532	**18.6**	84	170	**17.1**	24	0	0.0	
Metabolic/Nutritional/Immune	32	**1.1**		17	**1.7**		0	0	
Nervous System/Psychiatric	372	**13.0**	187	108	**10.9**	55	0	0	
Respiratory	1174	**41.0**	75	391	**39.4**	29	1	100	0
Skin	890	**31.1**	230	297	**29.9**	66	0	0	
Special Senses	517	**18.1**	10	164	**16.5**	3	1	100	0

8.3.7.10 Comparisons of Adverse Reactions

Comparisons were made of the number and percentage reporting one or more adverse experience, one or more injection site reactions and one or more systemic adverse events between each lot of ProQuad and the control group and between the combined lots of ProQuad and the control group (see Table 8.3.22). As expected, there was a lower rate of injection site reactions after ProQuad when compared to the control group although immunization with ProQuad was associated with a higher rate of systemic adverse experiences.

Similarly, comparisons were made of the number and percentage reporting one or more vaccine-related adverse experience, one or more vaccine-related injection site reactions and one or more vaccine-related

systemic adverse events as well as serious vaccine-related adverse events between each lot of ProQuad and the control group and between the combined lots of ProQuad and the control group. (Table 8.3.23)

Table 8.3.22 Comparison of Injection Site and Systemic Adverse Reactions after Each Lot of ProQuad vs. MMRII and VARIVAX

Term	Comparison Group A vs. Group B	Group A				Group B				Risk Difference† (Group A-Group B) Percentage Points (95% Confidence Interval)†	
		N	n	s	%	N	n	s	%		
Subjects with one or more adverse experiences	Lot 1 vs. Control	985	969	807	(83.3)	1012	993	799	(80.5)	2.8	(-0.6, 6.2)
	Lot 2 vs. Control	968	950	771	(81.2)	1012	993	799	(80.5)	0.7	(-2.8, 4.2)
	Lot 3 vs. Control	962	941	780	(82.9)	1012	993	799	(80.5)	2.4	(-1.0, 5.9)
	Combined Lots vs. Control‡	2915	2860	2358	(82.4)	1012	993	799	(80.5)	2.0	(-0.8, 4.9)
Subjects with one or more injection-site adverse experiences	Lot 1 vs. Control	985	969	318	(32.8)	1012	993	379	(38.2)	**-5.3**	(-9.6, -1.1)
	Lot 2 vs. Control‡	968	950	330	(34.7)	1012	993	379	(38.2)	-3.4	(-7.7, 0.9)
	Lot 3 vs. Control	962	941	329	(35.0)	1012	993	379	(38.2)	-3.2	(-7.5, 1.1)
	Combined Lots vs. Control‡	2915	2860	977	(34.2)	1012	993	379	(38.2)	-4.0	(-7.5, -0.6)
Subjects with one or more systemic adverse experiences	Lot 1 vs. Control‡	985	969	760	(78.4)	1012	993	728	(73.3)	**5.1**	(1.3, 8.9)
	Lot 2 vs. Control‡	968	950	717	(75.5)	1012	993	728	(73.3)	2.2	(-1.7, 6.0)
	Lot 3 vs. Control	962	941	737	(78.3)	1012	993	728	(73.3)	**5.0**	(1.2, 8.8)
	Combined Lots vs. Control‡	2915	2860	2214	(77.4)	1012	993	728	(73.3)	4.1	(1.0, 7.3)

Table 8.3.23 Comparison of Vaccine Related Adverse Reactions, Vaccine Related Injection Site Reactions and Vaccine Related Systemic Adverse Reactions after Each Lot of ProQuad and after Vaccination with MMRII and VARIVAX

Term	Comparison (Group A Versus Group B)	Group A				Group B				Risk Difference† (Group A-Group B) Percentage Points (95% Confidence Interval)†	
		N	n	s	%	N	n	s	%		
Subjects with vaccine-related adverse experiences	Lot 1 vs. Control‡	985	969	519	53.6	1012	993	518	(52.2)	1.4	(-3.0, 5.8)
	Lot 2 vs. Control‡	968	950	500	52.6	1012	993	518	52.2)	0.5	(-4.0, 4.9)
	Lot 3 vs. Control‡	962	941	504	53.6	1012	993	518	52.2)	1.4	(-3.1, 5.8)
	Combined Lots vs. Control‡	2915	2860	1523	53.3	1012	993	518	52.2)	1.1	(-2.5, 4.7)
Subjects with one or more vaccine-related injection-site adverse experiences_	Lot 1 vs. Control‡	985	969	317	(32.7)	1012	993	375	(37.8)	-**5.1**	(-9.3, -0.8)
	Lot 2 vs. Control‡	968	950	328	(34.5)	1012	993	375	(37.8)	-3.2	(-7.5, 1.0)
	Lot 3 vs. Control‡	962	941	326	(34.6)	1012	993	375	(37.8)	-3.1	(-7.4, 1.2)
	Combined Lots vs. Control‡	2915	2860	971	(34.0)	1012	993	375	(37.8)	-3.8	(-7.3, -0.4)
Subjects with one or more vaccine-related systemic adverse experiences_	Lot 1 vs. Control‡	985	969	312	(32.2)	1012	993	277	(27.9)	4.3	(0.3, 8.4)
	Lot 2 vs. Control‡	968	950	306	(32.2)	1012	993	277	(27.9)	4.3	(0.2, 8.4)
	Lot 3 vs. Control‡	962	941	313	(33.3)	1012	993	277	(27.9)	**5.4**	(1.3, 9.5)
	Combined Lots vs. Control‡	2915	2860	931	(32.6)	1012	993	277	(27.9)	4.7	(1.3, 7.9)
Subjects with serious vaccine-related adverse experiences	Lot 1 vs. Control‡	985	969	2	(0.2)	1012	993	1	(0.1)	0.1	(-0.4, 0.7)
	Lot 2 vs. Control‡	968	950	1	(0.1)	1012	993	1	(0.1)	0.0	(-0.5, 0.5)
	Lot 3 vs. Control‡	962	941	2	(0.2)	1012	993	1	(0.1)	0.1	(-0.4, 0.7)
	Combined Lots vs. Control‡	2915	2860	5	(0.2)	1012	993	1	(0.1)	0.1	(-0.4, 0.3)

Percentages are calculated base on the number of subjects with follow-up. † Risk differences and confidence intervals are based on pooled incidence rates across all study centers. ‡ Control is M-M-R™ II + VARIVAX™. § Combined Lots denote the combination of Lots 1, 2, and 3 of ProQuad™. _ Determined by the investigator to be possibly, probably, or definitely related to the vaccine. N = Number of subjects vaccinated. n = Number of subjects with safety follow-up. s = Number of subjects with adverse experience.

8.3.7.11 Comparison of Injection Site Reactions between ProQuad and MMRII or VARIVAX.

Analysis of injection site reactions after ProQuad vs. MMRII:
Days 0-4: There were no statistically significant increases in injection site reactions after ProQuad when compared to the MMR II injection site.
Days 0-42: Significantly more injection site rashes were reported after ProQuad (81/2860, 2.8%) than MMRII (7/993, 0.7%), two-sided p value < 0.001.

Analysis of injection site reactions after ProQuad vs. VARIVAX:
Day 0-4: There were no statistically significant increases in injection site reactions after ProQuad when compared to the VARIVAX injection site.
Day 0-42: Significantly more injection site rashes were reported after ProQuad (81/2860, 2.8%) than VARIVAX (17/993, 1.7%), two-sided p-value = 0.053

8.3.7.12 Systemic Adverse Experiences in ProQuad vs. MMRII + VARIVAX recipients.

Systemic adverse experiences that occurred 0 to 42 days after immunization were compared between each lot of ProQuad and the control group and between the combined lots of ProQuad and the control group. There were 172 comparisons. Fever and adverse reactions reported as “body as a whole” occurred more frequently in ProQuad recipients for each comparison. A few specific adverse reactions occurred more frequently after one of the three lots of ProQuad when compared to the controls including nervous system reactions, irritability, and URI. These data are compared in Table 8.3.24.

Table 8.3.24 Comparison of Systemic Adverse Experiences between ProQuad and MMRII and VARIVAX by Body System

	Comparison	**Group A**				**Group B**			**Risk Difference† (Group A-Group B) Percentage Points (95% Confidence Interval)†**	
	Group A vs. Group B	N	n		%	N	n			
Body as a Whole/Site Unspecified	Lot 1 vs. Control	985	969	415	(42.8)	1012	993	358 (36.1)	**6.8**	2.5, 11.1
	Lot 2 vs. Control	968	950	391	(41.2)	1012	993	358 (36.1)	**5.1**	0.8, 9.4
	Lot 3 vs. Control‡	962	941	409	(43.5)	1012	993	358 (36.1)	**7.4**	3.1, 11.8
	Combined Lots vs. Control‡	2915	2860	1215	(42.5)	1012	993	358 (36.1)	**6.4**	(2.9, 9.9)
Fever	Lot 1vs.Control	985	969	379	(39.1)	1012	993	328 (33.0)	**6.1**	(1.8, 10.3)
	Lot 2 vs. Control	968	950	362	(38.1)	1012	993	328 (33.0)	**5.1**	(0.8, 9.3)
	Lot 3 vs. Control	962	941	379	(40.3)	1012	993	328 (33.0)	**7.2**	(3.0, 11.5)
	Combined Lots vs. Control‡	2915	2860	1120	(39.2)	1012	993	328 (33.0)	**6.1**	(2.7, 9.5)
Digestive System	Lot 1 vs. Control‡	985	969	173	(17.9)	1012	993	170 (17.1)	0.7	(-2.6, 4.1)
	Lot2 +vs. Control	968	950	184	(19.4)	1012	993	170 (17.1)	2.2	(-1.2, 5.7)
	Lot 3 vs. Control	962	941	175	(18.6)	1012	993	170 (17.1)	1.5	(-1.9, 4.9)
	Combined Lots vs. Control‡	2915	2860	532	(18.6)	1012	993	170 (17.1)	1.5	(-1.3, 4.2)
Metabolic/Nutritional/ Immune	Lot 1 vs. Control	985	969	10	(1.0)	1012	993	17 (1.7)	-0.7	(-1.8, 0.4)
	Lot 2 vs. Control	968	950	8	(0.8)	1012	993	17 (1.7)	-0.9	(-2.0, 0.1)
	Lot 3 vs. Control	962	941	14	(1.5)	1012	993	17 (1.7)	0.2	(-1.4, 1.0)
	Combined Lots vs. Control‡	2915	2860	32	(1.1)	1012	993	17 (1.7)	-0.6	(-1.7,0.2)
Nervous System and Psychiatric	Lot 1 vs. Control	985	969	121	(12.5)	1012	993	108 (10.9)	1.6	(-1.2, 4.5)
	Lot 2 vs. Control	968	950	115	(12.1)	1012	993	108 (10.9)	1.2	(-1.6, 4.1)
	Lot 3 vs. Control	962	941	136	(14.5)	1012	993	108 (10.9)	3.6	(0.6, 6.6)
	Combined Lots vs. Control‡	2915	2860	372	(13.0)	1012	993	108 (10.9)	2.1	(-0.3, 4.3)
Respiratory System	Lot 1 vs. Control‡	985	969	407	(42.0)	1012	993	391 (39.4)	2.6	(-1.7, 7.0)
	Lot 2 vs. Control‡	968	950	376	(39.6)	1012	993	391 (39.4)	0.2	(-4.1, 4.6)
	Lot 3 vs. Control‡	962	941	391	(41.6)	1012	993	391 (39.4)	2.2	(-2.2, 6.6)
	Combined Lots vs. Control‡	2915	2860	1174	(41.0)	1012	993	391 (39.4)	1.7	(-1.9, 5.2)
Skin and Appendage	Lot 1 vs. Control	985	969	309	(31.9)	1012	993	297 (29.9)	2.0	(-2.1, 6.1)
	Lot 2 vs. Control	968	950	286	(30.1)	1012	993	297 (29.9)	0.2	(-3.9, 4.3)
	Lot 3 vs. Control	962	941	295	(31.3)	1012	993	297 (29.9)	1.4	(-2.7, 5.6)
	Combined Lots vs. Control‡	2915	2860	890	(31.1)	1012	993	297 (29.9)	1.2	(-2.2, 4.5)
Special Senses	Lot 1 vs. Control	985	969	191	(19.7)	1012	993	164 (16.5)	3.2	(-0.2, 6.6)
	Lot 2 vs. Control	968	950	155	(16.3)	1012	993	164 (16.5)	-0.2	(-3.5, 3.1)
	Lot 3 vs. Control	962	941	171	(18.2)	1012	993	164 (16.5)	1.7	(-1.7, 5.1)
	Combined Lots vs. Control‡	2915	2860	517	(18.1)	1012	993	164 (16.5)	1.6	(-1.2, 4.2)

8.3.7.13 Fever

The incidence of fever ≥102 F oral, oral equivalent temperature, or "abnormal" was significantly greater in the ProQuad group compared to MMRII + VARIVAX recipients for each comparison made. Fever was of short duration and lasted 1.7 days after ProQuad (median of 1.0 day, range of 1 to 43 days) and 1.7 days after MMRII + VARIVAX (median 1.0 day and range 1-13 days). There were more reports of high fever (≥104 F) days 5-12 after immunization in the ProQuad group (81/2834, 2.9%) than in the MMRII + VARIVAX group (17/978, 1.7%) but there was not a statistically significant difference (p= 0.056). These data are summarized and compared in Table 8.3.25.

Febrile seizure rates were not increased when the incidence after ProQuad immunization (8/2860, 0.3%) was compared to the incidence after immunization with MMRII + VARIVAX (3/993, 0.3%), p=0.909.

Table 8.3.25 Comparison of Fevers after ProQuad vs. MMRII and VARIVAX

	ProQuad								MMRII + VARIVAX		MMRII + ProQuad	
	Lot 1		Lot 2		Lot 3		Total					
	n	%	n	&	n	%	N	%	n	%	n	%
Number of Subjects	985		968		962		2915		1012		1	
With follow-up	965		940		935		2840		981		1	
Maximum Temperature ≥102 F	376	39.0	360	38.3	374	40.0	1110	39.1	325	33.1	0	0.0
Proquad vs. MMRII + VARIVAX p-Value	0.007		0.018		0.002		0.001					

8.3.7.14 Measles-Like And Rubella-Like Rashes:

Measles-like rashes reported 0-42 days after immunization occurred at a higher rate after each lot of ProQuad however when the data for each lot were combined the rate after ProQuad immunization (2.6%) was not significantly increased compared to the rate after MMRII + VARIVAX immunization (1.9%). In contrast, rubella-like rashes occurred significantly more frequently after Lot 1 (0.4% vs. 0.0%) and Lot 2 (0.4% vs. 0.0%) than after MMRII + VARIVAX immunization and this increase was statistically significant. However, the rate of rubella-like rashes was not significantly different when the data for ProQuad lots 1, 2 and 3 were combined and compared to the rate in the control group. (See Table 8.3.26)

8.3.7.15 Varicella-Like Rashes:

Varicella-like rashes were not significantly increased after ProQuad immunization for each individual lot tested or when the combined data for all lots were compared to the rate in the control group.

Of 96 subject who reported varicella or zoster-like rashes, 6 provided cultures of lesions for --- analysis. 2 samples were negative for varicella sequences, 2 were inadequate for testing, 1 subject (after lot 3 of ProQuad) had wild type varicella sequences detected and vaccine-type virus was recovered from another vaccinee after MMRII + VARIVAX immunization. (See Table 8.3.26)

Table 8.3.26 Rashes after Vaccination with ProQuad or MMRII and VARIVAX

Adverse Experience	Comparison Group A vs. Group B	Group A			Group B			Risk Difference† (Group A-Group B) Percentage Points (95% Confidence Interval)†		p-Value†
Measles-Like Rash‡	Lot 1 vs. Control	985	969	28 (2.9)	1012	993	19 (1.9)	1.0	(-0.4,2.4)	0.158
	Lot 2 vs. Control	968	950	22 (2.3)	1012	993	19 (1.9)	0.4	(-0.9,1.8)	0.537
	Lot 3 vs. Control	962	941	24 (2.6)	1012	993	19 (1.9)	0.6	(-0.7,2.0)	0.342
	Combined Lots vs. Control	2915	2860	74 (2.6)	1012	993	19 (1.9)	0.7	(-0.5,1.6)	0.233
Rubella-Like Rash‡	Lot 1 vs. Control	985	969	4 (0.4)	1012	993	0 (0.0)	0.4	(0.0,1.1)	0.043
	Lot 2 vs. Control	968	950	4 (0.4)	1012	993	0 (0.0)	0.4	(0.0,1.1)	0.041
	Lot 3 vs. Control	962	941	0 (0.0)	1012	993	0 (0.0)	0.0	(-0.4,0.4)	1.000
	Combined Lots vs. Control	2915	2860	8 (0.3)	1012	993	0 (0.0)	0.3	(-0.1,0.6)	0.095
Varicella-Like Rash	Lot 1 vs. Control	985	969	30 (3.1)	1012	993	24 (2.4)	0.7	(-0.8,2.2)	0.358
	Lot 2 vs. Control	968	950	18 (1.9)	1012	993	24 (2.4)	-0.5	(-1.9,0.8)	0.429
	Lot 3 vs. Control	962	941	15 (1.6)	1012	993	24 (2.4)	-0.8	(-2.2,0.5)	0.198
	Combined Lots vs. Control	2915	2860	63 (2.2)	1012	993	24 (2.4)	-0.2	(-1.5,0.8)	0.696

Percentages are calculated based on the number of subjects with follow-up after any visit. Although a subject may have had 2 or more of these systemic adverse experiences, the subject is counted only once in the overall total. † Risk differences and confidence intervals (CIs) are provided for events prompted for on the Vaccination Report Card (VRC) regardless of overall incidence rate, and are based on the incidence rates pooled across all study centers; corresponding p-values are calculated based on a test of difference between the 2 treatment groups. ‡ Includes rashes specifically reported as “measles/rubella-like” by ANs 05637 and 07313 (who received Lot 1 of ProQuad™) and ANs 01390 and 05651 (who received Lot 2 of ProQuad™). § Control is M-M-R™ II + VARIVAX™. _ Combined Lots denote the combination of Lots 1, 2, and 3 of ProQuad™. N = Number of subjects vaccinated. n = Number of subjects with follow-up. s = Number of subjects with adverse experience.

8.3.8 Comments & Conclusions (Study 012):

8.3.8.1 Data from this study demonstrate that the immune responses following immunization with the three consistency lots of ProQuad are comparable one to another for all 4 antigens. Vaccine response rates and GMTS were declared similar for measles, mumps, rubella, and varicella among the three consistency lots.

8.3.8.2 Data from this study demonstrate that when the immunogenicity data for all three ProQuad lots are pooled, the results are comparable to those seen after immunization with MMRII + VARIVAX for all 4 antigens. Vaccine response rates and GMTs were declared similar for measles, mumps, rubella, and varicella after ProQuad vs. MMRII + VARIVAX immunization

8.3.8.3 In addition, measles GMTs were significantly higher in Proquad recipients.

8.3.8.4 Vaccine response rates for measles, mumps, rubella, and varicella were found to be acceptable after ProQuad immunization because the lower bound of the 95% CI was above 90% for each MMR antigen and the lower bound of the 95% CI was at least 76% for varicella.

8.3.8.5 The fourth primary hypothesis stated that there would be no vaccine-related serious AEs in any vaccine group. However, 6 vaccine-related serious AEs were reported: reporting rates of 5/2860 (0.2%) in the ProQuad and 1/993 (0.1%) in the MMRII + VARIVAX group were comparable.

8.3.8.6 Injection site reactions (pain/tenderness/soreness and swelling) were less common in ProQuad than MMRII + VARIVAX recipients as one would expect when comparing a one vs. two injections. In this study measles- and rubella-like rashes occurred more frequently after ProQuad immunization than after MMRII + VARIVAX but the increase was not significant.

8.3.8.7 Rates of fever ≥102 F, oral equivalent or abnormal temperatures were reported significantly more frequently after each lot of ProQuad when compared to the control group. This increase was true when the rate of fevers was compared for the entire 42 day follow up period and when fever that occurred days 5-12 were compared between the investigational and control groups. Febrile seizures, however, occurred in 0.3% of vaccinees in each vaccine group.

8.3.8.8 In general the safety and tolerability profiles of all 3 consistency lots of ProQuad were comparable to those of concomitant MMRII + VARIVAX immunization.

8.4 Trial #013

An Open, Randomized, Multi-center Study of the Safety, Tolerability, and Immunogenicity of Frozen MMRV (ProQuad) Given Concomitantly Versus Non-concomitantly With Other Pediatric Vaccines in Healthy Children 12 to 15 Months of Age.

8.4.1 Objectives/Rationale:

The primary objectives of this study were: (1) to demonstrate that ProQuad can be administered concomitantly with TRIPEDIA and COMVAX without impairing immune responses to measles, mumps, rubella, varicella, diphtheria, tetanus, PT, FHA, hepatitis B, or *Hemophilus influenza* type b (Hib); (2) to demonstrate that concomitant administration of ProQuad, TRIPEDIA and COMVAX provides an acceptable immune response to measles, mumps, rubella and varicella; (3) to show that ProQuad is generally well tolerated when administered concomitantly with TRIPEDIA and COMVAX either at the same visit or separated by an interval of 6 weeks when compared to concomitant administration with MMRII and VARIVAX.

8.4.2 Design Overview:

Open-label, multi-center (48 sites), randomized, study in three groups of healthy children, 12-15 months old. Group 1 received ProQuad, TRIPEDIA and COMVAX concomitantly at separate injection sites. Group 2 received ProQuad on Day 0 and TRIPEDIA and COMVAX on Day 42. Group 3 received MMRII and VARIVAX on Day 0 and TRIPEDIA and COMVAX on Day 42. The targeted enrollment was 1600 children with 800 children in Group 1 and 400 children in each non-concomitant Group 2 and Group 3. The study began on **June 27, 2000** and ended on **October 23 2001**. Subjects were enrolled into Group 1, Group 2 and Group 3 in a 2:1:1 ratio using a computer generated allocation schedule that assigned subjects in groups of 4. [TRIPEDIA was administered to children 12 to 15 months of age because the ACIP schedule allows use of DtaP vaccine in children in this age group if 6 months has elapsed since completion of the primary series and if it is unlikely that they will return at 15 to 18 months for the booster dose.] The IRB at each study site reviewed and approved the protocol as well as the Informed Consent form used to enroll the subjects. Parents or legal guardians provided written informed consent and subjects were randomized and vaccinated on Study Day 0.

All subjects were followed for 56 days, i.e., 42 days after visit 1 and 14 days after visit 2. Subjects were seen 6 and 12 weeks after Day 0 and at these times the Vaccination Reports cards entries were reviewed and parents/guardians were asked about exposures to measles, mumps rubella, chickenpox or shingles. Subjects with measles or measles-like rashes were evaluated further. Whole blood samples were collected at the time of the rash and evaluated using RT-PCR for measles genome. Parents of children with varicella-like rashes were also asked to provide additional informed consent so that vesicular fluid could be collected and tested by --- for varicella genome to differentiate between wild type and vaccine virus associated lesions. In the event that a rash was noted, acute and convalescent blood samples were obtained and rash case report forms (CRFs) completed.

At the end of the study, subjects seronegative for any vaccine component were offered re-vaccination once the 56day safety follow-up period had been completed. This was an open label trial however, personnel performing the serology tests were blinded to group assignment but knew if they were testing

pre- or post- vaccination serum samples. The study design is summarized in Table 8.4.1.

Table 8.4.1 Summary of Study Design

	Procedures		
Time	**Group 1**	**Group 2**	**Group 3**
Day 0 (Visit 1)	Reviewed eligibility criteria. Obtained history/consent. Obtained 5-to 10-mL blood sample. Administered 0.5 mL of ProQuad™. Administered 0.5 mL of TRIPEDIA™. Administered 0.5 mL of COMVAX™. Handed out vaccination report cards (42-day follow-up period).	Reviewed eligibility criteria. Obtained history/consent. Obtained 5-to 10-mL blood sample. Administered 0.5 mL of ProQuad™. Handed out vaccination report cards (42-day follow-up period).	Reviewed eligibility criteria. Obtained history/consent. Obtained 5-to 10-mL blood sample. Administered 0.5-mL of M-M-R™ II. Administered 0.5-mL of VARIVAX™. Handed out vaccination report card (42-day follow-up period).
Days 0 to 42	Follow-up for adverse experiences.	Follow-up for adverse experiences	Follow-up for adverse experiences.
Day 42† (-7 days/+14 days) (Visit 2)	Obtained 5-to 10-mL blood sample. Collected vaccination report cards and reviewed with parent/guardian. Collected exposure survey information for measles, mumps, rubella, varicella, and/or zoster. Handed out vaccination report cards (14-day follow-up period).	Obtained 5-to 10-mL blood sample. Collected vaccination report cards and reviewed with parent/guardian. Collected exposure survey information for measles, mumps, rubella, varicella, and/or zoster. Administered 0.5 mL of TRIPEDIA™. Administered 0.5 mL of COMVAX™. Handed out vaccination report cards (14-day follow-up period).	Obtained 5-to 10-mL blood sample *(optional)*. Collected vaccination report cards and reviewed with parent/guardian. Collected exposure survey information for measles, mumps, rubella, varicella, and zoster. Administered 0.5 mL of TRIPEDIA™. Administered 0.5 mL of COMVAX™. Handed out vaccination report cards (14-day follow-up period).
Days 42 to 56	Follow-up for adverse experiences.	Follow-up for adverse experiences.	Follow-up for adverse experiences.
Day 84† (-7 days/+14 days)	Collected vaccination report cards and reviewed with parent/guardian. Collected exposure survey information for measles, mumps, rubella, varicella, and/or zoster.	Obtained 5-to 10-mL blood sample. Collected vaccination report cards and reviewed with parent/guardian. Collected exposure survey information for measles, mumps, rubella, varicella, and/or zoster.	Obtained 5-to 10-mL blood sample *(optional)*. Collected vaccination report cards and reviewed with parent/guardian. Collected exposure survey information for measles, mumps, rubella, varicella, and/or zoster.

† The -7 days/+14 days window surrounding the Day 42 time point and the Day 84 time point relates only to serologic follow-up. A different window (27 to 84 days post-vaccination) was used for statistical purposes. Group 1 = ProQuad™ + TRIPEDIA™ + COMVAX™ at Day 0. Group 2 = ProQuad™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later. Group 3 = M-M-R™ II + VARIVAX™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later. FOR ALL GROUPS, THE VACCINATION REPORT CARD WAS REQUIRED TO BE COMPLETED FOR A FULL 42 DAYS AFTER VISIT 1 AND FOR A FULL 14 DAYS AFTER VISIT 2. FOR GROUPS 2 AND 3 ONLY, A +14-DAY WINDOW WAS ALLOWED FOR ADMINISTRATION OF TRIPEDIA™ AND COMVAX™.

8.4.2.1 **Randomization:**

Subjects were randomly assigned 2:1:1 to treatment groups 1, 2 and 3, respectively, using computer generated allocation schedules that assigned subjects in blocks of 4. Allocation schedules were provided to each study site and numbers assigned sequentially. Numbers were not re-assigned for any reason.

8.4.2.2 **Interim analyses**:

No planned or unplanned interim analyses were performed with the exception that serological responses for Tripedia antigens were evaluated

after only a subset of the sera were tested due to constraints at the testing laboratory.

8.4.2.3 **Population:**

The vaccines were evaluated in healthy children 12-15 months of age who met the following criteria:

8.4.2.3.1 **Inclusion criteria**:

- Good health
- 12-15 months of age
- Negative history for varicella, shingles, measles, mumps, and rubella, diphtheria, tetanus, invasive Hib disease, and hepatitis B.
- Has completed either a 2-dose primary series of Pedvax/HIB or COMVAX or any 3 dose primary series of a licensed Hib vaccine.
- Has received 2 or 3 doses of any HepB vaccine or COMVAX prior to entry into the trial.
- Had previously received 3 doses of DTP or DtaP made by any manufacturer.

8.4.2.3.2 **Exclusion criteria**:

- Previous receipt of measles, mumps, rubella, or varicella vaccine either alone or in any combination.
- Immune impairment or deficiency, neoplastic disease, depressed immunity from steroid or other therapy.
- History of anaphylactic reaction to neomycin.
- History of anaphylactic or other immediate allergic reactions subsequent to egg ingestion or any component of the vaccine as stated in the package circulars.
- Any exposure to measles, mumps, rubella, varicella or shingles in the 4 weeks prior to each vaccination involving:
    - Continuous household contact
    - Playmate contact > 1 hour indoors
    - Hospital contact in the same room or prolonged face-to-face contact
    - Contact with a newborn whose mother had chickenpox 5 days or less prior to delivery or within 48 hours of delivery.
- Recent household, daycare or school exposure <14 days prior to study start to invasive Hib disease or Hepatitis B.
- Mother known to be Hepatitis B antigen positive.
- Vaccination with an inactivated vaccine within 14 days prior to receipt of each dose of vaccine or scheduled within 42 days thereafter.
- Vaccination with a live virus vaccine within 30 days of a dose of the study vaccine or scheduled within 42 days thereafter.
- Immune globulin or any blood product administered 3 months prior to or within 2 months after each vaccination.
- Any contraindication to either MMRII or VARIVAX or IM injection (such as thrombocytopenia) as stated in the package circulars.

- Any condition that in the opinion of the investigator might interfere with the evaluation of the study objectives.
- It was recommended that subjects not receive salicylates during the 6 weeks after vaccination because of aspirin use in children with varicella infection has been associated with Reye's syndrome.
- Recent (<72hours prior to study start) history of febrile illness ≥F oral temperature or equivalent) or underlying medical problem.

8.4.2.3.3 Subjects were discontinued from the study if they developed an anaphylactic reaction after vaccine administration or if they developed varicella, measles, mumps or rubella prior to the administration of the study vaccine. Subjects who received other vaccines or blood products before serologic follow-up samples were obtained were not necessarily discontinued from the study but their serology data may have been excluded from the group analyses.

8.4.3 Products used:

Products used in this study were manufactured by Merck with the exception of Tripedia. All clinical materials were supplied in single-dose vials. Study vaccines were re-supplied as needed throughout the study on a site-by-site basis. Doses were administered on Day 0, the day of entry into the study. Vaccine lot numbers and potencies are listed in Table 8.4.2.

Table 8.4.2 Vaccine lots numbers and potency.

Vaccine	Lot Number	Fill Number	Bulk Number(s)	Potency/0.5 mL Dose
ProQuad™	1593/WG699	-----	---------- ---------- ---------- ----------	3.97 (log10TCID50) 4.65 (log10TCID50) 4.25 (log10TCID50) 4.61 (log10 PFU)
M-M-R™ II	1676J	----	---------- ---------- ---------- ----------	3.8 (log10TCID50) 5.0 (log10TCID50) 3.7 (log10TCID50)
VARIVAX™	1681J	-----	----------	3.6 (log10 PFU)
	0443K	----	----------	3.5 (log10 PFU)
COMVAX™	1975J	----	---------- ----------	7.5 (mcg) 1.70 IVRP
	0112K	----	---------- ----------	7.1 (mcg) 1.83 IVRP
	0296K	---	---------- ----------	7.1 (mcg) 1.30 IVRP
	0104J	---	---------- ----------	8.1 (mcg) 2.24 IVRP
TRIPEDIA™	NA	---	NA	6.7 Lf Diphtheria Toxoid 5.0 Lf Tetanus Toxoid 23.4 (mcg) Pertussis Toxin
	NA	-----	NA	6.7 Lf Diphtheria Toxoid 5.0 Lf Tetanus Toxoid 23.4 (mcg) Pertussis Toxin
	NA	----	NA	6.7 Lf Diphtheria Toxoid 5.0 Lf Tetanus Toxoid 23.4 (mcg) Pertussis Toxin
	NA	----	NA	6.7 Lf Diphtheria Toxoid 5.0 Lf Tetanus Toxoid 23.4 (mcg) Pertussis Toxin
Diluent	0361K	NA	NA	NA
	1585J	NA	NA	NA
	0864H	NA	NA	NA
	1941J	NA	NA	NA

*PGS is phosphate, glutamate, and sorbitol stabilizer. It is reconstituted using the sterile diluent.
** Diluent: sterile water for injection.
N/A: not applicable.

8.4.4 Study Objectives:

8.4.4.1 Primary Hypothesis, Immunogenicity:

The antibody responses to measles, mumps, rubella, varicella, diphtheria, tetanus, pertussis toxin (PT), pertussis filamentous hemagglutinin (FHA), and Hib in subjects receiving ProQuad, TRIPEDIA and COMVAX (Group1) will be similar to those in subjects immunized with ProQuad followed by TRIPEDIA and COMVAX given 6 weeks later (Group 2).

The statistical criterion for showing similarity in response rates corresponded to the lower bound of the two-sided 90% confidence interval (CI) on the difference in response rates (Group 1 minus Group 2) excluding a decrease of δ* percentage points for each antigen where δ* is the non-inferiority margin as specified in the Table below based on the

method of Miettinen and Nurminen for testing the equivalence of two proportions.

The statistical criterion for showing similarity of GMTs for measles, mumps, rubella, and varicella responses corresponded to the lower bound of the two-sided 90% CI for the ratio of GMTs (Group1/Group2) being >0.67 for each antigen, i.e., excluding a decrease of 1.5 fold or more. The test of non-inferiority for GMTS was based on an ANOVA model for each antigen with $\alpha = 0.05$.

In summary, antibody responses to vaccine components measured 14 endpoints: seroconversion to each of the vaccine antigens (10 endpoints) and 4 GMTs for measles, mumps, rubella, and varicella antibody responses. (Table 3, below)

Rejection of all 14 null hypotheses (10 response rates and 4 GMTs) would result in the conclusion that concomitant administration of ProQuad TRIPEDIA and COMVAX elicited similar immune responses compared with ProQuad followed by TRIPEDIA and COMVAX immunization 6 weeks later. ***If similarity was not established for all 10 antigens then concomitant use might be claimed between vaccines for which similarity was established between groups.***

Immunogenicity endpoints are summarized below in Table 8.4.3.

Table 8.4.3 Summary of the immunogenicity endpoints, expected response rates and non-inferiority margins for antibody responses for each vaccine antigen

Vaccine Component	Primary Variable	Expected Response Rates in Each Group	Clinically Relevant Difference (δ*100 Percentage Points)
Measles	Seroconversion rate	95%†	5
Mumps	Seroconversion rate	95%†	5
Rubella	Seroconversion rate	95%†	5
Varicella	% with titer ≥5 gpELISA units	90%†	10
Diphtheria	% with titer ≥0.1 IU/mL	95%‡	10
Tetanus	% with titer ≥0.1 IU/mL	95%‡	10
Pertussis PT	% with ≥4-fold rise in titer	85%‡	15§
Pertussis FHA	% with ≥4-fold rise in titer	80%‡	15§
Hepatitis B	% with titer ≥10 mIU/mL	98%_	10
Haemophilus influenzae type B (Hib)	% with titer >1 mcg/mL	93%	10

† The expected seroconversion rates to measles, mumps, and rubella (as measured by EIA) are based upon clinical experience with M-M-R™ II from 1992 to 1997. The ranges for the seroconversion rates observed in clinical studies conducted during this period are 92.6 to 98.5% for measles, 97.3 to 100% for mumps, and 98.1 to 100% for rubella. The expected percentage of subjects with anti-VZV titers ≥5 gpELISA units is based on clinical experience with VARIVAX™ production lots dated 1991 or later. The range of observed varicella responses is 80.6 to 92.9%.
‡ The expected response rates for diphtheria, tetanus, pertussis PT, and pertussis FHA are based on data in the package circular for TRIPEDIA™ in which TRIPEDIA™ was used with ACTHib™ (Tetanus, Toxoid Conjugate, Aventis Pasteur MSD) as well as the Clinical Development Plan for ARBI (ACTHib™, RECOMBIVAX HB™, DTPBiken, Inactivated Polio).
§ A difference of 15 percentage points rather than 10 is hypothesized for the pertussis endpoints due to higher variability in the responses.
_ The expected response rates for hepatitis B and *Haemophilus influenzae* type B (Hib) are based on the COMVAX™ package circular.
PT = Pertussis toxin.
FHA = Filamentous hemagglutinin.
EIA = Enzyme immunoassay.
VZV = Varicella zoster virus.
gpELISA = Glycoprotein enzyme-linked immunoassay.

The following assumptions were made in calculating the sample size for this study: 5% of the subjects were expected to be seropositive for measles, mumps, or rubella antibodies or have a varicella gpELISA >1.25 at baseline; 10% of the subjects would be lost after visit 1 and another 5% lost after visit 2. The natural logarithm of the antibody titers was expected to vary by no more than 1.2 fold for each component based on responses seen in previous studies. The power calculations for each endpoint are listed in Table 4 below and the overall power of the study was 91.2%. The primary immunogenicity analyses were based on a per protocol approach using subjects with both pre- and post-vaccination serology results. Analyses of serological responses for measles, mumps, rubella, and varicella also had to meet baseline requirements such as < 120mIU/mL measles antibody, <10 ELISA Units/mL mumps antibody, <10IU/mL rubella antibody and <1.25 gpELISA units of varicella antibody.

Response rates between groups were compared using a statistical method designed to test non-zero difference in proportions stratified by study center. Centers with fewer than 10 evaluable patients per group

were pooled with the largest site in close geographic proximity to yield centers that met the minimum criteria. [Note bene: Study centers were combined differently for the evaluation of responses to D, T pertussis toxin (PT) and pertussis FHA. Study centers were combined to yield at least 10 evaluable subjects per group and included only those study centers that provided evaluable subjects for all 4 DTP endpoints]. Analysis of response rates was adjusted for study center.

Comparison of GMTs used an ANOVA method and adjusted for study center and treatment-by-center interaction.

A summary of the planned statistical comparisons is listed below in Table 8.4.4.

Table 8.4.4 Summary of vaccine antigens, expected response rates or expected standard deviation of titers, equivalence margins used for non-inferiority assessments, and statistical power for each comparison.

Antigen	Primary Variable	Expected Response Rate or Standard Deviation of Natural Log Titers in Each Group	Equivalence Margin	Power
Measles	% with titer ≥120 mIU/mL	95%	5 percentage points	97.1%
	Geometric mean titer	1.2	1.5-fold	>99.9%
Mumps	% with titer ≥10 Ab units	95%	5 percentage points	97.1%
	Geometric mean titer	1.2	1.5-fold	>99.9%
Rubella	% with titer ≥10 IU/mL	95%	5 percentage points	97.1%
	Geometric mean titer	1.2	1.5-fold	>99.9%
Varicella	% with titer ≥5 gpELISA units	90%	10 percentage points	>99.9%
	Geometric mean titer	1.2	1.5-fold	>99.9%
Diphtheria	% with titer ≥0.1 IU/mL	95%	10 percentage points	>99.9%
Tetanus	% with titer ≥0.1 IU/mL	95%	10 percentage points	>99.9%
Pertussis PT	% with ≥4-fold rise in titer	85%	15 percentage points	>99.9%
Pertussis FHA	% with ≥4-fold rise in titer	80%	15 percentage points	99.8%
Hepatitis B	% with titer ≥10 mIU/mL	98%	10 percentage points	>99.9%
Hib (Hib)	% with titer >1 mcg/mL	93%	10 percentage points	99.9%
Overall Power for Similarity Hypotheses				~91.2%

gpELISA = Glycoprotein enzyme-linked immunosorbent assay. PT = Pertussis toxin. FHA = Filamentous hemagglutinin.

8.4.4.2 Second Primary Hypothesis, Immunogenicity:

The concomitant administration of ProQuad with TRIPEDIA and COMVAX will demonstrate an acceptable immune response to measles, mumps, rubella, and varicella.

The statistical criterion for an acceptable immune response required that the lower bound of the two-sided 95% CI for measles, mumps, and rubella seroconversion rates be entirely above 90% for measles, mumps, and rubella and entirely above 76% for varicella.

Four, one-sided, 1-sample binomial tests were conducted, 1 for each antigen at the one-sided α=0.025 significance level. Sample size calculations: it was estimated that the drop-out rate of 15% would leave 85% of the subjects remaining and evaluable. Response rates were assumed to be 95% for measles, mumps, and rubella and 90% for varicella. The study had >99.9% power for each acceptability hypothesis.

The comparison of responses rates for acceptability used exact, one-sample binomial tests for each antigen. Two-sided, 95% confidence intervals on the response rate for each antigen were calculated using the exact CI method for a single binomial proportion.

8.4.4.3 Third Primary Hypothesis, Safety:

Concomitant administration of ProQuad, TRIPEDIA and COMVAX will be generally well tolerated.

All subjects were followed for a total of 56 days. Comparisons of adverse reactions were made between Group 1 and Group 2 (ProQuad followed by Tripedia plus Comvax).

Safety measures that were compared included:

1. Any adverse experience
2. Any injection site reaction
3. Any systemic reaction
4. Any vaccine related adverse experience

The following specific adverse reactions were also compared between groups:

1. Fever >102 F oral or equivalent
2. Measles-like rashes
3. Rubella-like rashes
4. Varicella-like rashes
5. Mumps symptoms

The incidence rates were compared between groups and the corresponding p-value, risk difference and 95% two-sided CIs were also provided. For systemic AEs occurring in 1% or more of the subjects in any group, the risk difference and 95% CIs were compared but p values were not provided.

The study had 86.6% power to detect a 5 percentage point increase in incidence rates from 5 to 10%.

8.4.4.4 Secondary Hypothesis:

ProQuad whether given concomitantly with TRIPEDIA and COMVAX at the same visit or separated by an interval of 6 weeks will be generally well tolerated when compared to adverse reactions reported for children in Group 3 who were immunized with MMRII plus VARIVAX.

All subjects were followed for a total of 56 days and comparisons were made between Group 1 and Group 3.

The study had 76.7% power to detect a 5-percentage point increase in incidence rates from 5 to 10%.

8.4.4.5 **Study Endpoints**:

Immunogenicity endpoints were measured using immunological assays that specifically measured IgG antibody responses to each vaccine antigen. **Safety endpoints** were assessed using the Vaccination Report Card that was completed by each subject's parent or legal guardian.

8.4.4.5.1 **Detection of Measles IgG Antibody (ELISA):**

The measles ELISA used measles antigen purchased from ---------
---

---------- The limit of detection of this assay was determined to be <12.3 mIU/mL corresponding to <120mIU/mL for a samples tested at a 1:1000 dilution. The assay precision was 23%. Samples were considered to be seronegative if they were below the OD cut-off and samples were considered to be seropositive if they had ≥12.13 ELISA antibody units (equivalent to 120mIU measles antibody/mL).

8.4.4.5.2 **Detection of Mumps IgG Antibody (ELISA):**

Mumps virus antigen used for this assay was produced at MRL. The mumps antigen was ----------
---

---------- The quantity of anti-mumps IgG was determined by comparing the response in the test sample to the standard curve. The cut-off was determined by running 72 known negative samples (12 samples in 6 assays). The assay cut-off was determined to be 10 Ab units. Samples with ODs less than or equal to the cut-off were serostatus negative and assigned a titer of < 10.0 Mumps AB units. Samples with OD values greater than the cut-off were quantified using the standard curve. The quantifiable range was 0.5 to 65 mumps Ab units/mL. Sera whose titers exceeded this range were re-analyzed at greater dilutions until an endpoint titer was obtained. The negative control for the assay was a pool of human sera known to be mumps negative. The low positive control was a pool of human sera while the high positive was also a pool of human sera. A single mumps positive serum was used to generate the standard curve. The standard curve data were fit using a quadratic polynomial. The LOD was <0.5 Ab units and the quantifiable range of the assays was 0.5 to 65 mumps Ab units/mL. Samples with medium and high titers vary 15.9% with each 10-fold dilution. Assay precision was 18.9-25.3%.

8.4.4.5.3 Detection of Rubella IgG (ELISA):

Inactivated rubella antigen purchased from ----------

---------- The cut-off for the assay was determined by determining the mean OD value for 10 known rubella negative control sera plus 5 times the mean of the negative control. Samples with OD values less than the cut-off were considered to be seronegative and were assigned a value of 10 AB units. Positive samples were quantitated relative to the standard curve. The negative control for this assay was a single human serum known to be negative for rubella antibody. The low positive and high positive controls were single human sera known to give responses at the low and high end of the curves receptively. A single human serum calibrated against the WHO reference serum was used to generate the standard curve. Standard curve data were fit using a quadratic polynomial. The LOD was <5IU rubella antibody/mL for a sample tested at 1:1000 dilution. The

quantifiable range of the assay was 0.005-0.32 IU/mL at a dilution of 1:1000 or 5-32 IU/mL. There was evidence of dilution bias between 1:100 and 1:800 dilutions with titers being almost 2 fold higher at the 1:800 dilution. Assay precision was 14%. A pre-vaccination sample was considered to be seronegative if it was below the OD cut-off and a post vaccination sample was considered to be seropositive if it contained ≥12.8 ELISA antibody units (=10 IU/mL).

8.4.4.5.4 Varicella IgG gp ELISA antibody:

The purpose of the glycoprotein enzyme-linked immunosorbent assay (gpELISA) was to detect IgG antibody to varicella-zoster virus (VZV) before and after vaccination with VZV-containing vaccine(s). This method detects antibodies to VZV glycoproteins (gp), which have been lectin affinity-purified from MRC-5 cells infected with the KMcC strain of VZV. The assay and the purification of the VZV gp from VZV-infected cells have been described ----------
---

Serum sample titers determined by gpELISA correlate with neutralizing antibody titers (Krah, DL, Cho I, Schofield T, et al. Comparison of gpELISA and neutralizing antibody responses to Oka/Merck live varicella vaccine in children and adults. Vaccine 1997 15(1):61-64) and with protective efficacy (White CJ, Kuter BJ, Ngai A, and et al. Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. Pediatr Infect Dis J 1992 11(1):19-23).

Results for the assay are reported as concentration of antibody in gpELISA units/mL. The negative control used for this assay was an individual human serum at a dilution of 1:50, found to be negative for anti-VZV. The high positive marker was a VZV-antibody-positive serum, diluted 1:15,000, which gave a response in the assay at the upper end of the standard curve. The low positive marker was a VZV-antibody-positive serum diluted 1:50,000, which gave a response in the assay at the lower end of the standard curve. A VZV-antibody-positive individual human serum was used to generate a standard curve (range of 0.625 to 20 gpELISA units/mL).

Prior to June-2001, the standard curve was approximated using a quadratic function fit to the 0.625 to 20 gpELISA units/mL concentration range of the standard. Since June-2001, the standard curve has been approximated using the four-parameter weighted logistic regression function. A statistical analysis

comparing the two fit procedures showed that the quadratic and logistic processing methods yield similar titers (generally within 3%) when interpolating from the 0.625 to 20 gpELISA units/mL region of the standard curve.

During validation, the limit of detection (LOD) was mathematically determined to be 0.3 gpELISA units/mL. However, because no standard concentrations below 0.625 gpELISA units/mL are run in the assay, the LOD is reported as <0.625 gpELISA units/mL. The quantifiable range of the assay is 0.625 to 20 gpELISA units/mL. Dilutability is defined as the attribute of a standard curve assay whereby it is demonstrated that a test sample can be diluted through a series, yielding equivalent titers across that series. The assay is dilutable for samples tested in the 1:500 to 1:40,000 dilution range. The precision of the assay for a sample titer was 11%. There was no statistical evidence of increased variability in test sample results due to different analysts performing the assay.

8.4.4.5.5 ANTI-PRP:

Anti-PRP was measured by ---- using the ---------- technique. Antibody concentrations were calculated relative to the standard curve generated in each run with the US FDA standard serum. The LOD was 125 ng/mL with a standard deviation of < 20%. The analytical LOD was 6.60 ng Ab/mL and the range was 100-11,600ng Ab/mL.

8.4.4.5.6 Antibodies to Hepatitis B Surface Antigen:

Antibodies to Hepatitis B surface antigen were measured by the quantitative ----------. The quantifiable range for the assay was 2 mIU/ml to 500 mIU/mL. The cut-off of 2 mIU/mL was set as 2.1 times the average count of the negative controls.

8.4.4.5.7 Detection of Diphtheria Antitoxin:

Diphtheria antitoxin was measured by ---------- culture assay using an in-house serum standard that had been previously calibrated against the WHO International Standard. The minimum level of antitoxin reported by this assay is 0.01 IU/mL.

8.4.4.5.8 Detection of Tetanus Antitoxin:

Tetanus IgG antitoxin is measured by an indirect noncompetitive ---------- using an in-house standard calibrated against the WHO International Standard for Tetanus. The limit of detection is 0.01 IU/mL.

8.4.4.5.9 Detection of anti-FHA:

Antibody to pertussis FHA is measured by an indirect noncompetitive ELISA using an in-house standard calibrated

against the US Reference Pertussis Antisera. The assay range is 0.8 to 97 u/ml.

8.4.4.5.10 Detection of anti-PT:

Antibody to pertussis antigen was measured using an indirect ELISA. An in-house serum previously calibrated against the US Reference Pertussis Antisera was used to quantitate antibody responses. The assay range is 0.8 to 50.5u/mL.

8.4.4.6 Changes in the Conduct of the Study:

The study followed the protocol and data analysis plan. Some additional analyses were performed at the conclusion of the study to understand the unexpected low results to pertussis FHA antigen.

The amended protocol was approved by CBER to allow:

1. An increase in sample size
2. Collection of blood samples for RT-PCR for measles genome.
3. Photography to document measles-like rashes
4. Additional days to extend the range for Visit #2
5. Include COMVAX as a vaccine given for the initial 2 dose series
6. Exclude the use of non-study vaccines during the study
7. Remove anemia as an exclusion criteria
8. Revisions to rubella serology
9. Include an acceptable immune response to varicella as a primary immunogenicity endpoint.
10. Various changes to serological cut-offs and criteria for response.

Notable issues in the conduct of the study:

1. At study site 013-012 subjects were randomized at the time of recruitment rather than at the time of vaccination. This did not impact on the study results.
2. Significant temperature excursions occurred in the refrigerator used to store COMVAX and TRIPEDIA below the recommended storage temperature at one study site. Immunogenicity data from subjects who received compromised vaccines was excluded from the immunogenicity analysis (see Table 8).
3. At study site 013-025, 10 subjects randomized to Group 1 or Group 2 received the alternate dosing (i.e., individuals randomized to concomitant vaccination in Group 1 were given ProQuad on Day 0 and TRIPEDIA and COMVAX on Day 42 while subject randomized to Group 2 were given all vaccines on Day 0). These subjects were excluded from the immunogenicity analysis. Subjects who received study vaccines and had safety follow-up were included in the safety analysis in the group whose treatment they received regardless of the protocol violation.

8.4.5 Surveillance

8.4.5.1 MRL conducts its own Quality Assurance and Quality Control Program and surveillance included on-site monitoring of investigators sites, on site and in-house review of clinical data and resultant databases, review of the clinical study reports and summary documents.

8.4.5.2 No formal interim analysis was performed.

8.4.5.3 Formal surveillance for cases of measles, mumps, rubella and varicella in the community was not performed but parents/guardians reported any known cases or exposures.

8.4.5.4 Follow-up visits for safety assessments and serology were as follows: Parents filled out the Vaccination Report Cards for 42 days after vaccination #1 and for 14 days after vaccination #2. They were required to note local and systemic AEs and record temperatures for 42 days after immunization #1 and 14 days after immunization #2. They were to contact study personnel immediately if any serious AEs were noted. Study personnel immediately evaluated children with rashes. Varicella-like lesions were cultured and tested by --- after informed consent was obtained from the parent/guardian.

8.4.6 Statistical considerations:

8.4.6.1 Statistical considerations are discussed under Study Objectives. See Section 8.4.4.

8.4.7 Results

8.4.7.1 Populations enrolled/analyzed

8.4.7.1.1 Multi-center Study:

The study was conducted at 48 study sites in the United States. Enrollment by study site and investigator is listed in Table 8.4.5 below:

Table 8.4.5 Summary of study sites, investigators and numbers of children enrolled at each site.

Study Number	Investigator	Location	Total (N=1915)
013001	Black, Steven	Oakland, CA	253
013002	Greenberg, David P.	Pittsburgh, PA	84
013003	Marshall, Gary	Louisville, KY	22
013004	Reisinger, Keith	Pittsburgh, PA	180
013005	Thompson, Steven M.	Little Rock, AR	69
013006	Rothstein, Edward	Sellersville, PA	101
013008	Vaughn, Katherine Fuelling	Vancouver, WA	67
013011	Ryan, Michael E.	Danville, PA	17
013012	Coury, Daniel L.	Columbus, OH	199
013015	Boehm, Frederick P.	Bend, OR	1
013016	Troutman, James L.	Bellingham, WA	67
013017	Edwards, Kathryn M.	Nashville, TN	2
013020	Asmar, Basim	Detroit, MI	7
013021	Sperling, Malcolm J.	Fountain Valley, CA	26
013023	Ball, Charles S.	Fayetteville, AR	11
013024	Daum, Robert S.	Chicago, IL	48
013025	Taylor, James A.	Seattle, WA	14
013026	Blumer, Jeffrey L	Cleveland, OH	8
013028	Rathore, Mobeen H.	Jacksonville, FL	13
013029	Luber, Stephen R.	Spokane, WA	26
013030	Walter, Emmanuel	Durham, NC	11
013031	Henderson, Frederick	Goldsboro, NC	57
013032	Senders, Shelly D.	University Heights, OH	22
013033	Bromberg, Kenneth	Brooklyn, NY	12
013035	Chatterjee, Archana	Omaha, NE	18
013036	Werzberger, Alan	Monroe, NY	66
013037	Block, Stan	Bardstown, KY	23
013038	Johnson, Candice E.	Denver, CO	4
013039	Andrews, Wilson P.	Marietta, GA	28
013040	Guerra, Fernando A.	San Antonio, TX	54
013041	Alvey, Justin C.	Layton, UT	58
013042	Sullivan, Bradley J.	Marshfield, WI	32
013043	Matson, David	Norfolk, VA	38
013044	Nachman, Sharon	Stony Brook, NY	13
013045	Lepow, Martha L.	Albany, NY	1
013046	Goessler, Mary C.	Bellevue, PA	2
013047	Jones, Ronald C.	Provo, UT	40
013048	Venters, Charmaine L.	Baton Rouge, LA	33
013049	Marchant, Colin D.	Boston, MA	51
013050	Yeiser, Michael F.	Owensboro, KY	61
013051	Leader, Joseph	Woburn, MA	10
013052	Yogev, Ram	Chicago, IL	4
013053	Azimi, Parvin H.	Oakland, CA	6
013054	Sher, Lawrence D.	Rolling Hills Estate, CA	8
013055	Fries, Stephen M.	Boulder, CO	6
013056	Conti, Ralph	Las Vegas, NV	35
013059	Eckert, Buckley	Kirkland, WA	5
013060	Bernstein, Jerry C.	Raleigh, NC	2

8.4.7.1.2 Subject accounting:

1915 subjects were enrolled with 1779 completing the study. Dropout rates were similar across treatment groups and no one discontinued due to an AE. Two subjects mistakenly received an incorrect combination of vaccines on Day 0 (either MMRII+ TRIPEDIA+ COMVAX or VARIVAX+ TRIPEDIA+ COMVAX). Three subjects were randomized but not vaccinated. Subject enrollment and dropouts are summarized in Table 8.4.6 below.

Table 8.4.6 Subject enrollment and dropouts by study group

	Concomitant Group (N=949)		**Nonconcomitant Group** (N=485)		**Control Group** (N=479)		**M-M-R™ II + TRIPEDIA™ + COMVAX™** (N=1)		**VARIVAX™ + TRIPEDIA™ + COMVAX™** (N=1)		**Total** (N=1915)	
	N	(%)	n	(%)	N	(%)	N	(%)	n	(%)	n	(%)
Entered:	949		485		479		1		1		1915	
Male (age range - months)	507	(11 to 15)	262	(11 to 16)	233	(12 to 15)	0		0		1002	(11 to 16)
Female (age range - months)	442	(12 to 15)	223	(12 to 15)	246	(11 to 15)	1	(14 to 14)	1	(15 to 15)	913	(11 to 15)
Vaccinated At:												
Vaccination Visit 1	949	(100)	485	(100)	479	(100)	1	(100)	1	(100)	1915	(100)
Vaccination Visit 2	909	(95.8)	468	(96.5)	453	(94.6)	0	(0.0)	0	(0.0)	1830	(95.6)
Completed:	884	(93.2)	453	(93.4)	442	(92.3)	0	(0.0)	0	(0.0)	1779	(92.9)
Discontinued:	65	(6.8)	32	(6.6)	37	(7.7)	1	(100)	1	(100)	136	(7.1)
Clinical adverse experience	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Deviation from protocol	2	(0.2)	1	(0.2)	4	(0.8)	1	(100)	0	(0.0)	8	(0.4)
Refused further participation	18	(1.9)	9	(1.9)	12	(2.5)	0	(0.0)	1	(100)	40	(2.1)
Lost to follow-up	31	(3.3)	12	(2.5)	13	(2.7)	0	(0.0)	0	(0.0)	56	(2.9)
Clinical adverse experience - Discontinued test vaccine	0	(0.0)	1	(0.2)	3	(0.6)	0	(0.0)	0	(0.0)	4	(0.2)
Missed one or more blood samplings	3	(0.3)	7	(1.4)	2	(0.4)	0	(0.0)	0	(0.0)	12	(0.6)
Incomplete safety follow-up	11	.2	2	.4	3	.6	0	.0	0	.0	16	.8

8.4.7.1.3 Protocol deviations

Protocol deviations or violations that resulted in data being excluded from the primary immunogenicity analysis are summarized in Tables 8.4.7 and 8.4.8, below:

Table 8.4.7 Protocol deviations that resulted in data being excluded from immunogenicity analysis

Description	Study Center	Allocation Number
Did not receive treatment assigned to the subject	0130025	11757, 11758, 11759, 11761, 11762, 11763, 11765, 11766, 11767, 11770
	013056	15301
Participated in an ear infection study	013040	10765†
Was younger than 12 months of age	013012	11644
	013032	11896
	013041	11971
Received M-M-R™ II instead of ProQuad™	013011	10917†
Received VARIVAX™ instead of ProQuad™	013037	12247
†Subjects who discontinued the study because of the protocol deviation.		

Table 8.4.8 Protocol violations that resulted in exclusion of serology results from the per protocol analysis of immunogenicity for the designated antigen

Serology Results Excluded	Description	Study Center	Allocation Number
Hepatitis B	Did not have required doses of Hepatitis B vaccine prior to Enrollment	013002	15258
		013004	10598, 10605†
		013008	10807
		013021	11696†
		013025	11760, 11759, 11761
		013026	12128
		013040	14725,10756, 10759, 10761, 10762, 14699, 14702, 14704, 14709, 14714, 14726
Hib	Did not have required doses of Hib vaccine prior to enrollment	013004	10713
		013005	10115, 10116, 10126, 15225
		013008	10855
		013012	11866
		013020	11662
		013024	11740
		013031	11974, 11976, 11980
		013037	12258
		013041	11967, 12369
		013040	14719
		013055	14732
Diphtheria, tetanus, pertussis PT, and pertussis FHA	Did not have required doses of DTP vaccine prior to enrollment	013008	10855
		013047	12487†
Hepatitis B, Hib, diphtheria, tetanus, pertussis PT, and pertussis FHA	COMVAX™ and TRIPEDIA™ had been frozen due to temperature control failure	013012	11595, 11597, 11598, 11599, 11600, 11606, 11605, 11607, 11608, 11609, 11610, 11611, 11612, 11645, 11646, 11647, 11648, 11650, 11651, 11649, 11652, 11653, 11654, 11655, 11656, 11657, 11658, 11659, 11660, 11838, 11837, 11839, 11840, 11009, 11010, 11011, 11012, 11589, 11590, 11591, 11592, 11637, 11638, 11639, 11640, 11641, 11642, 11643, 11644, 11853
†Subjects who discontinued the study because of the protocol deviation. PT = Pertussis toxin. FHA = Filamentous hemagglutinin.			

8.4.7.1.4 Subjects prematurely unblinded: none

8.4.7.1.5 Primary analysis of immunogenicity was based on the per protocol population.

8.4.7.1.6 Demographics:

Subjects in each group were comparable in terms of age, race, gender, and with regards to prior therapies or medications. 1915 healthy infants, 12-15 months of age were enrolled and vaccinated in this study. For a summary of subject demographics, see Table 8.4.9.

Table 8.4.9 Demographics of the study population by vaccine group

	Concomitant Group		**Nonconcomitant Group**		**Control Group**		**M-M-R™ II + TRIPEDIA™ + COMVAX™**		**VARIVAX™ + TRIPEDIA™ + COMVAX™**		**Total**	
	(N=949)		(N=485)		(N=479)		(N=1)		(N=1)		(N=1915)	
	n	(%)	n	(%)	n	(%)	N	(%)	n	(%)	n	(%)
Gender												
Male	507	(53.4)	262	(54.0)	233	(48.6)	0	(0.0)	0	(0.0)	1002	(52.3)
Female	442	(46.6)	223	(46.0)	246	(51.4)	1	(100)	1	(100)	913	(47.7)
Age (Months)												
Mean	12.4		12.3		12.4		14.0		15.0		12.4	
SD	0.7		0.7		0.7						0.7	
Median	12.0		12.0		12.0		14.0		15.0		12.0	
Range	11 to 15		11 to 16		11 to 15		14 to 14		15 to 15		11 to 16	
Male	11 to 15		11 to 16		12 to 15						11 to 16	
Female	12 to 15		12 to 15		11 to 15		14 to 14		15 to 15		11 to 15	
Race/Ethnicity												
African American	101	(10.6)	52	(10.7)	42	(8.8)	0	(0.0)	1	(100)	196	(10.2)
Asian/Pacific	103	(10.9)	54	(11.1)	57	(11.9)	0	(0.0)	0	(0.0)	214	(11.2)
Caucasian	678	(71.4)	336	(69.3)	342	(71.4)	1	(100)	0	(0.0)	1357	(70.9)
Hispanic	38	(4.0)	26	(5.4)	20	(4.2)	0	(0.0)	0	(0.0)	84	(4.4)
Native American	3	(0.3)	0	(0.0)	1	(0.2)	0	(0.0)	0	(0.0)	4	(0.2)
Other	26	(2.7)	17	(3.5)	17	(3.5)	0	(0.0)	0	(0.0)	60	(3.1)
Prior Therapy												
None	547	57.6	282	58.1	279	58.2	1	100	1	100	0	0

8.4.7.2 Efficacy endpoints: Immunogenicity

8.4.7.2.1 Primary Endpoint:

The primary endpoint for efficacy was the immune response to each of the 10 vaccine antigens: measles, mumps, rubella, varicella, diphtheria, tetanus, pertussis (PT and FHA), Hib, and HepB. Response rates and GMTs were compared in the per protocol population who were seronegative for measles, mumps and rubella antibody or had a varicella gpELISA titer <1.25 units at baseline.

Subjects excluded from the primary immunogenicity analyses of measles, mumps, rubella, and varicella are summarized in Table 8.4.10 below:

Table 8.4.10 Serostatus for measles, mumps, rubella and varicella antibody at baseline and reasons for exclusion from the immunogenicity analyses by vaccine antigen.

	Measles		Mumps		Rubella		Varicella	
	Concomitant Group	Nonconcomitant Group	Concomitant Group	Nonconcomitant Group	Concomitant Group	Nonconcomitant Group	Concomitant Group	Nonconcomitant Group
Subjects vaccinated at Visit 1:	949	485	949	485	949	485	949	485
Subjects included in the analysis:	**758**	**388**	**811**	**415**	**829**	**421**	**757**	**383**
Subjects excluded from the analysis:	191	97	138	70	120	64	192	102
Younger than 12 months at the first vaccination	1	1	1	1	1	1	1	1
Randomized to the wrong treatment group	3	8	3	8	3	8	3	8
Seropositive†or varicella titer ≥1.25 gpELISA units at baseline‡	79	37	25	8	4	1	83	41
Missing or not evaluable baseline‡ result	37	16	37	16	37	16	37	15
Missing or not evaluable post-vaccination result:§	82	42	83	41	83	41	82	42
Sampling outside the specified day range	27	9	27	9	27	9	27	9
Refused to provide blood sample	15	8	15	8	15	8	15	8
Difficult to obtain blood sample	30	18	30	18	30	18	30	18
Sample quantity not sufficient	2	2	3	1	1	1	2	1
Sample hemolyzed	2	1	2	1	2	1	2	1
Sample was not taken	1	1	1	1	1	1	1	1
Invalid assay	0	0	0	0	1	0	0	0
Discontinued test vaccine due to adverse experience	0	1	0	1	0	1	0	1
Lost to follow-up	20	8	20	8	20	8	20	8
Refused further participation	13	8	13	8	13	8	13	8
Result was not available_	0	0	0	0	1	0	0	1

† Seropositivity at baseline for measles corresponds to an antibody titer ≥120 mIU/mL, for mumps corresponds to an antibody titer ≥10 Ab unit, and for rubella corresponds to an antibody titer ≥10 IU/mL. ‡ Baseline sample is the sample taken immediately before the vaccination of the relevant component. § Including randomized to the wrong treatment group. _ Sample was not tested in the given assay. A subject may be counted in more than one category. Concomitant Group = ProQuad™ + TRIPEDIA™ + COMVAX™ at Day 0. Nonconcomitant Group = ProQuad™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later. GpELISA = Glycoprotein enzyme-linked immunosorbent assay.

Tests for interactions: Prior to the non-inferiority analysis there were comparisons of GMTs and response rates across study centers. No significant interactions were found when GMTs for measles, mumps, rubella, and varicella were compared across centers. There was also no treatment-by-center interaction found when the response rates for measles, rubella, varicella, diphtheria, tetanus, pertussis, and Hib were compared. However, response rates for mumps and hepatitis B varied significantly in this test. Therefore, the difference in responses rates for the treatment and control groups were calculated for each center for these two antigens. These tests showed that the variation was random and not qualitative and do not negate the finding of similarity between concomitant and nonconcomitant groups with respect to the mumps or hepatitis B antigens.

The evaluation for non-inferiority between the concomitant and non-concomitant groups was conducted in three stages to keep the overall type one error rate at $\alpha = 0.05$:

1. Responses (GMTs and responses rates) to the components of ProQuad
2. Responses to the 4 components of TRIPEDIA and two components of COMVAX.
3. Responses to the two components of COMVAX.

Responses to the four components of ProQuad were evaluated at $\alpha = 0.05$. With respect to the analysis of response rates to measles, mumps, and rubella, the analysis excluded a decrease of 5% or more and 10% or more for varicella. With respect to GMTs, the analysis excluded a decrease of 1.5 fold with the one-sided p value ≤ 0.05. All 8 CIs excluded the pre-specified decrease and the responses were declared similar between groups. The data are presented in Table 8.4.11 and the comparisons and statistical analyses are summarized in Table 8.4.12 below:

Table 8.4.11 Summary of antibody responses for the primary immunogenicity endpoints for measles, mumps, rubella and varicella following concomitant immunization with ProQuad plus Comvax plus Tripedia vs. nonconcomitant immunization with ProQuad followed by Tripedia and Comvax.

Vaccine (Assay)	Parameter	Concomitant Group (N=949)		Nonconcomitant Group (N=485)		Estimated Difference†‡ /Fold Difference†§ (90% CI)	Criterion	One-Sided p-Value	Conclusion_
		n	Response†	n	Response†				
Measles (ELISA)	% ≥120 mIU/mL	758	97.8%	388	98.7%	-0.9 (-2.3, 0.6)	LB>-5.0	<0.001	Immunogenicity for the components of ProQuad after concomitant immunization is similar to immunogenicity after nonconcomitant immunization.
	GMT (mIU/mL)	758	3505.0	388	3509.8	1.0 (0.9, 1.1)	LB>0.67	<0.001	
Mumps (ELISA)	% ≥10 ELISA Ab Units	811	95.4%	415	95.1%	0.3 (-1.7, 2.6)	LB>-5.0	<0.001*	
	GMT (ELISA Ab Units)	811	89.5	415	83.9	1.1 (1.0, 1.2)	LB>0.67	<0.001	
Rubella (ELISA)	% ≥10 IU/mL	829	98.6%	421	99.3%	-0.7 (-1.8, 0.5)	LB>-5.0	<0.001	
	GMT (IU/mL)	829	98.9	421	99.9	1.0 (0.9, 1.1)	LB>0.67	<0.001	
Varicella (gpELISA)	% ≥5 gpELISA Units	757	89.6%	383	90.8%	-1.2 (-4.1, 2.0)	LB>-10.0	<0.001	
	GMT (gpELISA Units)	757	13.8	383	15.3	0.9 (0.8, 1.0)	LB>0.67	<0.001	

Table 8.4.12 Summary of the comparison of the primary immunogenicity endpoints for measles, mumps, rubella and varicella responses following concomitant immunization with ProQuad plus Comvax plus Tripedia vs. nonconcomitant immunization with ProQuad followed by Tripedia and Comvax and compared to immune responses in the Control Group following immunization with MMRII plus VARIVAX.

Vaccine Component (Assay)	Parameter	Concomitant Group (N=949)		Nonconcomitant Group (N=485)		Control Group (N=479)	
		n	Observed Response (95% CI)	n	Observed Response (95% CI)	n	Observed Response (95% CI)
Measles (ELISA)	% ≥120 mIU/mL	758	97.8% (741/758) (96.4%, 98.7%)	388	98.7% (383/388) (97.0%, 99.6%)	126	98.4% (124/126) (94.4%, 99.8%)
	GMT (mIU/mL)	758	3504.9 (3269.7, 3757.2)	388	3506.2 (3195.7, 3846.9)	126	2562.1 (2172.2, 3022.0)
Mumps (ELISA)	% ≥10 ELISA Ab Units	811	95.4% (774/811) (93.8%, 96.8%)	415	95.2% (395/415) (92.7%, 97.0%)	145	98.6% (143/145) (95.1%, 99.8%)
	GMT (ELISA Ab Units)	811	89.4 (83.5, 95.7)	415	84.1 (76.2, 92.8)	145	98.1 (85.7, 112.3)
Rubella† (ELISA)	% ≥10 IU/mL	829	98.6% (817/829) (97.5%, 99.2%)	421	99.3% (418/421) (97.9%, 99.9%)	148	100% (148/148) (97.5%, 100%)
	GMT (IU/mL)	829	98.7 (92.8, 105.0)	421	99.9 (91.8, 108.7)	148	126.3 (111.9, 142.6)
Varicella (gpELISA)	% ≥5 gpELISA Units	757	89.7% (679/757) (87.3%, 91.8%)	383	90.9% (348/383) (87.5%, 93.6%)	139	93.5% (130/139) (88.1%, 97.0%)
	GMT (gpELISA Units)	757	13.8 (12.8, 14.8)	383	15.4 (13.8, 17.0)	139	15.8 (13.8, 18.0)

† Rubella titers obtained by the legacy format were converted to their corresponding titers in the modified format. Rubella serostatus was determined after conversion to IU/mL; seronegative corresponds to an antibody
Titer <10 IU/mL and seropositive corresponds to an antibody titer ≥10 IU/mL.
Percentages were calculated as the number of subjects who met the criterion divided by the number of subjects contributing to the per-protocol analysis.
Seronegative to measles corresponds to an antibody titer <120 mIU/mL, to mumps corresponds to an antibody titer <10 ELISA Ab units, to rubella corresponds to an antibody titer <10 IU/mL.
Concomitant Group = ProQuad™ + TRIPEDIA™ + COMVAX™ at Day 0.
Nonconcomitant Group = ProQuad™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later.
Control Group = M-M-R™ II + VARIVAX™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later.
N = Number of subjects vaccinated in each treatment group.
N = Number of subjects contributing to the per-protocol analysis.
gpELISA = Glycoprotein enzyme-linked immunosorbent assay.
ELISA = Enzyme-linked immunosorbent assay.
CI = Confidence interval.
GMT = Geometric mean titer.

With respect to the 4 components of TRIPEDIA and two components of COMVAX, non-inferiority testing was performed at the one-sided, α=0.05 level. The 90% CIs for the differences in response rates for diphtheria, tetanus, pertussis PT, hepatitis B, and Hib excluded a decrease of 10%. However, the 90%CI for pertussis FHA responses included a 15% difference. Therefore, a conclusion of a similar response to all antigens in COMVAX and TRIPEDIA could not be made based on this analysis. Antibody responses to Tripedia antigens following concomitant immunization with ProQuad, and following non-concomitant immunization are summarized in Table 8.4.13 and antibody response to COMVAX antigens are summarized in Table 8.4.14.

The tests for non-inferiority for antibody responses for each vaccine antigen are listed in Table 8.4.15.

Table 8.4.13 Summary of antibody responses to Tripedia antigens by vaccine group

Vaccine Antigen (Assay)	End-point	ProQuad™ + TRIPEDIA™ + COMVAX™ (N=949)				ProQuad™ followed by TRIPEDIA™ + COMVAX™ (N=468)				M-M-R™ II + VARIVAX™ followed by TRIPEDIA™ + COMVAX™ (N=452)			
		Pre-Vaccination		Post-Vaccination		Pre-Vaccination		Post-Vaccination		Pre-Vaccination		Post-Vaccination	
		N	Observed Response (95% CI)	N	Observed Response (95% CI)	N	Observed Response (95% CI)	N	Observed Response (95% CI)	n	Observed Response (95% CI)	N	Observed Response (95% CI)
Diphtheria	**% ≥0.1 IU/mL**	596	70.5% (420/596) (66.6%, 74.1%)	675	98.8% (667/675) (97.7%, 99.5%)	292	66.1% (193/292) (60.4%, 71.5%)	337	98.8% (333/337) (97.0%, 99.7%)	43	55.8% (24/43) (39.9%, 70.9%)	95	98.9% (94/95) (94.3%, 100%)
	GMT	596	0.19 (0.17, 0.21)	675	1.33 (1.24, 1.43)	292	0.15 (0.13, 0.16)	337	1.72 (1.55, 1.89)	43	0.15 (0.11, 0.21)	95	1.59 (1.29, 1.97)
Tetanus	**% ≥0.1 IU/mL**	747	85.0% (635/747) (82.2%, 87.5%)	803	99.1% (796/803) (98.2%, 99.6%)	356	83.1% (296/356) (78.8%, 86.9%)	387	99.7% (386/387) (98.6%, 100%)	59	55.9% (33/59) (42.4%, 68.8%)	114	100% (114/114) (96.8%, 100%)
	GMT	747	0.40 (0.37, 0.45)	803	3.93 (3.59, 4.31)	356	0.31 (0.27, 0.35)	387	5.74 (5.13, 6.42)	59	0.13 (0.09, 0.19)	114	4.36 (3.67, 5.17)
Pertussis PT	**% ≥ 4-fold rise in titer**	NA	NA	748	74.1% (554/748) (70.8%, 77.2%)	NA	NA	355	90.4% (321/355) (86.9%, 93.3%)	NA	NA	59	94.9% (56/59) (85.9%, 98.9%)
	GMT	748	5.85 (5.36, 6.38)	748	42.9 (39.5, 46.5)	355	4.52 (3.97, 5.15)	355	55.7 (49.7, 62.4)	59	2.20 (1.58, 3.05)	59	46.3 (35.0, 61.4)
Pertussis FHA	**% ≥ 4-fold rise in titer**	NA	NA	748	67.1% (502/748) (63.6%, 70.5%)	NA	NA	356	86.8% (309/356) (82.8%, 90.1%)	NA	NA	59	89.8% (53/59) (79.2%, 96.2%)
	GMT	748	9.37 (8.59, 10.2)	748	58.1 (53.7, 62.9)	356	7.38 (6.45, 8.43)	356	81.2 (73.5, 89.7)	59	3.59 (2.60, 4.96)	59	65.0 (49.6, 85.2)

Percentages were calculated as the number of subjects who met the criterion divided by the number of subjects contributing to the per-protocol analysis. Only subjects who had valid antibody results at both pre-vaccination and 6 weeks
Post-vaccination are included in the calculation of subjects with 4-fold rise.

N = Number of subjects vaccinated in each treatment group.

N = Number of subjects contributing to the per-protocol analysis.

GMT = Geometric mean titer.

CI = Confidence interval.

Table 8.4.14 Summary of antibody responses to COMVAX antigens by vaccine group

Vaccine Antigen (Assay)	Endpoint	ProQuad™ + TRIPEDIA™ + COMVAX™ (N=949)				ProQuad™ followed by TRIPEDIA™ + COMVAX™ (N=468)				M-M-R™ II + VARIVAX™ followed by TRIPEDIA™ + COMVAX™ (N=452)			
		Pre Vaccination		Post Vaccination		Pre Vaccination		Post Vaccination		Pre Vaccination		Post Vaccination	
		N	Observed Response (95% CI)	n	Observed Response (95% CI)	N	Observed Response (95% CI)	n	Observed Response (95% CI)	n	Observed Response (95% CI)	N	Observed Response (95% CI)
Hepatitis B	% ≥10 mIU/mL	780	79.7% (622/780) (76.7%, 82.5%)	824	95.9% (790/824) (94.3%, 97.1%)	371	74.4% (276/371) (69.6%, 78.8%)	396	98.7% (391/396) (97.1%, 99.6%)	63	84.1% (53/63) (72.7%, 92.1%)	122	99.2% (121/122) (95.5%, 100%)
	GMT (mIU/mL)	780	54.0 (45.6, 64.0)	824	759 (659, 874)	371	41.8 (32.3, 54.2)	396	996 (828, 1199)	63	47.7 (28.0, 81.2)	122	1135 (836, 1541)
	% ≥1 mcg/mL	782	47.2% (369/782) (43.6%, 50.8%)	821	94.6% (777/821) (92.9%, 96.1%)	376	39.4% (148/376) (34.4%, 44.5%)	398	96.5% (384/398) (94.2%, 98.1%)	64	29.7% (19/64) (18.9%, 42.4%)	124	96.0% (119/124) (90.8%, 98.7%)
Hib	% ≥0.15 mcg/mL	782	89.8% (702/782) (87.4%, 91.8%)	821	99.5% (817/821) (98.8%, 99.9%)	376	87.2% (328/376) (83.4%, 90.4%)	398	99.7% (397/398) (98.6%, 100%)	64	93.8% (60/64) (84.8%, 98.3%)	124	99.2% (123/124) (95.6%, 100%)
	GMT (mcg/mL)	782	0.92 (0.83, 1.01)	821	11.1 (10.1, 12.3)	376	0.68 (0.59, 0.78)	398	12.1 (10.6, 13.7)	64	0.65 (0.48, 0.87)	124	12.9 (10.4, 16.1)

Percentages were calculated as the number of subjects who met the criterion divided by the number of subjects contributing to the per-protocol analysis.
For pertussis PT and pertussis FHA, only subjects who had valid antibody results at both pre-vaccination and 6 weeks post-vaccination are included in the calculation of percent with ≥4-fold rise.
Concomitant Group = ProQuad™ + TRIPEDIA™ + COMVAX™ at Day 0.
Nonconcomitant Group = ProQuad™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later.
Control Group = M-M-R™ II + VARIVAX™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later.
N = Number of subjects vaccinated in each treatment group.
n = Number of subjects contributing to the per-protocol analysis.
GMT, geometric mean titer,
CI, confidence interval

Table 8.4.15 Summary of the comparison of antibody responses to Tripedia and COMVAX

Vaccine (Assay)	Parameter	Concomitant ProQuad™ + TRIPEDIA™ +COMVAX™ (N=949) n	Concomitant ProQuad™ + TRIPEDIA™ +COMVAX™ (N=949) Estimated Response†	Nonconcomitant ProQuad™ followed by TRIPEDIA™ + COMVAX™ (N=468) n	Nonconcomitant ProQuad™ followed by TRIPEDIA™ + COMVAX™ (N=468) Estimated Response†	Estimated Difference† ‡ (90% CI)§	Criterion	1-Sided p-Value§	Conclusion§
Diphtheria	% ≥0.1 IU/mL	675	98.8%	337	98.8%	-0.0 (-1.2, 1.5)	LB>-10.0	<0.001*	Similar
Tetanus	% ≥0.1 IU/mL	803	99.1%	387	99.7%	-0.6 (-1.5, 0.4)	LB>-10.0	<0.001*	Similar
Pertussis PT	**% ≥4-fold rise in titer**	**748**	**74.0%**	**355**	**90.1%**	**-16.1 (-19.7, -12.3)**	**LB>-15.0**	**0.686**	***Not similar***
Pertussis FHA	**% ≥4-fold rise in titer**	**748**	**67.1%**	**356**	**86.7%**	**-19.6 (-23.6, -15.4)**	**LB>-15.0**	**0.963**	***Not similar***
Hepatitis B	%≥10 mIU/mL	824	95.9%	396	98.8%	-2.8 (-4.8, -0.8)	LB>-10.0	<0.001	Similar
Hib	%≥1 mcg/mL	821	94.6%	398	96.5%	-1.9 (-4.1, 0.8)	LB>-10.0	<0.001	Similar

* A 1-sided p-value ≤0.05 implies the difference is statistically significantly less than the pre-specified clinically relevant decrease.
† Responses and their differences are based on a statistical analysis model adjusting for combined study center.
‡ [ProQuad™ + TRIPEDIA™ + COMVAX™] - [ProQuad™ followed by TRIPEDIA™+ COMVAX™]. § The conclusion of similarity (non-inferiority) is based on the lower bound of the 2-sided 90% CI on difference excluding a decrease of less than pre-specified criterion of either 10.0 or 15.0 percentage points, which implies that the difference is statistically significantly less than the pre-specified clinically relevant decrease N =Number of subjects vaccinated in each treatment group. n =Number of subjects contributing to the Per-Protocol analysis CI=Confidence interval. LB=Lower Bound (of 2-sided 90% confidence interval).

Since COMVAX did not contribute to the failure to declare similarity at the second stage of testing, non-inferiority testing was performed to compare immune responses to the components in COMVAX at an adjusted type 1 error rate of α =0.025. Both 95% CIs for the immune responses to Hepatitis B and Hib excluded a decrease of 10%. It was concluded that the immune responses to COMVAX were similar in the concomitant and nonconcomitant groups. This analysis supports the conclusion that ProQuad and COMVAX can be administered concomitantly

8.4.7.2.2 Co-Primary Endpoint for Immunogenicity:

The co-primary endpoint for immunogenicity was the demonstration that an acceptable immune response was elicited in the concomitant group in at least 90% for measles, mumps, and rubella and in at least 76% for varicella. This analysis is summarized in Table 8.4.16 below.

Table 8.4.16 Antibody responses to measles, mumps, rubella, and varicella are acceptable.

Vaccine Component (Assay)	Parameter	N	n	Observed Response (95% CI)	Criterion†	One-Sided p-Value†	Conclusion†
Measles (ELISA)	%≥120 mIU/mL	949	758	97.8% (96.4%, 98.7%)	LB >0.90	<0.001*	Acceptable
Mumps (ELISA)	%≥10 ELISA Ab Units	949	811	95.4% (93.8%, 96.8%)	LB >0.90	<0.001*	Acceptable
Rubella (ELISA)	%≥10 IU/mL	949	829	98.6% (97.5%, 99.2%)	LB >0.90	<0.001*	Acceptable
Varicella (gpELISA)	%≥5 gpELISA units	949	757	89.7% (87.3%, 91.8%)	LB >0.76	<0.001*	Acceptable

The lower bound of the two-sided 95% CIs was above 90% for measles, mumps, and rubella and above 76% for varicella. It was concluded that concomitant immunization of ProQuad with TRIPEDIA and COMVAX provided and acceptable immune response to each antigen in ProQuad.

8.4.7.2.3 Additional immunogenicity endpoints that were evaluated include

8.4.7.2.3.1 Comparisons of reverse cumulative distribution curves of post-vaccination antibody titers demonstrated that the immune responses to measles, mumps, rubella, varicella, HepB, and Hib were similar in the concomitant and non-concomitant groups (data not shown).

8.4.7.2.3.2 An analysis of <u>all subjects with serology</u> was consistent with the results of the per protocol analysis for each vaccine antigen (data not shown).

8.4.7.2.3.3 Analysis of immunogenicity in <u>subjects with all</u> serology was consistent with the analysis presented above for the per protocol population for each vaccine antigen (data not shown).

8.4.7.2.3.4 Exploratory analyses of the immune response to pertussis FHA indicated that the lower responses seen in children receiving vaccines concomitantly as due to the younger age at the time of vaccination. In addition, children were less likely to respond to pertussis FHA if 180 days or 6 months had not elapsed since the time of the last immunization.

In this post hoc analysis, children ≥ 13.5 months who received DTaP immunization 6 months or more prior to boosting had immune response to pertussis FHA that were similar to the immune responses elicited in infants immunized in the non concomitant group (data not shown).

8.4.7.3 Safety endpoints.

8.4.7.4 Summary of Clinical Adverse Experiences:

Children in each group were followed for 56 days after immunization for adverse reactions. Follow-up was obtained on 929 of 949 subjects (97.9%) in the concomitant group and in 479 of 485 (98.8%) in the nonconcomitant group and in 467 of 479 (97.5%) in the control group. Overall, 90.2% of subjects in the concomitant group reported at least one AE with 92.5% in the nonconcomitant group and 89.3% in the control group. Rates of injection site reactions were 62.0, 58.9 and 59.5% in the concomitant, nonconcomitant and control groups, respectively. Vaccine related AEs were reported in 69.1, 72.2 and 68.1% of subjects in the concomitant, non-concomitant and controls groups, respectively. 15 subjects reported serious AES but none of these were thought to be vaccine-related (with 11 in the concomitant group, 3 in the nonconcomitant group and 1 in the control group). Table 8.4.17 provides a summary of clinical adverse experiences (AEs) by vaccine group.

Table 8.4.17 Summary of clinical adverse experiences reported by vaccine group

	Concomitant Group (N=949)		Nonconcomitant Group (N=485)		Control Group (N=479)	
	n	(%)	n	(%)	n	(%)
Number of subjects	949		485		479	
Subjects without follow-up	20		6		12	
Subjects with follow-up	929		479		467	
Number (%) of subjects:						
With no adverse experience	91	(9.8)	36	(7.5)	50	(10.7)
With one or more adverse experiences	838	(90.2)	443	(92.5)	417	(89.3)
Injection-site adverse experiences	576	(62.0)	282	(58.9)	277	(59.3)
Systemic adverse experiences	736	(79.2)	386	(80.6)	359	(76.9)
With vaccine-related adverse experiences	642	(69.1)	346	(72.2)	318	(68.1)
Injection-site adverse experiences	576	(62.0)	281	(58.7)	277	(59.3)
Systemic adverse experiences	269	(29.0)	154	(32.2)	136	(29.1)
With serious adverse experiences	11	(1.2)	3	(0.6)	1	(0.2)
With serious vaccine-related adverse experiences	0	(0.0)	0	(0.0)	0	(0.0)
Who died	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to an adverse experience	0	(0.0)	1	(0.2)	3	(0.6)
Discontinued due to a vaccine-related adverse experience	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a serious adverse experience	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a serious vaccine-related adverse experience	0	(0.0)	0	(0.0)	0	(0.0)

8.4.7.5 Safety Outcomes:

8.4.7.6 Serious Vaccine Related Adverse Reactions:

The primary endpoint for safety stated that there would be no serious vaccine related serious adverse reactions.

There were no deaths in this study.

There were 15 serious adverse reactions with 11 in the concomitant group, 3 in the nonconcomitant group and 1 in the control group. None of these AEs were judged to be vaccine related. In the children given vaccines concomitantly the serious adverse reactions included afebrile seizure (1), pneumonia (3), asthma/wheezing (2), laryngotracheobronchitis (2) febrile seizure (1), orbital cellulitis (1) and food allergy (1). In the group immunized with vaccines non-concomitantly, the adverse reactions included pneumonia (1) inguinal hernia and necrosis of the testicle (1), adenovirus gastroenteritis (1). The one control child with a serious AE had an afebrile seizure. All of these children completed the study.

4 additional children who experienced seizures that were not judged to be serious did not complete the study including one child in the nonconcomitant group and 3 children in the control group.

One additional child experienced a serious adverse reaction (severe rotavirus gastroenteritis) prior to immunization but after randomization and the case is reported here for completeness.

8.4.7.7 Injection Site Reactions:

Injection site reactions were compared at the ProQuad injection site days 0-42 after vaccination. 42.4% in the concomitant group reported injection site reactions at the ProQuad site vs. 37.6% in the nonconcomitant group. In the control group, 33.2% reported injection site reactions at the MMRII site and 31.9% at the VARIVAX site. On days 0-4 after immunization there was significantly more erythema (15.7% vs. 11.1%, p=0.041) and swelling (9.6% vs. 5.6%, p=0.019) at the ProQuad injection site when compared to the VARIVAX injection site. Rash occurred significantly more frequently at the ProQuad injection site when compare to the MMRII injection site day 0-42 after immunization (1.9% vs. 0.2%, p=0.012) although the rates in both groups were very low.

The most common injection site reaction at the ProQuad site in each group was pain, tenderness, and soreness.

In the concomitant group, data for TRIPEDIA and COMVAX injection site reactions were summarized. Following TRIPEDIA, 47.9% reported an injection site AE with 35.2% reporting paint tenderness and soreness, 26.3% reported erythema, and 21.9% reporting swelling. At the COMVAX site, 49.9% reported an injection site AE with 35.2% reporting pain, tenderness, and soreness, 28.6% reported erythema and 22.0% reported swelling.

In the nonconcomitant and control group injection site reactions at the TRIPEDIA and COMVAX sites were compared. 40.6% reported injection site AEs in the concomitant group vs. 41.4% in the control group at the

TRIPEDIA site while 40.2% and 41.6% reported injection site AEs at the COMVAX site in the nonconcomitant and control groups, respectively.

8.4.7.8 Systemic Adverse Reactions:

Systemic reactions reported days 0-56 after immunization were summarized and compared. In the concomitant group, 79.2% reported at least one systemic AE while 80.6% reported a systemic AE in the nonconcomitant group and 76.7% in the control group.

Following immunization with ProQuad, TRIPEDIA and COMVAX, the most common systemic AEs were fever (31.8%), upper respiratory infection (27.8%), otitis media (18.8%), irritability (11.7%), diarrhea (9.5%), cough (8.9%), diaper rash (8.8%), viral exanthem (7.6%), rhinorrhea (6.9%), otitis (6.5%), vomiting (6.0%), rash (4.8%), milaria rubra (4.7%), viral infection (4.4%) and respiratory congestion (3.1%).

Following immunization with ProQuad followed 42 days later by TRIPEDIA and COMVAX, the most common systemic AEs were fever (29.9%), upper respiratory infection (27.3%), otitis media (17.3%), irritability (10.9%), cough (9.8%), diaper rash (7.7%), diarrhea (6.9%), rhinorrhea (6.7%), vomiting (6.5%), viral infection (5.8%) viral exanthema (5.2%), rash (4.6%), conjunctivitis (4.4%), otitis (4.0%), measles like rash (3.3%), milaria rubra (3.3%), and respiratory congestion (3.3%).

In the control group, the most common AEs after immunization with MMRII + VARIVAX followed 42 days later by TRIPEDIA and COMVAX, were fever (29.1%), upper respiratory infection (23.1%), otitis media (16.5%), irritability (14.3%), diarrhea (10.3%), cough (9.2%), diaper rash (9.2%), rhinorrhea (8.8%), vomiting (6.4%), rash (5.4%), viral exanthema (4.9%), rash (4.6%), conjunctivitis (3.9%), viral infection (3.9%), milaria rubra (3.6%), sinusitis (3.6%), and varicella-like rash (3.2%).

Systemic adverse reactions are summarized in Table 8.4.18.

Table 8.4.18 Summary of systemic adverse reactions reported days 0 to 56 following concomitant immunization with ProQuad plus Comvax plus Tripedia, or following nonconcomitant immunization with ProQuad followed by Comvax plus Tripedia or after MMRII plus VARIVAX immunization

Days 0-56 after immunization	ProQuad Concomitant Group N=949		ProQuad Nonconcomitant Group N=485		MMRII + VARIVAX Control Group N=479	
	N	%	N	%	n	%
Number of Subjects						
Without follow-up	20		6		12	
With follow-up	929		479		467	
With one or more AE	736	79.2	386	80.6	359	76.9
With no AE	193	20.8	93	19.4	108	23.1
Body as Whole	347	37.4	169	35.3	157	33.6
Digestive	181	19.5	88	18.4	92	19.7
Metabolic/Nutritional/Immune	21	2.3	5	1.0	9	1.9
Nervous System/Psychiatric	125	13.5	54	11.3	77	16.5
Respiratory	420	45.2	221	46.1	198	42.2
Skin	322	34.7	157	32.8	153	32.8
Special Senses	250	26.9	117	24.4	103	22.1

Adverse reactions were compared between the concomitant group and both the nonconcomitant group and the control group over two study periods: Days 0 to 56 after immunization and over Days 0-42 post-vaccination 1. The rate of subjects discontinuing due to an AE was lower in the concomitant group (0.0%) compared to the control group (0.6%). The rate of subjects with one or more AEs reported was higher in the group given ProQuad alone (85.2%) vs. the rate in the control group (80.3%) days 0-42 after immunization.

When individual AEs were compared across groups, the safety profile of ProQuad administered concomitantly with TRIPEDIA and COMVAX was judged to be comparable in safety to ProQuad given alone or to MMRII + VARIVAX followed 6 weeks later by TRIPEDIA and COMVAX. A few AEs were reported more frequently after concomitant immunization vs. the control group: upper respiratory infection (27.8% vs. 23.1%), bronchiolitis (1.2% vs. 0.0%), and otitis (6.5% vs. 2.8%), while a few AEs were reported less frequently in the concomitant group than controls: excoriation (0.1% vs. 1.1%) and varicella-like rash (1.4% vs. 3.2%).

When ProQuad was administered alone there was a higher rate of anorexia in the group given vaccines concomitantly vs. control immunized children (1.9% vs. 0.4% respectively) while there were lower rates of nervous system complaints (7.9% vs. 14.6%) and irritability (7.1% vs. 12.6%) vs. control immunized children.

There were 156 comparisons; observed differences between groups were small, occurred in both directions and there was no noticeable trend towards higher AE incidence rates in one group or another.

Comparisons of AEs by body system are summarized by vaccine group in Table 8.4.19 below:

Table 8.4.19 Comparison of AEs by body system for Group A (concomitant immunization) versus Group B (non-concomitant immunization)

AE	Comparison	Group A			Group B			Risk Difference† (Group A-Group B) Percentage Points (95% CI)†
		N	n	S(%)	N	n	S(%)	
Body as a Whole	Concomitant Versus Nonconcomitant (Day 0 to 56)	949	929	347 (37.4)	485	479	169 (35.3)	2.1 (-3.3, 7.3)
	Concomitant Versus Control (Day 0 to 56)	949	929	347 (37.4)	479	467	157 (33.6)	3.7 (-1.6, 9.0)
	Nonconcomitant Versus Control (Day 0 to 42)	485	479	145 (30.3)	479	467	131 (28.1)	2.2 (-3.6, 8.0)
Digestive System	Concomitant Versus Nonconcomitant (Day 0 to 56)	949	929	181 (19.5)	485	479	88 (18.4)	1.1 (-3.3, 5.3)
	Concomitant Versus Control (Day 0 to 56)	949	929	181 (19.5)	479	467	92 (19.7)	-0.2 (-4.8, 4.1)
	Nonconcomitant Versus Control (Day 0 to 42)	485	479	79 (16.5)	479	467	81 (17.3)	-0.9 (-5.7, 3.9)
Metabolic/ Nutritional/Immune	Concomitant Versus Nonconcomitant (Day 0 to 56)	949	929	21 (2.3)	485	479	5 (1.0)	1.2 (-0.3, 2.6)
	Concomitant Versus Control (Day 0 to 56)	949	929	21 (2.3)	479	467	9 (1.9)	0.3 (-1.5, 1.8)
	Nonconcomitant Versus Control (Day 0 to 42)	485	479	3 (0.6)	479	467	6 (1.3)	-0.7 (-2.2, 0.7)
Nervous System and Psychiatric	Concomitant Versus Nonconcomitant (Day 0 to 56)	949	929	125 (13.5)	485	479	54 (11.3)	2.2 (-1.6, 5.7) –
	Concomitant Versus Control (Day 0 to 56)	949	929	125 (13.5)	479	467	77 (16.5)	3.0 (-7.2, 0.9)
	Nonconcomitant Versus Control (Day 0 to 42)	485	479	38 (7.9)	479	467	68 (14.6)	6.6 (-10.7, -2.6)
Respiratory System	Concomitant Versus Nonconcomitant (Day 0 to 56)	949	929	420 (45.2)	485	479	221 (46.1)	-0.9 (-6.4, 4.5)
	Concomitant Versus Control (Day 0 to 56)	949	929	420 (45.2)	479	467	198 (42.4)	2.8 (-2.7, 8.3)
	Nonconcomitant Versus Control (Day 0 to 42)	485	479	191 (39.9)	479	467	173 (37.0)	2.8 (-3.4, 9.0)
Skin and Skin Appendage	Concomitant Versus Nonconcomitant (Day 0 to 56)	949	929	322 (34.7)	485	479	157 (32.8)	1.9 (-3.4, 7.0)
	Concomitant Versus Control (Day 0 to 56)	949	929	322 (34.7)	479	467	153 (32.8)	1.9 (-3.4, 7.1) –
	Nonconcomitant Versus Control (Day 0 to 42)	485	479	143 (29.9)	479	467	141 (30.2)	0.3 (-6.2, 5.5)
Special Senses	Concomitant Versus Nonconcomitant (Day 0 to 56)	949	929	250 (26.9)	485	479	117 (24.4)	2.5 (-2.4, 7.2)
	Concomitant Versus Control (Day 0 to 56)	949	929	250 (26.9)	479	467	103 (22.1)	4.9 (0.0, 9.5)
	Nonconcomitant Versus Control (Day 0 to 42)	485	479	95 (19.8)	479	467	84 (18.0)	1.8 (-3.2, 6.9)

8.4.7.9 Fever:

Fever was defined as a temperature ≥102 F (or oral equivalent) during Days 0-56 after immunization. Fever occurred more frequently after concomitant immunization with ProQuad, TRIPEDIA and COMVAX (31.9%) than in the non-concomitant group (29.8%) or the control group (29.0%). However, when groups were compared there were no significant differences in the incidence of fevers.

In general, fevers were of short duration lasting a mean of 1 day with a median duration of 1.5 days (days 0-42 after immunization) irrespective of vaccine group.

Table 8.4.20 below summarizes the data on fevers reported in the concomitant, non-concomitant and control groups for days 0 to 42 after immunization and Table 8.4.21 provides the analysis of these data.

Table 8.4.20 Summary of fevers days 0 to 42 after immunization

	Concomitant Group (N=949)		Nonconcomitant Group (N=485)		Control Group (N=479)	
	n	(%)	n	(%)	n	(%)
Any Vaccination Visit						
Number of subjects	949		485		479	
Subjects with no follow-up	25		8		17	
Subjects with follow-up	924		477		462	
Maximum temperature (oral equivalent):						
<102°F (38.9°C) or Normal	629	(68.1)	335	(70.2)	328	(71.0)
≥102°F (38.9°C) or Abnormal‡	295	(31.9)	142	(29.8)	134	(29.0)

† Days 0 to 56 Post vaccination Visit 1 means Days 0 to 42 Post vaccination Visit 1 and Days 0 to 14 post Visit 2. ‡Sixty-six (66), 30, and 32 subjects reported a maximum temperature of abnormal in the concomitant, nonconcomitant, and control groups, respectively, during Days 0 to 42 Post vaccination Visit 1.
Eighteen (18), 5, and 14 subjects reported a maximum temperature of abnormal in the concomitant, nonconcomitant, and control groups, respectively, during Days 0 to 14 post Visit 2. Percentages are calculated based on the number of subjects with follow-up after any visit.
All temperatures have been converted to oral equivalent by adding 1°F to axillary temperatures or subtracting 1°F from rectal temperatures. Temperatures reported as otic were not converted and were entered as oral equivalent. Two (2) subjects in this trial were incorrectly vaccinated.
One (1) of these subjects received M-M-R™ II + TRIPEDIA™ + COMVAX™ on Day 0 (Allocation Number 10917) instead of ProQuad™ + TRIPEDIA™ + COMVAX™ and the other received VARIVAX™ + TRIPEDIA™ + COMVAX™ on Day 0 (Allocation Number 12247) instead of ProQuad™ + TRIPEDIA™ + COMVAX™. Each of these subjects represents a deviation from the protocol. Data from these subjects are not included in this table.
Concomitant Group = ProQuad™ + TRIPEDIA™ + COMVAX™ at Day 0. Nonconcomitant Group = ProQuad™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later. Control Group = M-M-R™ II+ VARIVAX™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later.

Table 8.4.21 Comparison of fevers between vaccine and control groups

Elevated Temperature	Comparison	Group A			Group B			Risk Difference‡ (Group A-Group B) Percentage Points (95% CI)‡	p-Value‡
Maximum temperature (oral equivalent) ≥102°F (38.9°C) or abnormal§	Concomitant Vs. Nonconcomitant (Days 0 to 56)	949	924	295 (31.9)	485	477	142 (29.8)	2.2 (-3.0, 7.2)	0.409
	Concomitant Vs. Control (Days 0 to 56)	949	924	295 (31.9)	479	462	134 (29.0)	2.9 (-2.3, 8.0)	0.267
	Nonconcomitant Vs. Control (Days 0 to 42)	485	476	125 (26.3)	479	462	109 (23.6)	2.7 (-2.9, 8.2)	0.345

† Concomitant = ProQuad™ + TRIPEDIA™ + COMVAX™; Nonconcomitant = ProQuad™ followed by TRIPEDIA™ + COMVAX™ 6 weeks later; Control = M-M-R™ II + VARIVAX™ followed by TRIPEDIA™ + COMVAX™ 6 weeks later. ‡ Risk differences and confidence intervals (CIs) based on the pooled incidence rates across all study centers; corresponding p-values are calculated based on a test of difference between the 2 treatment groups. § Sixty-six (66), 30, and 32 subjects reported a maximum temperature of abnormal in the concomitant, nonconcomitant, and control groups, respectively, during Days 0 to 42 post-vaccination Visit 1. Eighteen (18), 5, and 14 subjects reported a maximum temperature of abnormal in the concomitant, nonconcomitant, and control groups, respectively, during Days 0 to 14 post Visit 2. Percentages are calculated based on the number of subjects with temperature follow-up in the day range. All temperatures have been converted to oral equivalent by adding 1°F to axillary temperatures or subtracting 1°F from rectal temperatures. Temperatures reported as otic were not converted and were entered as oral equivalent. N = Number of subjects vaccinated in each treatment group. n = Number of subjects with follow-up in each treatment group. s = Number of subjects with indicated adverse experience in each treatment group.

When fevers ≥102 F were compared for post-vaccination days 5-12, fevers occurred significantly more frequently after ProQuad immunization than in the control group immunized with MMRII + VARIVAX. These results are summarized in Table 8.4.22 below:

Table 8.4.22 Comparison of fevers reported days 5-12 after immunization

Elevated Temperature Definition	Post vaccination Visit 1 Day Range	Comparison (Group A Versus Group B)†	Group A			Group B			Risk Difference‡ (Group A-Group B) Percentage Points (95% CI)‡		p-Value‡
	Days 5 to 12	Concomitant Vs Nonconcomitant Concomitant Vs. Control	949	922	127 (13.8)	479	461	45 (9.8)	4.0	(0.3, 7.4)	0.033
		Concomitant Vs Control	949	922	127 (13.8)	485	473	74 (15.6)	-1.9	(-6.0, 2.0)	0.347
		Nonconcomitant Vs. Control	485	473	74 (15.6)	479	461	45 (9.8)	5.9	(1.6, 10.2)	0.007

Although the rate of fevers ≥102 F were increased between days 5-12 the percent of subjects with febrile seizures (Days 0-42 post vaccination) were not significantly different between groups. This information is summarized in Table 8.4.23.

Table 8.4.23 Summary of febrile seizures days 0-42 after immunization

	Comparison	**Group A**			**Group B**			**Risk Difference† (Group A - Group B) (Percentage Points) (95% Confidence Interval)†**	**p-Value†**
Febrile Seizure	Concomitant Vs. Nonconcomitant	949	929	2 (0.2)	485	479	1 (0.2)	0.0 (-1.0, 0.6)	0.980
	Concomitant Vs. Control	949	929	2 (0.2)	479	467	3 (0.6)	-0.4 (-1.7, 0.3)	0.208
	Nonconcomitant Vs. Control	485	479	1 (0.2)	479	467	3 (0.6)	-0.4 (-1.7, 0.6)	0.304

†Risk differences and confidence intervals are based on the pooled incidence rates across all study centers; corresponding p-values are calculated based on a test of differences between the 2 treatment groups.
Percentages are calculated based on the number of subjects with temperature follow-up.
Concomitant Group = ProQuad™ + TRIPEDIA™ + COMVAX™ at Day 0.
Nonconcomitant Group = ProQuad™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later.
Control Group = M-M-R™ II + VARIVAX™ at Day 0 followed by TRIPEDIA™ and COMVAX™ 6 weeks later.
N = Number of subjects vaccinated.
n = Number of subjects with safety follow-up.
s = Number of subjects with febrile seizure.

8.4.7.10 **Measles-Like Rashes, Rubella-Like Rashes, Measles/Rubella-Like Rashes, Injection Site Reactions and injection Site Rashes:**

The incidences of measles- and rubella-like rashes were not significantly different between groups. In contrast, varicella-like rashes were reported significantly more frequently after MMRII + VARIVAX immunization (15/467, 3.2%) than after ProQuad (13/929, 1.4%, p=0.023). (See Table 8.4.24)

Table 8.4.24 Summary of measles and rubella-like rashes after concomitant and non-concomitant immunization

Adverse Experience	Comparison (Group A Versus Group B)†	Group A N	Group A n	Group A s (%)	Group B N	Group B n	Group B s (%)	Risk Difference‡ (Group A-Group B) Percentage Point (95% Confidence Interval)‡		p-Value‡
Measles-like rash§	Concomitant Vs. Nonconcomitant	949	929	23 (2.5)	485	479	15 (3.1)	-0.7	(-2.8, 1.1)	0.472
	Concomitant Vs. Control	949	929	23 (2.5)	479	467	9 (1.9)	0.5	(-1.3, 2.1)	0.518
	Nonconcomitant Vs. Control	485	479	15 (3.1)	479	467	9 (1.9)	1.2	(-0.9, 3.4)	0.239
Rubella-like rash§	Concomitant Vs. Nonconcomitant	949	929	5 (0.5)	485	479	3 (0.6)	-0.1	(-1.3, 0.7)	0.835
	Concomitant Vs. Control	949	929	5 (0.5)	479	467	2 (0.4)	0.1	(-1.0, 0.9)	0.784
	Nonconcomitant Vs. Control	485	479	3 (0.6)	479	467	2 (0.4)	0.2	(-1.0, 1.5)	0.675
Varicella-like rash	Concomitant Vs. Nonconcomitant	949	929	13 (1.4)	485	479	12 (2.5)	-1.1	(-3.0, 0.3)	0.137
	Concomitant Vs. Control	949	929	13 (1.4)	479	467	15 (3.2)	-1.8	(-3.9, -0.2)	0.023
	Nonconcomitant Vs. Control	485	479	12 (2.5)	479	467	14 (3.0)	-0.5	(-2.8, 1.7)	0.643

† Concomitant = ProQuad™ + TRIPEDIA™ + COMVAX™; Nonconcomitant = ProQuad™ followed by TRIPEDIA™ + COMVAX™ 6 weeks later; Control = M-M-R™ II + VARIVAX™ followed by TRIPEDIA™ + COMVAX™ 6 weeks later. ‡ Risk differences and confidence intervals are provided for events prompted for on the Vaccination Report Card (VRC) regardless of overall incidence rate, and are based on the pooled incidence rates across all study centers; corresponding p-values are calculated based on a test of difference between the 2 treatment groups. § Includes rashes specifically reported as “measles/rubella-like” by Allocation Number (AN) 10604 (concomitant group), ANs 11375 and 12443 (nonconcomitant group), and AN 11837 (control group). Percentages are calculated based on the number of subjects with follow-up in the day range. Although a subject might have multiple episodes of the indicated adverse experience, the subject is counted only once in the overall total for that adverse experience. N = Number of subjects vaccinated in each treatment group. n = Number of subjects with follow-up in each treatment group. s = Number of subjects with indicated adverse experience in each treatment group.

Of the 41 subjects who reported a varicella-like rash, zoster, or zoster-like rash or varicella infection after immunization, 4 subjects provided cultures of lesion for --- analysis. One subject was in the concomitant group and this sample was inadequate for analysis. Two subjects were from the non-concomitant group; of these, one sample was negative for VZV genome and the other sample was inadequate for analysis. One control subject tested positive for VZV vaccine virus genome.

In subjects who reported measles-like rashes, 22 of 38 provided a blood sample within 7 days of rash onset for evaluation by RT-PCR. 19 of the subjects with measles-like rashes that provided blood samples were immunized with ProQuad while 3 of these subjects were immunized with MMRII + VARIVAX. No samples were positive for measles virus genome.

The incidences of these reactions were also calculated based on the numbers of subjects with follow-up for the population quadruple-negative at baseline and the results are presented in Table 8.4.25 below:

Table 8.4.25 Summary of injection site reactions and measles-like, rubella-like and varicella-like rashes reported by vaccine group in the subset of vaccinees who were quadruple negative pre-vaccination

Baseline Serostatus	Clinical Complaint Reported (Days 0 to 42 Post-vaccination)	Concomitant Group (N=949) N	(%)	Nonconcomitant Group (N=485) n	(%)	Control Group (N=479) n	(%)
Quadruple Seronegative†	Number of subjects vaccinated	737		385		365	
	Number with safety follow-up	721		380		355	
	Measles-like rash‡	18	(2.5)	12	(3.2)	7	(2.0)
	Rubella-like rash‡	4	(0.6)	3	(0.8)	1	(0.3)
	Varicella-like rash	7	(1.0)	11	(2.9)	11	(3.1)
	Injection-site of ProQuad™						
	Any injection-site adverse experience	318	(44.1)	144	(37.9)	NA	NA
	Erythema	128	(17.8)	66	(17.4)	NA	NA
	Pain/tenderness/soreness	254	(35.2)	95	(25.0)	NA	NA
	Rash	10	(1.4)	9	(2.4)	NA	NA
	Swelling	98	(13.6)	43	(11.3)	NA	NA
	Injection-site of M-M-R™ II						
	Any injection-site adverse experience	NA	NA	NA	NA	117	(33.0)
	Erythema	NA	NA	NA	NA	52	(14.6)
	Pain/tenderness/soreness	NA	NA	NA	NA	95	(26.8)
	Rash	NA	NA	NA	NA	1	(0.3)
	Swelling	NA	NA	NA	NA	29	(8.2)
	Injection-site of VARIVAX™						
	Any injection-site adverse experience	NA	NA	NA	NA	115	(32.4)
	Erythema	NA	NA	NA	NA	47	(13.2)
	Pain/tenderness/soreness	NA	NA	NA	NA	95	(26.8)
	Rash	NA	NA	NA	NA	2	(0.6)
	Swelling	NA	NA	NA	NA	25	(7.0)
	Injection-site of TRIPEDIA™						
	Any injection-site adverse experience	356	(49.4)	NA	NA	NA	NA
	Erythema	191	(26.5)	NA	NA	NA	NA
	Pain/tenderness/soreness	261	(36.2)	NA	NA	NA	NA
	Rash	6	(0.8)	NA	NA	NA	NA
	Swelling	163	(22.6)	NA	NA	NA	NA
	Injection-site of COMVAX™						
	Any injection-site adverse experience	375	(52.0)	NA	NA	NA	NA
	Erythema	214	(29.7)	NA	NA	NA	NA
	Pain/tenderness/soreness	266	(36.9)	NA	NA	NA	NA
	Rash	5	(0.7)	NA	NA	NA	NA
	Swelling	169	(23.4)	NA	NA	NA	NA
	Number with temperature follow-up	715		377		351	
	Temperature ≥102°F (38.9°C), oral equivalent or Abnormal	203	(28.4)	100	(26.5)	84	(23.9)

8.4.8 Comments & Conclusions (Study 013):

8.4.8.1 The seroresponse rates to measles, mumps, rubella, varicella, *Hemophilus influenza* type b and hepatitis B are similar in the concomitant group compared with the nonconcomitant group indicating that ProQuad and COMVAX may be administered simultaneously.

8.4.8.2 The antibody responses to pertussis FHA were not similar between the concomitant group and nonconcomitant group therefore the data do not support simultaneous administration of ProQuad and Tripedia in children 12-15 months old.

8.4.8.3 Immune responses to measles, mumps, rubella, and varicella were found to be acceptable when ProQuad was given with TRIPEDIA and COMVAX.

8.4.8.4 In general, the safety and tolerability profiles of each vaccine were similar in both the concomitant and nonconcomitant groups.

8.4.8.4.1 Injection site reactions were reported slightly more frequently in the concomitant immunization group than in the group given vaccines non-concomitantly.

8.4.8.4.2 There is a higher incidence of fevers ≥102 F oral equivalent or abnormal after concomitant immunization however the fevers are transient and not associated with long term sequelae.

8.4.8.4.3 No serious vaccine related AEs were reported.

8.4.8.5 The post hoc analysis that indicated that pertussis anti-PT and anti-FHA antibody responses in children > 13.5 months old who were immunized concomitantly with ProQuad, Tripedia, and Comvax were similar to those seen in children in the nonconcomitant group will not be used to support concurrent administration of these vaccines in the product label for the following reasons:

8.4.8.5.1 Children enrolled in this study received previous doses of DTaP from any manufacturer and because there are differences in the content of vaccine antigens, the differences in priming history could potentially contribute to differences in the results,

8.4.8.5.2 Also, the package inserts for DTaP vaccines do not allow for a mix and match of products when a series of doses are given. Interchanging DTaP vaccines from different manufacturers for successive doses of vaccines is not acceptable.

8.4.8.5.3 Children immunized in this study were younger than the age described in the label for Tripedia vaccine and dosing intervals between the third and fourth doses varied considerably. Use of Tripedia was inconsistent with the approved schedule/label that recommends that the fourth dose be administered between 15 and 18months of age. The effect of age and dosing interval confound the interpretation of pertussis immunogenicity data in this study.

8.4.8.5.4 Validation data for the assays used to detect antigen specific antibodies have not been submitted.

8.4.8.5.5 The post-hoc, age stratified analysis cannot be mentioned in the label.

8.4.8.5.6 If the sponsor desires a label claim to support concomitant use of ProQuad with DTaP vaccine, then they will need to perform an appropriately designed controlled immunogenicity study. All subjects should receive the same DTaP vaccine for their entire series of doses including the dose administered with ProQuad. The fourth dose should be administered on a schedule consistent with the ages listed in the package insert (15 to 20 months of age) and with a minimum interval between doses. Immune responses should compare both GMTs and seroresponse rates for each pertussis antigen in the vaccine using validated assays.

8.5 Trial # 014

Administration of Frozen Measles, Mumps and Rubella and Varicella Vaccine to Healthy Children at 4 to 6 years of Age

8.5.1 Objectives/Rationale:

The primary objective was (1) to show that the antibody responses to measles, mumps, and rubella following a dose of ProQuad at 4 to 6 years of age were similar to antibody responses after the recommended second dose of MMRII vaccine (2) to show that the antibody responses to measles, mumps, rubella, and varicella following a dose of ProQuad at 4 to 6 years of age were similar to the antibody responses after a second dose of MMRII + VARIVAX administered concomitantly at separate injection sites (3) to show that a dose of ProQuad was generally well tolerated and (4) to summarize the following immunogenicity parameters by group: seroconversion rates to measles, mumps, and rubella in subjects seronegative to each antigen, seropositive rates to measles, mumps, and rubella in all subjects, the percent of subjects with post-vaccination varicella titers of ≥5 gpELISA units in subjects initially seronegative to varicella, in subjects with a pre-dose titer of <1.25 gpELISA units and in all subjects; for each vaccine antigen, summarize the percent of subjects achieving a ≥four fold antibody rise.

8.5.2 Design Overview:

Study 014 was a double blind, multi-center study conducted at 17 sites. Healthy children 4-6 years of age were enrolled. Children were stratified at the time of enrollment based on whether their primary dose of MMRII and VARIVAX were given concomitantly or non- concomitantly into one of three vaccine groups. Group 1 received one dose each of ProQuad and placebo at separate injection sites; Group 2 received MMRII + placebo at separate injection sites, Group 3 received MMRII + VARIVAX at separate injection sites.

Targeted enrollment was for 700 healthy subjects 4 to 6 years of age with 350 subjects in the investigational group and 175 subjects each in Group 2 or Group 3. The study was initiated on **August 24, 2000** and was completed on **May 6, 2002**. Subjects were enrolled and randomized in a 2:1:1 ratio based on whether their primary doses of MMRII and VARIVAX had been given concomitantly or not. Vaccines and placebo were physically different therefore un-blinded personnel reconstituted vaccine and placebo and then delivered blinded syringes (labeled vial 1 or vial 2) to study personnel for administration. Vial 1 doses were given in the right arm while vial 2 doses were administered into the left arm, always. Parents/guardians, subjects, personnel administering the vaccines and performing follow-up evaluations, and all MRL personnel performing serology were blinded to group assignment

The IRB at each study site reviewed and approved the protocol and Informed Consent form used to enroll subjects. Parents or legal guardians provided written informed consent and subjects were randomized and vaccinated on the first study day (N.B. in previous studies the first study day has been called, Day 0. In this study, because data was collected using a new database, the first study day is called, Day 1).

Pre-vaccination blood samples were obtained on Day 1, subjects were vaccinated and then followed through Day 43 at which time the post vaccination blood samples were obtained, the Vaccination Report Card was turned in and reviewed with study personnel and the parent/legal guardians were asked about

any exposures to measles, mumps, rubella, varicella, or shingles. Group 2 subjects immunized with MMRII + placebo were offered VARIVAX at the completion of the study and after unblinding had occurred. If vaccinated, they were also followed for an additional 42 days after VARIVAX immunization and optional blood samples were collected at that time.

Any subject with no detectable antibodies to vaccine antigens upon completion of the study was offered vaccination with the viral component to which they did not respond.

Subjects with measles or measles like rashes were evaluated further. Whole blood samples were obtained at the time of the rash and evaluated by RT-PCR for measles virus genome.

Immunogenicity analyses were based on a per protocol approach. A summary of the Study Design is in Table 8.5.1 below:

Table 8.5.1 Summary of the Study Design for Protocol #014

Time	Procedures		
	ProQuad™ and Placebo (Group 1)	M-M-R™II and Placebo (Group 2)	M-M-R™II and VARIVAX™ (Group 3)
Day 1	Reviewed eligibility criteria. Obtained history/consent. Obtained 5- to 10-mL blood sample. Administered 0.5 mL each of ProQuad™ and placebo by subcutaneous injection. Distributed and reviewed instructions for vaccination report card (VRC).	Reviewed eligibility criteria. Obtained history/consent. Obtained 5- to 10-mL blood sample. Administered 0.5 mL each of M-M-R™II and placebo by subcutaneous injection. Distributed and reviewed instructions for VRC.	Reviewed eligibility criteria. Obtained history/consent. Obtained 5- to 10-mL blood sample. Administered 0.5 mL each of M-M-R™II and VARIVAX™ by subcutaneous injection. Distributed and reviewed instructions for VRC.
Days 1 to 43	Follow-up for adverse experiences using VRC.	Follow-up for adverse experiences using VRC.	Follow-up for adverse experiences using VRC.
Day 43† (-7/+14 days)	Obtained 5- to 10-mL blood sample. Collected VRC and review with parent/legal guardian. Collected exposure/disease survey information for measles, mumps, rubella, varicella and/or zoster.	Obtained 5- to 10-mL blood sample. Collected VRC and reviewed with parent/legal guardian. Collected exposure/disease survey information for measles, mumps, rubella, varicella and/or zoster.	Obtained 5- to 10-mL blood sample. Collected VRC and reviewed with parent/legal guardian. Collected exposure/disease survey information for measles, mumps, rubella, varicella and/or zoster.
Post Day 43, after data are unblinded	N/A	Offered optional second dose of VARIVAX™	N/A
Days 1 to 43 after receipt of optional dose of VARIVAX™	N/A	Follow-up for adverse experiences using VRC.	N/A
Day 43† (-7/+14 days) after receipt of optional dose of VARIVAX™	N/A	Obtained 5- to 10-mL blood sample (optional [upon parent/legal guardian request]). Collected VRC and reviewed with parent/legal guardian. Collected exposure/disease survey information for measles, mumps, rubella, varicella and/or zoster.	N/A

† The 7-day/+14-day window surrounding the Day 43 time point relates only to serologic follow-up. N/A = Not applicable.

8.5.2.1 **Randomization:**

Subjects were divided into two strata using their prior immunization history based on whether prior MMRII and VARIVAX were given on the same or different days. Separate allocation schedules were used for each stratum and assignments to treatment groups were made using a computer generated allocation schedule. A statistician not otherwise affiliated with the study provided the allocation schedules. An un-blinded study person at each study center was given a set of unique allocation numbers for each treatment group (i.e., one schedule for children who received the first doses of MMRII + VARIVAX concomitantly and another for children given these vaccines on different days) in sealed envelopes. Children at each site were randomized in blocks of 4. Allocation numbers were not re-assigned for any reason.

8.5.2.2 **Interim analyses**:

An interim analysis was not performed.

8.5.2.3 **Study Population:**

Healthy children, 4 to 6 years of age were enrolled.

8.5.2.3.1 **Inclusion criteria**:

- Good health
- 4 to 6 years of age
- Negative history for varicella, shingles, measles, mumps and rubella
- Documentation of primary dose of MMRII at ≥12 months of age and at least one month prior to enrollment.
- Documentation of VARIVAX at ≥12 months of age and at least three months prior to enrollment.

8.5.2.3.2 **Exclusion criteria**:

- Previous receipt of more than one dose of measles, mumps rubella or varicella vaccine.
- Immune impairment or deficiency, neoplastic disease, depressed immunity from steroid or other therapy
- History of anaphylactic reaction to neomycin, gelatin or any other vaccine component.
- History of anaphylactic or other immediate allergic reactions subsequent to egg ingestion.
- Any exposure to measles, mumps, rubella, varicella or shingles in the 4 weeks prior to each vaccination involving:
    - Continuous household contact
    - Playmate contact > 1 hour indoors
    - Hospital contact in the same room or prolonged face-to-face contact
    - Contact with a newborn whose mother had chickenpox 5 days or less prior to delivery or within 48 hours of delivery.
- Vaccination with an inactivated vaccine within 14 days prior to receipt of each dose of vaccine or scheduled within 42 days thereafter.

- Vaccination with a live virus vaccine within 30 days of a dose of the study vaccine or scheduled within 42 days thereafter.
- Immune globulin or any blood products administered 3 months (150 days) prior to or within 2 months after each vaccination.
- Any contraindications to either MMRI or VARIVAX as stated in the package circulars.
- Any condition that in the opinion of the investigator might interfere with the evaluation of the study objectives.
- It was recommended that subjects not receive salicylates during the 6 weeks after vaccination because aspirin use in children with varicella infection has been associated with Reye's syndrome.

8.5.2.3.3 Subjects were discontinued from the study if they developed an anaphylactic reaction after vaccine administration or if they developed varicella, measles, mumps, or rubella prior to the administration of the study vaccine. Subjects who received other vaccines or blood products before serologic follow-up samples were obtained were not necessarily discontinued from the study but their serology data may have been excluded from the group analyses.

8.5.3 **Products used**

Products used in this protocol were manufactured by Merck. All clinical materials were supplied in 0.7mL single-dose vials. Study vaccines were re-supplied as needed throughout the study on a site-by-site basis. Doses were administered on Day 1, the day of entry into the study. The lot numbers and potencies of the vaccines used in Study 014 are summarized in Table 8.5.2 below.

Table 8.5.2 Summary of vaccines used in Study 014, potency and lot numbers

Vaccine	Formulation/ Clinical Packaging Order Number	Fill Number	Bulk Number(s)	Potency/ 0.5-mL Dose
ProQuad™	1592W-G698/ WP-511	-------	---------- ---------- ---------- ----------	4.10 log10TCID50 4.97 log10TCID50 4.22 log10TCID50 4.40 log10 PFU†
M-M-R II	1676J/ WP-H513	---------	---------- ---------- ----------	3.8 log10TCID50 5.0 log10TCID50 3.7 log10TCID50
	V205CVA1005 A001/ WP-J594	---------	---------- ---------- ----------	4.0 log10TCID50 4.7 log10TCID50 4.0 log10TCID50
VARIVAXTM	1681J/ WP-H514	------	---------- ---------	3.6 log10 PFU†
	1469K/ WP-J179	----------	-------- ---------	3.6 log10 PFU†
	0443K/ WP-J346	----------	---------- --------	3.5 log10 PFU
	1533W-D940/ WP-H512	----------	N/A	0 PFU
Placebo	1533W-D940/ WP-J488	--------	N/A	0 PFU
	PV211-HLS 002 P002/ WP-J593	N/A	N/A	0 PFU
Diluent	0398K/ WP-515	N/A	N/A	N/A

† Upon release, varicella potencies for VARIVAX™ were reported in plaque-forming units (PFU)/mL. The release potency values were converted to log10 PFU/0.5-mL dose in this table for consistency of presentation with measles, mumps, and rubella release potencies. N/A = Not applicable.

*PGS is phosphate, glutamate, and sorbitol stabilizer. It is reconstituted using the sterile diluent.
** Diluent: sterile water for injection.
N/A: not applicable.

8.5.4 Study Objectives:

8.5.4.1 Primary Hypotheses, Immunogenicity

The first primary immunogenicity hypothesis: GMTs for measles, mumps, and rubella antibodies (adjusted by the pre-dose titer and stratified by primary vaccination history) in subjects immunized with ProQuad and placebo will be similar to the antibody responses in subjects receiving MMRII and placebo at separate injection sites.

GMTs will be considered similar if the lower bound of the 90% confidence on the fold difference in GMTs excludes a decrease of 2-fold or more.

The second primary immunogenicity hypothesis: GMT antibody titers for measles, mumps, rubella and varicella (adjusted for pre-dose titer and stratified by vaccination history) in subjects receiving ProQuad plus placebo will be similar to the antibody responses in subjects receiving MMRII + VARIVAX at separate injection sites.

GMTS will be considered similar if the lower bound of the 90% confidence on the fold difference in GMTs excludes a decrease of 2-fold or more.

8.5.4.2 Primary Hypothesis for Safety:

A dose of ProQuad at 4 to 6 years of age instead of a dose of MMRII will be generally well tolerated.

The safety profile of group 1 (ProQuad plus placebo) was compared to the safety profile of Group 2 (MMRII plus placebo) or Group 3 (MMRII plus VARIVAX). All vaccinated subjects with safety follow-up were included in the analyses.

Success of the study required success on all primary hypotheses.

8.5.4.3 Secondary hypothesis:

8.5.4.3.1 The antibody response to measles, mumps and rubella as measured by GMT adjusted for pre-dose titer and stratified by primary vaccination history in subjects immunized with MMRII + VARIVAX will be similar to the antibody responses in subjects receiving MMRII + placebo.

8.5.4.4 (Not used)

8.5.4.5 Study Endpoints:

Immunogenicity endpoints were measured using immunological assays that specifically measured IgG antibody responses to each vaccine virus. **Safety endpoints** were assessed using the Vaccination Report Card that was completed by each subject's parent or legal guardian.

8.5.4.5.1 **Detection of Measles IgG Antibody (ELISA):**

The measles ELISA used measles antigen purchased from ---------
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8.5.4.5.2 **Detection of Mumps IgG Antibody (ELISA):**

Mumps virus antigen used for this assay was produced at MRL. The mumps antigen was ---
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---------- The quantity of anti-mumps IgG was determined by comparing the response in the test sample to the standard curve. The cut-off was determined by running 72 known negative samples (i.e., 12 samples in 6 assays). The assay cut-off was determined to be 10 Ab units. Samples with ODs less than or equal to the cut-off were serostatus negative and assigned a titer of < 10.0 Mumps Ab units. Samples with OD values greater than the cut-off were quantified using the standard curve. The quantifiable range was 0.5 to 64 mumps Ab units/mL. Sera whose titers exceeded this range were re-analyzed at greater dilutions until an endpoint titer was obtained. The negative control for the assay was a pool of human sera known to be mumps negative. The low positive control was a pool of human sera while the high positive was also a pool of human sera. A single mumps positive serum was used to generate the standard curve. The standard curve data were fit using a quadratic polynomial. The LOD was <0.5 Ab units and the quantifiable range of the assays was 0.5 to 65 mumps Ab units/mL. Samples with medium and high titers vary 15.9% with each 10-fold dilution. Assay precision was 18.9-25.3%.

8.5.4.5.3 Detection of Rubella IgG (ELISA):

Inactivated rubella antigen purchased from ----------
---------- The cut-off for the assay was determined by determining them mean OD value for 10 known rubella negative control sera plus 5 times the S.D. of the negative controls. Samples with OD values less than the cut-off were considered to be seronegative and were assigned a value of 10 Ab units. Positive samples were quantitated relative to the standard curve. The negative control for this assay was a single human serum known to be negative for rubella antibody. The low positive and high positive controls were the WHO International Standard diluted to 40 and 160 mIU/mL. The WHO reference serum was also used to generate the standard curve. Standard curve data were fit using a quadratic polynomial. The LOD was 0.91 rubella antibody units/mL. The quantifiable range of the assay was 1-32 antibody units /mL. There was no evidence of significant dilution bias and the overall assay variability was 22.4%. A pre-vaccination sample was considered to be seronegative if it was below the OD cut-off and a post-vaccination sample was considered to be seropositive if it contained ≥12.8 ELISA antibody units (=10 IU/mL).

8.5.4.5.4 Varicella IgG gpELISA antibody:

The purpose of the glycoprotein enzyme-linked immunosorbent assay (gpELISA) was to detect IgG antibody to varicella-zoster virus (VZV) before and after vaccination with VZV-containing vaccine(s). This method detects antibodies to VZV glycoproteins (gp), which have been lectin affinity-purified from MRC-5 cells

infected with the KMcC strain of VZV. The assay and the purification of the VZV gp from VZV-infected cells have been described ----------

Serum sample titers determined by gpELISA correlate with neutralizing antibody titers (Krah, DL, Cho I, Schofield T, et al. Comparison of gpELISA and neutralizing antibody responses to Oka/Merck live varicella vaccine in children and adults. Vaccine 1997 15(1):61-64.) and with protective efficacy (White CJ, Kuter BJ, Ngai A, et al. Modified cases of chickenpox after varicella vaccination: correlation of protection with antibody response. Pediatr Infect Dis J 1992 11(1):19-23.).

Results for the assay are reported as concentration of antibody in gpELISA units/mL. The negative control used for this assay was an individual human serum at a dilution of 1:50, found to be negative for anti-VZV. The high positive marker was a VZV-antibody-positive serum, diluted 1:15,000, which gave a response in the assay at the upper end of the standard curve. The low positive marker was a VZV-antibody-positive serum diluted 1:50,000, which gave a response in the assay at the lower end of the standard curve. A VZV-antibody-positive individual human serum was used to generate a standard curve (range of 0.625 to 20 gpELISA units/mL).

Prior to June-2001, the standard curve was approximated using a quadratic function fit to the 0.625 to 20 gpELISA units/mL concentration range of the standard. Since June-2001, the standard curve has been approximated using the four-parameter weighted logistic regression function. A statistical analysis comparing the two fit procedures showed that the quadratic and logistic processing methods yield similar titers (generally within 3%) when interpolating from the 0.625 to 20 gpELISA units/mL region of the standard curve.

During the validation, the limit of detection (LOD) was mathematically determined to be 0.3 gpELISA units/mL. However, because no standard concentrations below 0.625 gpELISA units/mL are run in the assay, the LOD is reported as <0.625 gpELISA units/mL. The quantifiable range of the assay is 0.625 to 20 gpELISA units/mL. Dilutability is defined as the attribute of a standard curve assay whereby it is demonstrated that a test sample can be diluted through a series, yielding equivalent titers across that series. The assay is dilutable for samples tested in the 1:500 to 1:40,000 dilution range. The precision of the assay for a

sample titer was 11%. There was no statistical evidence of increased variability in test sample results due to different analysts performing the assay.

8.5.4.5.5 Detection of Virus in subjects with Rashes or other serious Adverse reactions:

No samples were submitted for RT-PCR (for measles detection) or --- (for detection of varicella) analysis.

8.5.4.6 Changes in the Conduct of the Study:

8.5.4.6.1 Merck evaluated measles serology using 120mIU/mL as the sero-protective cut-off as requested by CBER.

8.5.4.6.2 Merck evaluated rubella serology using 10 IU/mL as the sero-protective cut-off as requested by CBER.

8.5.4.6.3 The primary varicella immunogenicity analysis was performed on subjects with a baseline antibody titer < 1.25 gpELISA units instead of on subjects with baseline titers < 5gpELISA units as requested by CBER.

8.5.4.6.4 Data on adverse reactions was entered into a new database using preferred terms from the Medical Dictionary for Regulatory Activities (MedRA). CBER approved this change.

8.5.5 Surveillance

8.5.5.1 MRL conducts its own Quality Assurance and Quality Control Program and surveillance included on-site monitoring of investigators sites, on site and in-house review of clinical data and resultant databases, review of the clinical study reports and summary documents.

8.5.5.2 Parents/guardians completed the VRC for 42 days after immunization (Days 1-43). They recorded systemic reactions, injection site reactions, temperatures, and any other vaccines or medications given. Swelling and redness were evaluated by size. Fevers were not graded. Other adverse reactions were graded by the investigator according to the intensity rating criteria outlined in Table 8.5.3 below:

Table 8.5.3 Rating criteria for adverse reactions

Intensity	Rating Criteria
None	No signs or symptoms of intolerance.
Mild	Subject is aware of symptoms but they are easily tolerated.
Moderate	Subject is definitely acting like something is wrong.
Severe	Subject appears extremely distressed or unable to do usual activities.

8.5.5.3 Serious adverse experiences were the same as in the other studies and were reported to study personnel immediately.

8.5.5.4 No formal interim analysis was performed

8.5.5.5 Formal surveillance for cases of measles, mumps, rubella and varicella in the community was not done; parents and guardians reported any known cases or exposures that occurred during the study and prior to obtaining the post vaccination blood sample.

8.5.5.6 Follow-up visits for safety assessments and serology were as follows:

Parents filled out the Vaccination Report Cards for 42 days after each vaccination. They were required to note local and systemic AEs and record temperatures for 42 days after immunization.

Children with a rash or with symptoms of mumps infection were evaluated immediately. Varicella-like lesions were cultured and tested by --- after informed consent was obtained from the parent/guardian.

Blinded study personnel provided follow-up and collected information regarding adverse reactions.

8.5.6 Statistical considerations:

8.5.6.1 The primary purpose of the study was to show that ProQuad could be used in place of MMRII to fulfill the recommendation for a second dose of measles containing vaccine in childhood.

8.5.6.1.1 First Primary Immunogenicity Hypothesis:

The first primary hypothesis proposed that the GMTs for measles, mumps, and rubella antibodies (adjusted by the pre-dose titer and stratified by primary vaccination history) in subjects immunized with ProQuad and placebo would be similar to the antibody responses in subjects receiving MMRI and placebo at separate injection sites.

GMTS were considered similar if the lower bound of the 90% confidence on the fold difference in GMTs excluded a decrease of 2-fold or more.

Measles, mumps, and rubella GMTs 6 weeks post vaccination were compared between Group 1 (ProQuad plus placebo)) and Group 2 (MMRII + placebo) using a one-sided, non-inferiority test for each antigen at $\alpha=0.05$. H_O: GMTa/GMTb ≤ 0.5 where a and b represent subjects in groups 1 and 2 respectively. Testing at the one sided, 0.05 level was equivalent to requiring that the two sided 90% CI for the ratios of GMTs (Group1/Group2) exclude a fold difference of 0.5 or smaller. Rejecting the one-sided hypothesis of a greater than 2 fold difference led to the conclusion that the immune responses to measles, mumps and rubella were similar 6 weeks after immunization.

The sample size calculations were based on the following assumptions: (1) 10% would be lost to follow up or excluded due to protocol violations and (2) the standard deviation of the natural log of the post vaccination titer was 1.2 (estimated from previous studies).

350 subjects were enrolled in Group 1 and 175 enrolled in Group 2 and Group 3 to give 315, 157 and 157 evaluable subjects in each group, respectively. The study had 99.9% power to exclude a 2 fold difference in GMTs between groups at α=0.05.

Similarly, the study had 99.9% power to exclude a two-fold or greater difference in varicella GMTs based on a one sided test at α=0.05.

8.5.6.1.2 Second Primary Immunogenicity Hypothesis:

The second primary hypothesis proposed that the GMT antibody titers for measles, mumps, rubella, and varicella (adjusted for pre-dose titer and stratified by vaccination history) in subjects receiving ProQuad plus placebo will be similar to the antibody responses in subjects receiving MMRII + VARIVAX at separate injection sites.

GMTs were considered similar if the lower bound of the 90% confidence on the fold difference in GMTs excluded a decrease of 2-fold or more.

The methodology used to compare GMTs was the same as that described above under 8.1.5.1.1

8.5.6.1.3 Primary Endpoint for Safety:

The primary endpoint for safety was the incidence of vaccine related serious adverse experiences. In addition, for adverse reactions occurring in at least 1% of subjects in any treatment group, the risk difference and 95% confidence interval for the risk difference were compared.

The safety profile of group 1 (ProQuad plus placebo) was compared to the safety profile of Group 2 (MMRII plus placebo) and to the safety profile of Group 3 (MMRII plus VARIVAX). All vaccinated subjects with safety follow-up were included in the analyses.

The risk differences and incidence rates between Group 1 and Group 2 and between Group 1 and Group 3 were estimated and 95% two-sided CIs provided. The following were compared:

- Incidence of adverse experiences
- Incidence of injection site reactions
- Incidence of systemic adverse reactions
- Incidence of vaccine related adverse reactions.

The incidence rates of specific adverse reactions (fever, measles-like rashes, rubella-like rashes, varicella-like rashes, mumps-like symptoms) were tabulated and tests of significance used to compare rates between groups using two-sided 95% CIs.

Similarly, the incidence of redness, swelling, and the incidence of pain and tenderness at the injection site were compared between

Group 1 and Group 2 and between Group 1 and the MMRII injection site in Group 3 as well as between Group 1 and the VARIVAX injection site also in Group 3.

Risk differences and incidence rates for AEs not solicited on the VRC were also compared for those AEs that occurred at a rate of greater than or equal to 1% in any group. The method for comparison was the same as that described above.

This study had 85.0% power to detect a 10 percent increase in the incidence rate of a vaccine adverse reaction between Groups 1 and 2 or between Groups 1 and 3.

Success of the study required success on all primary hypotheses.

8.5.6.1.4 Multi-center Study:

The clinical trial used 17 study sites. If there were fewer than 44 subjects at a site, this center was pooled with a larger center and pooling continued until at least 44 subjects were combined. Pooling took place prior to study unblinding and was done to yield at least 10 evaluable subjects per group at each center.

8.5.6.1.5 Treatment by Center Interactions:

A test for treatment-by-center interactions was conducted to evaluate whether GMT ratios were consistent across combined study centers. The test used ANOVA with the log titer as the response variable and treatment group, combined study center, primary vaccination history, pre-dose titer and treatment by center as fixed effects at α=0.10 significance level.

8.5.6.1.6 Multiplicity Adjustments:

No multiplicity adjustments were made. Each of the 7 hypotheses was tested at the one-sided, 0.05 level.

8.5.6.1.7 Confounding Factors:

One lot of MMRII used in this study had a mumps potency of 4.7log10pfu/dose at the time of release and an estimated potency of 4.2log10pfu/dose at the time of use. Mumps GMTs were lower in children immunized with this lot when compared to children immunized with the other lot that had a release titer of 5.0 log10 PFU/dose but the difference was not significant.

8.5.7 Results

8.5.7.1 Populations enrolled/analyzed

8.5.7.1.1 The study was conducted at 17 study sites in the United States and the sites, investigators, and numbers of children enrolled at each site are summarized in Table 8.5.4.

Table 8.5.4 Summary of study sites, principal investigators, and number of subjects enrolled per group at each site.

Study Number	Investigator	ProQuad™ + Placebo (N=399)†	M-M-R™II + Placebo (N=205)	M-M-R™II + VARIVAX™ (N=195)
014001	Milnes, Philip	4	3	2
014003	Sullivan, Bradley	84	42	42
014002	Bernstein, Henry ‡	0	0	0
014004	Reisinger, Keith	137	70	65
014005	Marshall, Gary	31	17	16
014006	Henderson, Frederick	6	4	3
014007	Barone, Stephen	3	2	1
014008	Nauert, Beth	20	11	9
014010	Iwaishi, Louise	7	5	4
014011	Senders, Shelly	8	3	4
014012	Marchant, Colin	15	7	8
014014	Pollara, Bernard	10	4	4
014015	Silas, Peter	17	8	9
014016	Matson, David	18	8	8
014017	Sher, Lawrence	16	9	8
014018	Conti, Ralph	13	7	6
014019	Rothstein, Edward	9	5	4
014020	Greenberg, David	1	0	2

8.5.7.1.2 Study enrollment and drop-outs:

802 subjects were enrolled in the study however two subjects withdrew prior to vaccination leaving 800 vaccinated study subjects. 781 (97.4%) completed the study. 21 subjects discontinued the study and the proportion of subjects who discontinued was comparable among the three groups with 9 subjects (2.2%) in Group 1, 4 subjects (2.0%) in Group 2 and 7 subjects (3.6%) in Group 3. One subject (25842) was randomized to received ProQuad plus placebo but instead received two doses of placebo and this subject discontinued the study as soon as the mistake was identified. 11 additional subjects were screened for the study but were not randomized because 2 parents withdrew consent and 9 did not meet the inclusion/exclusion criteria. The numbers of subjects enrolled and drop-outs by group are listed in Table 8.5.5 below.

Table 8.5.5 Enrollment and study dropouts by vaccine group

	ProQuad™ + Placebo		M-M-R™II + Placebo		M-M-R™II + VARIVAX		Diluent		Total	
	n	(%)†	N	(%)	N	(%)	n	(%)	n	(%)
RANDOMIZED	401‡		205		195		1§		802	
Male (age range—years)	208 (4 to 6)		100 (4 to 6)		118 (4 to 6)				426 (4 to 6)	
Female (age range—years)	191 (4 to 6)		105 (4 to 5)		77 (4 to 5)				374 (4 to 6)	
Vaccinated	399		205		195		1		800	
COMPLETED	392	(97.8)	201	(98.0)	188	(96.4)			781	(97.4)
DISCONTINUED	9	(2.2)	4	(2.0)	7	(3.6)	1	(100)	21	(2.6)
Lost to follow-up	3	(0.7)	2	(1.0)	2	(1.0)			7	(0.9)
Subject discontinued for other reasons	1	(0.2)			1	(0.5)			2	(0.2)
Subject withdrew consent	3	(0.7)	2	(1.0)					5	(0.6)
Protocol deviation							1	(100)	1	(0.1)
Specimen or test not done	2	(0.5)			4	(2.1)			6	(0.7)
Clinical adverse experience										

† Percentage based on number of subjects vaccinated.
‡ Includes 2 subjects (ANs 20341 and 20342) who withdrew consent prior to vaccination.
§ Subject was randomized to receive ProQuad™ and placebo, but was inadvertently administered 2 doses of diluent. The subject was considered discontinued due to a protocol
Deviation. The subject's parent/legal guardian withdrew consent immediately following vaccination.

8.5.7.1.3 Protocol Deviations:

Protocol deviations that resulted in data being excluded from the primary immunogenicity analysis after dose 1 included subjects who were not vaccinated (2), improper labeling of the post vaccination sample (2), hemolyzed pre-vaccination sample (1), sample lost (1), received primary MMRII or VARIVAX prior to 12 months (9), received 2 doses of MMR (1) or 2 doses of VARIVAX (1) or was exposed to varicella within 4 weeks of the study (1), subjects received MMRII and VARIVAX not supplied by the study (1), received two doses of diluent (1), chickenpox (1), unblinded prematurely (30). Subjects excluded for protocol deviations are summarized by group in Table 8.5.6 below.

8.5.7.1.4 Six children had protocol deviations but were not excluded from the primary immunogenicity analyses because the reason for exclusion would not have interfered with the immune responses to the vaccines.

Table 8.5.6 Subjects excluded due to protocol deviations by group

	ProQuad™+ Placebo	M-M-R™II + Placebo	M-M-R™II + VARIVAX™
Subjects vaccinated	399	205	195
Subjects included in the analysis	367	185	171
Subjects excluded from the analysis	32	20	24
Subject received incorrect doses of vaccine prestudy per inclusion criteria	4	3	3
Subject received incorrect test product administration	0	0	1
Subject recently exposed to disease of interest	1	0	0
Subject was diagnosed with a medical condition excluded by protocol†	0	1	0
Subject was inadvertently unblinded during the trial‡	15	6	7
Missing or not evaluable pre-vaccination result:	1	1	1
Other technical difficulty	1	1	0
Specimen damaged	0	0	1
Missing or not evaluable post-vaccination result:	13	9	12
Post-vaccination sampling outside the specified day range	3	1	2
Sample identity questionable	1	1	0
Lost to follow-up	3	2	2
Other technical difficulty	3	4	3
Patient uncooperative	3	1	4
Specimen lost	0	0	1

† It was discovered after enrollment that this subject was diagnosed with a clinical case of varicella prior to study enrollment. ‡ The current version of the Clinical Trials System (CTS) database allowed only 1 protocol violation flag. Two (2) subjects, Allocation Number (AN) 20266 and AN 25005, were not accounted in this category but were accounted for in other violation categories. A subject may be counted in more than 1 category.

8.5.7.1.5 All vaccinated subjects with safety follow-up were included in the evaluation of safety.

8.5.7.1.6 The primary analysis of immunogenicity was based on the per protocol population. Serostatus for each vaccine antigen at baseline is listed in Table 8.5.8 below:

8.5.7.1.7 Demographics:

Subjects in each group were comparable in terms of age, race, gender, primary vaccination history and with regards to prior therapies or medications. This information is summarized in Table 8.5.7 below.

Table 8.5.7 Subject demographics by vaccine group

	ProQuad™ + Placebo (Group 1) (N=399)	MMR™ II + Placebo (Group 2) (N=205)	MMR™ II + VARIVAX™ (Group 3) (N=195)	Diluent (N=1)	TOTAL (N=800)
	n (%)	N (%)	n (%)	N (%)	n (%)
Gender					
Male	208 (52.1)	100 (48.8)	118 (60.5)	0 (0.0)	426 (53.3)
Female	191 (47.9)	105 (51.2)	77 (39.5)	1 (100.0)	374 (46.8)
Age (Years)					
Mean	4.3	4.3	4.3	5.0	4.3
SD	0.5	0.5	0.5	0.0	0.5
Median	4.0	4.0	4.0	5.0	4.0
Range	4 to 6	4 to 6	4 to 6	5 to 5	4 to 6
Male	4 to 6	4 to 6	4 to 6	NA	4 to 6
Female	4 to 6	4 to 5	4 to 5	NA	4 to 6
Race					
African American	49 (12.3)	19 (9.3)	25 (12.8)	0 (0.0)	93 (11.6)
Asian/Pacific	8 (2.0)	5 (2.4)	3 (1.5)	0 (0.0)	16 (2.0)
Caucasian	313 (78.4)	162 (79.0)	153 (78.5)	1 (100.0)	629 (78.6)
Hispanic	15 (3.8)	10 (4.9)	7 (3.6)	0 (0.0)	32 (4.0)
Other	14 (3.5)	9 (4.4)	7 (3.6)	0 (0.0)	30 (3.8)
Primary Vaccination History Status					
Concomitant	258 (64.7)	134 (65.4)	126 (64.6)	0 (0.0)	518 (64.8)
Nonconcomitant	141 (35.3)	71 (34.6)	69 (35.4)	1 (100.0)	282 35.3)
Prior Therapy					
One or more	148 (37.1)	78 (38.0)	79 (40.5)		
None	251 (62.9)	127 (62.0)	116 (59.5)		
Concomitant Therapy					
One or more	234 (58.6)	126 (61.5)	118 (60.5)		
None	165 (41.4)	79 (38.5)	77 (39.5)		

Concomitant = Subjects received their primary M-M-R™II and VARIVAX™ on the same day.
Nonconcomitant = Subjects received their primary M-M-R™II and VARIVAX™ on different days.
N = Total number of subjects vaccinated.
SD = Standard deviation.

The three treatment groups were generally comparable with respect to baseline serostatus measles, mumps, rubella and varicella. (See Table 8.5.8)

Table 8.5.8 Serostatus for each vaccine antigen at baseline (pre-vaccination)

Initial Serostatus and GMTs:	**ProQuad™ + Placebo (Group 1)** (N=399)		**M-M-R™II + Placebo (Group 2)** (N=205)		**M-M-R™II + VARIVAX™ (Group 3)** (N=195)	
	n		n		n	
MEASLES (ELISA)						
Negative (<120 mIU/mL) (%)	8	(2.0)	2	(1.0)	3	(1.5)
Positive (≥120 mIU/mL) (%)	390	(97.7)	202	(98.5)	191	(97.9)
Unknown (%)	1	(0.3)	1	(0.5)	1	(0.5)
GMT (95% CI)	**398**	**1616.6 (1463.0, 1786.3)**	**204**	**1632.4 (1411.2, 1888.1)**	**194**	**1611.3 (1398.3, 1856.8)**
MUMPS (ELISA)						
Negative (<10 ELISA Ab units/mL) (%)	15	(3.8)	11	(5.4)	4	(2.1)
Positive (≥10 ELISA Ab units/mL) (%)	383	(96.0)	193	(94.1)	190	(97.4)
Unknown (%)	1	(0.3)	1	(0.5)	1	(0.5)
GMT (95% CI)	**398**	**84.1 74.9, 94.3)**	**204**	**84.4 (71.4, 99.8)**	**194**	**85.3 (73.4, 99.2)**
RUBELLA†(ELISA)						
Negative (<10 IU/mL) (%)	8	(2.0)	10	(4.9)	8	(4.1)
Positive (≥10 IU/mL) (%)	390	(97.7)	194	(94.6)	186	(95.4)
Unknown (%)	1	(0.3)	1	(0.5)	1	(0.5)
GMT (95% CI)	**398**	**70.1 (63.9, 76.9)**	**204**	**61.4 (53.2, 70.9)**	**194**	**58.8 (51.4, 67.2)**
VARICELLA (GPELISA)						
<1.25 gpELISA units/mL (%)	15	(3.8)	6	(2.9)	5	(2.6)
≥1.25 gpELISA units/mL (%)	383	(96.0)	198	(96.6)	189	(96.9)
Unknown (%)	1	(0.3)	1	(0.5)	1	(0.5)
GMT (95% CI)	**398**	**25.3 (21.4, 29.9)**	**204**	**25.4 \(20.0, 32.3)**	**194**	**25.1 (19.6, 32.2)**

† Rubella titers obtained by the legacy format assay were converted to their corresponding titers in the modified format. n = Number of subjects contributing to analysis ELISA = Enzyme-linked immunosorbent assay. gpELISA = Glycoprotein enzyme-linked immunosorbent assay. GMT = Geometric mean titer. CI = Confidence interval.

8.5.7.2 Efficacy endpoints

8.5.7.2.1 Tests for interaction: There was no significant treatment-by-primary vaccination history interaction when GMTs 6 weeks after immunization were compared for measles, mumps, rubella, or varicella. Likewise, there was no significant treatment-by-center interaction when GMTs 6 weeks after immunization were compared across centers.

8.5.7.2.2 First Primary Immunogenicity Endpoint:

The first primary immunogenicity endpoint was to compare the measles, mumps, and rubella antibody responses after ProQuad plus placebo to those elicited by a second dose of MMRII plus placebo. Six weeks after immunization all subjects were seropositive for antibody against measles, mumps, and rubella with 2 exceptions: 2 individuals in the ProQuad plus placebo

group were seronegative for mumps antibody. GMTs were compared as the primary endpoint for this study and the data are summarized in Tables 9, 10, 11, 12 and 13 below (Group1, ProQuad plus placebo vs. Group 2, MMRII plus placebo):

Measles responses: All children had measles antibody >120mIU/mL 6 weeks after immunization. When the lower limit of the 90% CI for GMTs were compared for Group 1/Group 2, the ratio was greater than 0.5 and it was concluded that the responses after ProQuad were not inferior to those seen after MMRII + placebo immunization. (See Tables 8.5.9 and 8.5.10)

Mumps responses: 2 of 367 children in the ProQuad plus placebo group did not develop mumps antibody while all children in Group 2 had mumps antibody 6 weeks after immunization. When the lower limit of the 90% CI for GMTs were compared Group 1/Group 2, the ratio was greater than 0.5 and it was concluded that the responses after ProQuad were not inferior to those seen after MMRII plus placebo. (See Tables 8.5.9 and 8.5.11)

Rubella responses: All children had rubella antibody 6 weeks after immunization. When the lower limit of the 90% CI for GMTs were compared for Group 1/Group 2, the ratio was greater than 0.5 and it was concluded that the responses after ProQuad were not inferior to those seen after MMRII plus placebo. (See Tables 8.5.9 and 8.5.12)

8.5.7.2.3 Second Primary Immunogenicity Endpoint:

The second primary immunogenicity endpoint was to compare measles, mumps, rubella, and varicella antibody responses after ProQuad plus placebo (group 1) to those elicited by a second dose of MMRII + VARIVAX (Group 3). Six weeks after immunization all subjects were seropositive against the vaccine antigens with 4 exceptions: 1 individual immunized with MMRII +VARIVAX was seronegative to measles and 1 individual in this same group was seronegative to rubella. As noted above, two individuals immunized with ProQuad were seronegative to mumps. GMTs are listed in Table 8.5.9 below for Group 1 and Group 3.

Measles responses: 1/171 failed to develop measles antibody >120mIU/mL after MMRII +VARIVAX. When the lower limit of the 90% CI for GMTs were compared Group 1/Group 3, the ratio was greater than 0.5 and it was concluded that the measles antibody responses after ProQuad immunization were non-inferior to the control group. (See Tables 8.5.9 and 8.5.10)

Mumps responses: 2 of 367 children immunized with ProQuad plus placebo did not develop mumps antibody. When the lower limit of the 90% CI for GMTs were compared Group 1/Group 3, the ratio was greater than 0.5 and it was concluded that the mumps antibody responses after ProQuad immunization were non-inferior to the control group. (See Tables 8.5.9 and 8.5.11)

Rubella responses: 1 of 171 children immunized with MMRII +VARIVAX failed to develop rubella antibody >10IU/mL. When the

lower limit of the 90% CI for GMTs were compared Group 1/Group 3, the ratio was greater than 0.5 and it was concluded that the rubella antibody responses after ProQuad immunization were non-inferior compared to the control. (See Tables 8.5.9 and 8.5.12)

Varicella responses: 6 weeks after immunization with ProQuad, 98.9% of the children had varicella antibody >5gpELISA units/mL while 99.4% of those immunized with MMRII + VARIVAX were seropositive. When the lower limit of the 90% CI for GMTs were compared Group 1/Group 3, the ratio was greater than 0.5 and it was concluded that the varicella antibody responses after ProQuad were non-inferior. Also, varicella gpELISA GMTs in Group 1 were significantly higher than GMTs in Group 3. (See Tables 8.5.9 and 8.5.13)

8.5.7.2.4 Secondary Immunogenicity Endpoint:

A secondary endpoint for the study was to compare measles, mumps, and rubella responses between Group 2 (immunized with MMRII+ placebo) and Group 3 (immunized with MMRII + VARIVAX).

Measles responses: Only 1 of 171 children immunized with MMRII +VARIVAX failed to develop measles antibody >120mIU/mL. When the lower limit of the 90% CI for GMTs were compared Group 2/Group 3, the ratio was greater than 0.5 and it was concluded that the measles antibody responses after MMRII plus placebo were non-inferior to those after MMRII plus VARIVAX. (See Table 8.5.10)

Mumps responses: All children developed mumps antibody by 6 weeks after immunization. When the lower limit of the 90% CI for GMTs were compared Group 2/Group 3, the ratio was greater than 0.5 and it was concluded that the mumps antibody responses after MMRII plus placebo were non-inferior to those after MMRII plus VARIVAX. (See Table 8.5.11)

Rubella responses: Only 1 child immunized with MMRII and VARIVAX failed to develop rubella antibody 6 weeks after immunization. When the lower limit of the 90% CI for GMTs were compared Group 2/Group 3, the ratio was greater than 0.5 and it was concluded that the rubella antibody responses after MMRII plus placebo were non-inferior to those after MMRII plus VARIVAX. (See Table 8.5.12)

Table 8.5.9 Summary of antibody responses for measles, mumps, rubella and varicella following immunization with ProQuad, MMRII, or MMRII and VARIVAX at 4-6 years of age

Time Point	Parameter	ProQuad™+ Placebo (Group 1) (N=399) n	ProQuad™+ Placebo (Group 1) (N=399) Observed Response (95% CI)	M-M-R™II + Placebo (Group 2) (N=205) n	M-M-R™II + Placebo (Group 2) (N=205) Observed Response (95% CI)	M-M-R™II + VARIVAX™ (Group 3) (N=195) N	M-M-R™II + VARIVAX™ (Group 3) (N=195) Observed Response (95% CI)
MEASLES							
Pre-vaccination	GMT (mIU/mL)		1634.9 (1472.2, 1815.6)		1597.9 (1370.8, 1862.6)		1592.9 (1368.1, 1854.7)
	Seropositivity rate†		97.8% (95.8%, 99.1%)		98.9% (96.1%, 99.9%)		98.2% (95.0%, 99.6%)
6 weeks post-vaccination	GMT (mIU/mL)	367	**1985.9** (1817.6, 2169.9)	185	**2046.9** (1815.2, 2308.2)	171	**2084.3** (1852.3, 2345.5)
	Seropositivity rate†		**100%** (99.0%, 100%)		**100%** (98.0%, 100%)		**99.4%** (96.8%, 100%)
	% ≥4-fold rise in titer		4.9% (2.9%, 7.6%)		4.3% (1.9%, 8.3%)		4.7% (2.0%, 9.0%)
MUMPS							
Pre-vaccination	GMT (ELISA Ab units/mL)		84.9 (75.3, 95.7)		83.7 (69.8, 100.3)		88.1 (75.4, 102.9)
	Seropositivity rate†		96.2% (93.7%, 97.9%)		94.1% (89.6%, 97.0%)		98.2% (95.0%, 99.6%)
6 weeks post-vaccination	GMT (ELISA Ab units/mL)	367	**206.0** (188.2, 225.4)	185	**308.5** (269.6, 352.9)	171	**295.9** (262.5, 333.5)
	Seropositivity rate†		**99.5%** (98.0%, 99.9%)		**100%** (98.0%, 100%)		**100%** (97.9%, 100%)
	% ≥4-fold rise in titer		27.2% (22.8%, 32.1%)		41.1% (33.9%, 48.5%)		41.5% (34.0%, 49.3%)
RUBELLA							
Pre-vaccination	GMT (IU/mL)	367	72.4 (65.7, 79.8)	185	62.0 (53.4, 72.0)	171	62.4 (54.5, 71.3)
	Seropositivity rate†	367	98.1% (96.1%, 99.2%)	185	95.7% (91.7%, 98.1%)	171	98.2% (95.0%, 99.6%)
6 weeks post-vaccination	GMT (IU/mL)	367	**217.3** (200.1, 236.0)	185	**174.0** (157.3, 192.6)	171	**154.1** (138.9, 170.9)
	Seropositivity rate†	367	**100%** (99.0%, 100%)	185	**100%** (98.0%, 100%)	171	**99.4%** (96.8%, 100%)
	% ≥4-fold rise in titer	367	32.7% (27.9%, 37.8%)	185	31.9% (25.2%, 39.1%)	171	26.9% (20.4%, 34.2%)
VARICELLA							
Pre-vaccination	GMT (gpELISA units/mL)	367	25.9 (21.7, 31.0)		NA	171	24.6 (19.1, 31.8)
	Seropositivity Rate	367	88.0% (84.2%, 91.2%)		NA	171	88.9% (83.2%, 93.2%)
6 weeks post-vaccination	GMT (gpELISA Units/mL)	367	**322.2** (278.9, 372.2)		NA	171	**209.3** (171.2, 255.9)
	%>5 gpELISA units/mL	367	98.9% (97.2%, 99.7%)		NA	171	99.4% (96.8%, 100%)
	% ≥4-fold rise in titer	367	80.7% (76.2%, 84.6%)		NA	171	71.9% (64.4%, 78.5%)

Table 8.5.10 Comparison of measles antibody responses following immunization at 4 to 6 years of age

MEASLES Comparison (Treatment A vs. Treatment B)	Treatment A			Treatment B			Estimated Fold Difference†‡ (90% CI)†	One-Sided p-Values†	Conclusion
	N	n	Estimated GMT†	N	n	Estimated GMT†			
ProQuad™+ placebo (Group 1) (Treatment A) vs. M-M-R™II + placebo (Group 2) (Treatment B)	399	367	1971.8	205	185	2062.4	0.96 (0.88, 1.04)	<0.001*	Similar
ProQuad™+ placebo (Group 1) (Treatment A) vs. M-M-R™II + VARIVAX™ (Group 3) (Treatment B)	399	367	1971.8	195	171	2099.4	0.94 (0.86, 1.02)	<0.001*	Similar
M-M-R™II + VARIVAX™ (Group 3) (Treatment A) vs. M-M-R™II + placebo (Group 2) (Treatment B)	195	171	2099.4	205	185	2062.4	1.02 (0.92, 1.12)	<0.001*	Similar

Table 8.5.11 Comparison of mumps antibody responses following immunization at 4 to 6 years of age

MUMPS Comparison (Treatment A vs. Treatment B)	Treatment A			Treatment B			Estimated Fold Difference†‡(90% CI)†	One-Sided p-Values†	Conclusion
	N	N	Estimated GMT†	N	n	Estimated GMT†			
ProQuad™+ placebo (Group 1) (Treatment A) vs. M-M-R™II + placebo (Group 2) (Treatment B)	399	367	206.4	205	185	310.9	0.66 (0.59, 0.74)	<0.001*	Similar
ProQuad™+ placebo (Group 1) (Treatment A) vs. M-M-R™II + VARIVAX™ (Group 3) (Treatment B)	399	367	206.4	195	171	292.0	0.71 (0.63, 0.79)	<0.001*	Similar
M-M-R™II + VARIVAX™ (Group 3) (Treatment A) vs. M-M-R™II + placebo (Group 2) (Treatment B)	195	171	292.0	205	185	310.9	0.94 (0.82, 1.07)	<0.001*	Similar

Table 8.5.12 Comparison of rubella antibody responses following immunization at 4 to 6 years of age

RUBELLA **Comparison (Treatment A vs. Treatment B)**	**Treatment A**			**Treatment B**					
	N	**n**	**Estimated GMT†**	**N**	**n**	**Estimated GMT†**	**Estimated Fold Difference†‡ (90% CI)†**	**One-Sided p-Values†**	**Conclusion**
ProQuad™ + placebo (Group 1) (Treatment A) vs. M-M-R™II + placebo (Group 2) (Treatment B)	399	367	212.1	205	185	178.9	1.19 (1.07, 1.31)	<0.001*	Similar
ProQuad™ + placebo (Group 1) (Treatment A) vs. M-M-R™II + VARIVAX™ (Group 3) (Treatment B)	399	367	212.1	195	171	157.5	1.35 (1.21, 1.50)	<0.001*	Similar
M-M-R™II + VARIVAX™ (Group 3) (Treatment A) vs. M-M-R™II + placebo (Group 2) (Treatment B)	195	171	157.5	205	185	178.9	0.88 (0.78, 0.99)	<0.001*	Similar

Table 8.5.13 Comparison of varicella antibody responses after immunization at 4 to 6 years of age

VARICELLA Comparison (Treatment A vs. Treatment B)	**Treatment A**			**Treatment B**			**Estimated Fold Difference†‡ (90% CI)†**	**One-Sided p-Values†**	**Conclusion**
	N	**N**	**GMT†**	**N**	**n**	**GMT†**			
ProQuad™ + placebo (Group 1) (Treatment A) vs. M-M-R™II + VARIVAX™ (Group 3) (Treatment B)	399	367	317.0	195	171	212.4	1.49 (1.26, 1.76)	<0.001*	Similar

8.5.7.2.5 Additional immunogenicity endpoints that were evaluated included:

8.5.7.2.5.1 The data for the fold rise in GMTs from post dose 1 to post dose 2 are summarized in Table 8.5.14 below.

Table 8.5.14 Summary of Observed Fold Rise from Post Dose 1 to Post Dose 2 in Subjects who Received 2 Doses of ProQuad (Per Protocol Analysis)

			ProQuad™+ Placebo (Group 1) (N=399)		M-M-R™II + Placebo (Group 2) (N=205)		M-M-R™II + VARIVAX™ (Group 3) (N=195)	
Antigen	**Time Point**	**Parameter**	**n**	**Observed Response (95% CI)**	**n**	**Observed Response (95% CI)**	**n**	**Observed Response (95% CI)**
Measles	Pre vaccination	GMT (mIU/mL)	367	1634.9 (1472.2, 815.6)	185	1597.9 (1370.8, 1862.6)	171	1592.9 (1368.1,1854.7)
	6 weeks Post-vaccination	GMT (mIU/mL)	367	1985.9 (1817.6,2169.9)	185	2046.9 (1815.2, 2308.2)	171	2084.3 (1852.3, 2345.5)
		Geometric mean fold rise	367	1.21 (1.13, 1.30)	185	1.28 (1.17, 1.40)	171	1.31 (1.17, 1.46)
Mumps	Pre vaccination	GMT (ELISA Ab nits/mL)	367	84.9 (75.3, 95.7)	185	83.7 (69.8, 100.3)	171	88.1 (75.4, 102.9)
	6 weeks Post-vaccination	GMT (ELISA Ab units/mL)	367	206.0 (188.2, 225.4)	185	308.5 (269.6, 352.9)	171	295.9 (262.5, 333.5)
		Geometric mean fold rise	367	2.43 (2.19, 2.69)	185	3.69 (3.14, 4.32)	171	3.36 (2.84, 3.97)
Rubella	Pre vaccination	GMT (IU/mL)	367	72.4 (65.7, 79.8)	185	62.0 (53.4, 72.0)	171	62.4 (54.5, 71.3)
	6 weeks Post-vaccination	GMT (IU/mL)	367	217.3 (200.1, 236.0)	185	174.0 (157.3, 192.6)	171	154.1 (138.9, 170.9)
		Geometric mean fold rise	367	3.00 (2.72, 3.31)	185	2.81 (2.41, 3.27)	171	2.47 (2.17, 2.81)
Varicella	Pre vaccination	GMT (gpELISA units/mL)	367	25.9 (21.7, 31.0)	N/A		171	24.6 (19.1, 31.8)
	6 weeks Post-vaccination	GMT (gpELISA units/mL)	367	322.2 (278.9, 372.2)			171	209.3 (171.2, 255.9)
		Geometric mean fold rise	367	12.43 (10.63, 14.53)			171	8.50 (6.69, 10.81)

8.5.7.2.5.2 Comparisons of reverse cumulative distribution of post vaccination antibody titers also demonstrated that antibody titers were similar between groups (data not shown).

8.5.7.2.5.3 An analysis of all subjects with serology was consistent with the results of the per protocol analysis (data not shown).

8.5.7.3 **Safety endpoints.**

8.5.7.4 **Summary of Clinical Adverse Experiences:**

Overall, the proportion experiencing AEs in each group was similar with 77.6% of subjects in Group 1 reporting at least one AE compared with 78.0% in Group 2 and 75.6% of subjects in Group 3. Injection site reactions were reported in 50.6% of subjects in Group 1 at the ProQuad injection site, 40.8% of subjects in Group 2 at the MMRII injection site, in 42.0% of subjects in Group 3 at the MMRII injection site and in 40.4% at the VARIVAX injection site. In Group 1, 54.7% reported one or more systemic AEs while 60.0% of subjects in Group 2 and 59.1% of subjects in Group 3 reported systemic AEs after immunization.

There were no deaths in this study and no vaccine related serious adverse experiences in any group. There was one serious AE reported after ProQuad + placebo immunization but this was not judged to be vaccine related. No subjects discontinued the study due to an AE.

Clinical Adverse Experience reporting is summarized in Table 8.5.15 below:

Table 8.5.15 Summary of clinical adverse reactions reported following immunization with ProQuad plus placebo (Group 1), MMRII plus placebo (Group 2), or MMRII + VARIVAX (Group 3)

	ProQuad™ + Placebo (Group 1) N=399		**M-M-R™II + Placebo (Group 2) N=205**		**M-M-R™II + VARIVAX™ (Group 3) N=199**	
	n	(%)	n	(%)	N	(%)
Number of subjects vaccinated	399		205		195	
Subjects without follow-up	2		0		2	
Subjects with follow-up	397		205		193	
Number (%) of subjects:						
With no adverse experience	89	(22.4)	45	(22.0)	47	(24.4)
With one or more adverse experiences	308	(77.6)	160	(78.0)	146	(75.6)
Injection-site adverse experiences	223	(56.2)	104	(50.7)	99	(51.3)
Systemic adverse experiences	217	(54.7)	123	(60.0)	114	(59.1)
With vaccine-related† adverse experiences	231	(58.2)	110	(53.7)	105	(54.4)
Injection-site adverse experiences	223	(56.2)	103	(50.2)	99	(51.3)
Systemic adverse experiences	32	(8.1)	19	(9.3)	18	(9.3)
With serious adverse experiences	1	(0.3)	0	(0.0)	0	(0.0)
With serious vaccine-related adverse experiences	0	(0.0)	0	(0.0)	0	(0.0)
Who died	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued‡ due to an adverse experience	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a vaccine-related AE	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a serious adverse experience	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a serious vaccine-related AE	0	(0.0)	0	(0.0)	0	(0.0)

† Determined by the investigator to be possibly, probably, or definitely related to the vaccine. ‡ Discontinued = Subject discontinued from study. Percentages are calculated based on the number of subjects with follow-up after any visit. One (1) subject in this trial was incorrectly vaccinated. The subject (AN 25842) was randomized to Group 1 but inadvertently received 2 doses of diluent. This subject withdrew consent immediately after vaccination, and did not participate in the safety follow-up period. Therefore, there are no additional data from this subject; they are not included in this table. N = Number of subjects vaccinated. n = the number of subjects with one or more of the specified adverse experiences.

Risk differences and 95% CIs for clinical adverse reactions were used to compare reactogenicity of Group 1 to Group 2 and Group 1 to Group 3. All 95% confidence intervals of the risk differences contained 0 which demonstrated that the safety profile of Group1 was comparable to the safety profiles of Group 2 and Group 3. These comparisons are summarized in Table 8.5.16 below.

Table 8.5.16 Summary of the comparison of clinical adverse experiences between groups

	Comparison	Group A			Group B			Risk Difference† (Group A—Group B) Percentage Points (95% CI)†
		N	n	S (%)	N	n	S (%)	
With one or more adverse experiences	ProQuad™ + placebo vs. M-M-R™II + placebo	399	397	308 (77.6)	205	205	160 (78.0)	-0.5 (-7.2, 6.8)
	ProQuad™ + placebo vs. M-M-R™II + VARIVAX™	399	397	308 (77.6)	195	193	146 (75.6)	1.9 (-5.1, 9.5)
Injection-site adverse experiences	ProQuad™ + placebo vs. M-M-R™II + placebo	399	397	223 (56.2)	205	205	104 (50.7)	5.4 (-2.9, 13.8)
	ProQuad™ + placebo vs. M-M-R™II + VARIVAX™	399	397	223 (56.2)	195	193	99 (51.3)	4.9 (-3.7, 13.4)
Systemic adverse experiences	ProQuad™ + placebo vs. M-M-R™II + placebo	399	397	217 (54.7)	205	205	123 (60.0)	-5.3 (-13.5, 3.0)
	ProQuad™ + placebo vs. M-M-R™II + VARIVAX™	399	397	217 (54.7)	195	193	114 (59.1)	-4.4 (-12.8, 4.2)
With vaccine-related‡ adverse experiences	ProQuad™ + placebo vs. M-M-R™II + placebo	399	397	231 (58.2)	205	205	110 (53.7)	4.5 (-3.8, 12.9)
	ProQuad™ + placebo vs. M-M-R™II + VARIVAX™	399	397	231 (58.2)	195	193	105 (54.4)	3.8 (-4.7, 12.3)
Injection-site adverse experiences	ProQuad™ + placebo vs. M-M-R™II + placebo	399	397	223 (56.2)	205	205	103 (50.2)	5.9 (-2.5, 14.3)
	ProQuad™ + placebo vs. M-M-R™II + VARIVAX™	399	397	223 (56.2)	195	193	99 (51.3)	4.9 (-3.7, 13.4)
Systemic adverse experiences	ProQuad™ + placebo vs. M-M-R™II + placebo	399	397	32(8.1)	205	205	19(9.3)	-1.2 (-6.5, 3.3)
	ProQuad™ + placebo vs. M-M-R™II + VARIVAX™	399	397	32(8.1)	195	193	18(9.3)	-1.3 (-6.7, 3.3)

8.5.7.5 Adverse experiences in all randomized and vaccinated subjects were collected for 6 weeks (42 days) after immunization and included in the safety analysis. The safety profile of Group1 (ProQuad + placebo) was compared to both Group 2 (MMRII + placebo) and Group 3 (MMRII + VARIVAX).

Follow-up was obtained from 397 of 299 (99.5%) in Group 1, 205 of 205 (100%) in Group 2 and from 193 of 195 (99.0%) in Group 3.

8.5.7.6 **Serious Vaccine Related Adverse Reactions:**

The primary endpoint for safety was any observation of vaccine-related serious adverse reactions. No serious vaccine-related adverse reactions were expected

One child in the ProQuad plus placebo group had a serious adverse reaction after immunization. This subject (#20317) was a four year old female who developed pyelonephritis 20 days after immunization and presented with fever, vomiting and urinary incontinence and was admitted to the hospital for IV fluids and antibiotics. This AE was classified as definitely not related to the study vaccine.

8.5.7.7 Injection Site Reactions:

The most commonly reported injection site adverse experiences were injection site pain, erythema, and swelling in each vaccine group. The incidence rate for pain in Group 1 was 41.1% at the ProQuad site and 34.5% at the placebo site; in Group 2, the incidence was 36.6% at the MMRII injection site and 34.6% at the placebo site; in Group 3 the incidence of pain was 35.2% at the MMRII injection site and 36.8% at the VARIVAX injection site. The incidence rate for erythema in Group 1 was 24.7% at the ProQuad site and 13.4% at the placebo site; in Group 2, the incidence was 15.6% at the MMRII injection site and 14.1% at the placebo site; in Group 3 the incidence of erythema was 14.5% at the MMRII injection site and 16.1% at the VARIVAX injection site. The incidence rate for swelling in Group 1 was 15.6% at the ProQuad site and 8.1% at the placebo site; in Group 2, the incidence was 10.2% at the MMRII injection site and 8.8% at the placebo site; in Group 3 the incidence of pain was 7.8% at the MMRII injection site and 10.9% at the VARIVAX injection site.

Injection site reactions at the vaccine and at the placebo injection site for each group are summarized in Table 8.5.17 below:

Table 8.5.17. Summary of injection site reactions by vaccine group

	ProQuad™ + Placebo (Group 1)						**M-M-R™II + Placebo (Group 2)**						**M-M-R™II + VARIVAX™ (Group 3)**					
	ProQuad™ (N=399)			**Placebo (N=399)**			**M-M-R™II (N=205)**			**Placebo (N=205)**			**M-M-R™II (N=195)**			**VARIVAX™ (N=195)**		
	All Adverse Experiences		**VR**	**All Adverse Experiences**		**VR**	**All Adverse Experiences**		**VR**	**All Adverse Experiences**		**VR**	**All Adverse Experiences**		**VR**	**All Adverse Experiences**		**VR**
	n	**(%)**		**N**	**(%)**		**n**	**(%)**		**n**	**(%)**		**n**	**(%)**		**n**	**(%)**	
Number of subjects	399			399			205			205			195			195		
Subjects without follow-up	2			2			0			0			2			2		
Subjects with follow-up	397			397			205			205			193			193		
Number(%) of subjects with one or more injection site adverse experiences	201	(50.6)		162	(40.8)		94	(45.9)		86	(42.0)		76	(39.4)		78	(40.4)	
Injection-site bruising	14	(3.5)	14	15	(3.8)	15	5	(2.4)	5	7	(3.4)	7	3	(1.6)	3	4	(2.1)	4
Injection-site erythema	98	(24.7)	98	53	(13.4)	53	32	(15.6)	32	29	(14.1)	29	28	(14.5)	28	31	(16.1)	31
Injection-site nodule	0	(0.0)		0	(0.0)		0	(0.0)		0	(0.0)		0	(0.0)		2	(1.0)	2
Injection-site pain†	163	(41.1)	163	137	(34.5)	137	75	(36.6)	75	71	(34.6)	70	68	(35.2)	68	71	(36.8)	71
Injection-site pruritus	4	(1.0)	4	1	(0.3)	1	0	(0.0)		0	(0.0)		0	(0.0)		2	(1.0)	2
Injection-site rash	7	(1.8)	7	5	(1.3)	5	0	(0.0)		0	(0.0)		1	(0.5)	1	0	(0.0)	
Injection-site swelling	62	(15.6)	62	32	(8.1)	32	21	(10.2)	21	18	(8.8)	18	15	(7.8)	15	21	(10.9)	21

Analysis: Group 1 had a significantly higher incidence of erythema at the ProQuad injection site when compared to the MMRII injection site of group 2 (p = 0.012) and when compared to either the MMRII (p = 0.006) or the VARIVAX (p = 0.014) injection sites in Group 3 for Days 1 to 5 after vaccination. Group 1 also had a higher incidence of swelling when compared to the incidence at the MMRII injection site in Group 3 (p = 0.008). Erythema and swelling were of short duration (≤ 3 days) and were the smallest size (≤ 1 inch).

8.5.7.8 Systemic Adverse Reactions:

The most commonly reported systemic adverse reactions in Group 1 were nasopharyngitis (13.1%), cough (12.8%), pyrexia (10.3%), vomiting (5.8%), headache (5.5%), upper respiratory tract infection (4.5%), rhinorrhea (4.3%), diarrhea (3.5%), and otitis media (3.0%). In Group 2 the most common systemic AEs were nasopharyngitis (12.7%), pyrexia (9.8%), cough (8.8%), rhinorrhea (8.8%), nasal congestion (5.4%), upper respiratory tract infection (3.4%), vomiting (3.4%), pharyngitis (3.4%), and pharyngolaryngeal pain (3.4%). In Group 3 the most common systemic AEs were nasopharyngitis (13.5%), cough (10.9%), pyrexia (9.8%), headache (6.2%), otitis media (5.2%), upper respiratory tract infection (4.7%), vomiting (4.1%), pharyngeal pain (4.1%) and rhinorrhea (3.6%).

Systemic adverse reactions by body system for Group 1, Group 2 and Group 3 are summarized in Table 8.5.18 below:

Table 8.5.18 Summary of systemic adverse reactions by body system

	ProQuad™ + Placebo (Group 1) (N=399)			M-M-R™II + Placebo (Group 2) (N=205)			M-M-R™II + VARIVAX™ (Group 3)(N=195)		
	All Adverse Experiences		VR	All Adverse Experiences		VR	All Adverse Experiences		VR
	N	(%)	n	n	(%)	n	n	(%)	n
Number of subjects	399			205			195		
Subjects without follow-up Subjects with follow-up	2 397			0 205			2 193		
Number (%) of subjects with one or more systemic adverse experiences	217	(54.7)		123	(60.0)		114	(59.1)	
Number (%) of subjects with no systemic adverse experience	180	(45.3)		82	(40.0)		79	(40.9)	
Ear and Labyrinth Disorders	9	(2.3)		2	(1.0)	1	4	(2.1)	
Eye Disorders	7	(1.8)	1	4	(2.0)		6	(3.1)	1
Gastrointestinal Disorders	45	(11.3)	3	18	(8.8)	2	17	(8.8)	1
General Disorders and Injection Site AEs	54	(13.6)	11	29	(14.1)	5	23	(11.9)	8
Immune System Disorders	3	(0.8)		2	(1.0)	1	2	(1.0)	
Infections and Infestations	112	(28.2)	1	63	(30.7)	5	57	(29.5)	4
Injury, Poisoning and Procedural Complications	13	(3.3)	1	8	(3.9)		9	(4.7)	
Metabolism and Nutrition Disorders	4	(1.0)		0	(0.0)		1	(0.5)	
Musculoskeletal and Connective Tissue Disorders	13	(3.3)	1	3	(1.5)	1	2	(1.0)	
Nervous System Disorders	23	(5.8)	3	10	(4.9)	3	15	(7.8)	3
Psychiatric Disorders	10	(2.5)	4	3	(1.5)	1	6	(3.1)	2
Respiratory, Thoracic, and Mediastinal Disorders	79	(19.9)	10	43	(21.0)	3	41	(21.2)	2
Skin and Subcutaneous Tissue	30 (7.6)		6	15 (7.3)		1	17 (8.8)		2

Analysis: There were 126 comparisons of systemic AEs between Group 1 and Group 2 and between Group 1 and Group 3. Most (114 of 126) 95% confidence intervals of the risk differences contained 0, which suggests that the safety profiles of the vaccines are similar. In 11 comparisons, the incidence in Group 1 was lower than that seen in either Group 2 or Group 3. However, diarrhea occurred more frequently after ProQuad + placebo immunization than after MMRII + VARIVAX immunization. With as many as 126 comparisons it is expected that some incidence rates will be significantly different by chance alone. Each of the significant differences was small and there was no obvious pattern or noticeable trend toward a higher incidence in ProQuad recipients and the clinical significance of the differences is unknown.

Analysis of risk differences and 95% CIs by body system is summarized in Table 8.5.19 below:

Table 8.5.19 Comparison of systemic adverse reactions following ProQuad immunization versus MMRII or versus MMRII and VARIVAX

Study 014: ProQuad given at 4-6 years vs. MMRII or MMRII+ VARIVAX	Comparison ProQuad + Placebo (Group A) vs. Group B:	Group A			Group B			Risk Difference† (Group A—Group B) Percentage Points (95% CI)†
Ear and Labyrinth Disorders	M-M-R™II + placebo	399	397	9 (2.3)	205	205	2 (1.0)	1.3 (-1.4, 3.5)
	M-M-R™II + VARIVAX™	399	397	9 (2.3)	195	193	4 (2.1)	0.2 (-3.1, 2.6)
Eye Disorders	M-M-R™II + placebo	399	397	7 (1.8)	205	205	4 (2.0)	-0.2 (-3.3, 2.0)
	M-M-R™II + VARIVAX™	399	397	7 (1.8)	195	193	6 (3.1)	-1.3 (-5.0, 1.1)
Gastrointestinal Disorders	M-M-R™II + placebo	399	397	45 (11.3)	205	205	18 (8.8)	2.6 (-2.9, 7.3)
	M-M-R™II + VARIVAX™	399	397	45 (11.3)	195	193	17 (8.8)	2.5 (-3.0, 7.4)
General Disorders and	M-M-R™II + placebo	399	397	54 (13.6)	205	205	29 (14.1)	-0.5 (-6.8, 5.0)
	M-M-R™II + VARIVAX™	399	397	54 (13.6)	195	193	23 (11.9)	1.7 (-4.4, 7.1)
Immune System Disorders	M-M-R™II + placebo	399	397	3 (0.8)	205	205	2 (1.0)	-0.2 (-2.8, 1.4)
	M-M-R™II + VARIVAX™	399	397	3 (0.8)	195	193	2 (1.0)	-0.3 (-3.0,1.3)
Infections and Infestations	M-M-R™II + placebo	399	397	112 (28.2)	205	205	63 (30.7)	-2.5 (-10.4, 5.0)
	MMRII +VARIVAX	399	397	112 (28.2)	195	193	57 (29.5)	-1.3 (-9.3,6.3)
Injury, Poisoning and Procedural Complications	M-M-R™II + placebo	399	397	13 (3.3)	205	205	8 (3.9)	-0.6 (-4.5, 2.3)
	M-M-R™II + VARIVAX™	399	397	13 (3.3)	195	193	9 (4.7)	-1.4 (-5.6, 1.8)
Metabolism and Nutrition	M-M-R™II + placebo	399	397	4 (1.0)	205	205	0 (0.0)	1.0 (-0.8, 2.6)
	M-M-R™II + VARIVAX™	399	397	4 (1.0)	195	193	1 (0.5)	0.5 (-1.9, 2.1)
Musculoskeletal and Connective Tissue	M-M-R™II + placebo	399	397	13 (3.3)	205	205	3 (1.5)	1.8 (-1.2, 4.3)
	M-M-R™II + VARIVAX™	399	397	13 (3.3)	195	193	2 (1.0)	2.2 (-0.7, 4.7)
Nervous System Disorders	M-M-R™II + placebo	399	397	23 (5.8)	205	205	10 (4.9)	0.9 (-3.4, 4.5)
	M-M-R™II + VARIVAX™	399	397	23 (5.8)	195	193	15 (7.8)	-2.0 (-7.0, 2.1)
Psychiatric Disorders	M-M-R™II + placebo	399	397	10 (2.5)	205	205	3 (1.5)	1.1 (-1.9, 3.4)
	M-M-R™II + VARIVAX™	399	397	10 (2.5)	195	193	6 (3.1)	-0.6 (-4.3, 2.1)
Respiratory, Thoracic and Mediastinal AEs	M-M-R™II + placebo	399	397	79 (19.9)	205	205	43 (21.0)	-1.1 (-8.2, 5.5)
	M-M-R™II + VARIVAX™	399	397	79 (19.9)	195	193	41 (21.2)	-1.3 (-8.6, 5.4)
Skin and Subcutaneous Tissue	M-M-R™II + placebo	399	397	30 (7.6)	205	205	15 (7.3)	0.2 (-4.7, 4.4)
	M-M-R™II + VARIVAX™	399	397	30 (7.6)	195	193	17 (8.8)	-1.3 (-6.6, 3.2)

8.5.7.9 Fever:

On days 1 to 42 post immunization similar percentages of children reported fever ≥102 F in each group: 10.2% in Group 1, 9.9% in Group 2 and 9.4% in Group 3. Two additional children had fever ≥102 F on day 43 post immunization (one in Group 1 and one in Group 3). The investigators caring for these two children felt that their fevers were not due to prior vaccination and these two cases were not counted in the total reported above. Fevers were of short duration and the average fever episode lasted 1.5 days in Group 1, 1.4 days in Group 2, and 1.6 days in Group 3.

8.5.7.10 Measles-Like Rash:

In this study, the overall incidence of measles-like rash was low, with <1% in each treatment group. Incidence rates were similar when Group 1 was compared to Group 2 or to Group 3. Only two measles rashes were reported with one in Group 1 and one in Group 3. No blood samples for RT-PCR testing for measles genome were provided from subjects with measles-like rashes.

8.5.7.11 Varicella Rash:

In this study the overall incidence of varicella-like rashes were low, with < 1% in each treatment group. Incidence rates were similar when Group 1 was compared to Group 2 or to Group 3. Only one culture was obtained for --- testing and this sample was inadequate for testing.

8.5.8 Comments & Conclusions:

ProQuad may be administered in place of a second dose of MMRII alone or in place of MMRII + VARIVAX based on the following:

8.5.8.1 The immunogenicity data demonstrated non-inferiority in antibody responses to measles, mumps, and rubella 6 weeks post vaccination between the group that received ProQuad + placebo and the group immunized with MMRII plus placebo.

8.5.8.2 The immunogenicity data demonstrated non-inferiority in antibody responses to measles, mumps, rubella, and varicella 6 weeks post-vaccination between the group that received ProQuad + placebo and the group immunized with MMRII plus VARIVAX.

8.5.8.3 Non-inferiority was based on a comparison of GMTs post immunization and excluded the two fold or greater decrease in antibody responses after ProQuad for each vaccine antigen.

8.5.8.4 Seropositivity rates after ProQuad immunization were very high and at nearly 100% for measles, mumps, and rubella and >98% for varicella following a dose at 4 to 6 years.

8.5.8.5 There were no deaths or vaccine related serious AEs after ProQuad immunization at 4-6 years of age. Rates of non-serious AEs after ProQuad immunization at 4-6 years of age were similar to the incidence seen in the control groups. There was one exception: diarrhea occurred significantly more frequently after ProQuad immunization than after MMRII + VARIVAX. However, the incidence was not increased when the rate after ProQuad was compared with the rate after MMRII plus placebo.

8.5.8.6 These data support the use of ProQuad in place of MMRII at 4 to 6 years of age.

9 Overview of Efficacy Across Trials

9.1 Indication # I: ProQuad™ is indicated for simultaneous vaccination against measles, mumps, rubella, and varicella in children 12 months to 12 years of age.

9.1.1 Methods:

This overview of product efficacy summarizes serological data from children seronegative for each respective vaccine antigen which were derived from clinical trials 009, 011, 012 and 013 that evaluated ProQuad™ formulated to contain varicella at a potency ≥3.97 $\log_{10}$ PFU/dose.

9.1.2 General Discussion of Efficacy Endpoints:

Clinical efficacy of ProQuad™ is inferred from studies performed using the monovalent components. In these studies, there was a good correlation between clinical efficacy and the presence of virus specific antibody. For example, in the studies that demonstrated protection against measles disease, there was a good correlation with the presence of measles -- or neutralizing antibody. In studies that demonstrated efficacy of mumps vaccine there was a good correlation with the presence of mumps neutralizing antibody. Likewise in the studies performed in support of licensure of RA27/3 rubella vaccine strain there was a good correlation between clinical efficacy and the presence of serum rubella --- antibody. Protection against clinical varicella disease has been correlated with varicella antibody titers that are ≥5 gpELISA units. The varicella gpELISA used to assess antibody responses following ProQuad is the same assay used to assess antibody responses in previous studies of VARIVAX. Merck also demonstrated a good correlation between measles -- antibody, mumps neutralizing antibody and rubella -- antibody with each respective ELISA. These studies indicated that the ELISAs used to assess antibody response to each of the vaccine antigens in ProQuad would parallel responses that correlated with protection in other studies.

ELISA assays used to evaluate immunogenicity of ProQuad™ were validated by Merck Research Laboratories. Please see each clinical study or Mr. Steven Rubin's review for additional information pertaining to the validation of these assays.

Antibody response rates and GMTs ~6 weeks after vaccination were used as the primary serologic endpoints in each study. Statistical analyses were based on comparisons of antibody responses in the initially seronegative population. The response rate for varicella was defined as the percent of subjects with a post-vaccination VZV antibody titer ≥ 5gpELISA units/mL. The response rate for measles was defined as ≥207.5 mIU/mL for studies 009, 011 and ≥120mIU for studies 012 and 013 and 014. However, the summary data for measles antibody responses used 255mIU/mL as the cut-off. In studies 009 and 011 a mumps ELISA used the vaccine strain as antigen while studies 012, 013 and 014 used an ELISA based on wild type mumps antigen as requested by CBER. The response rate for mumps was the percent of subjects with post vaccination mumps titers ≥ 10 ELISA units in all studies. The response rate for rubella was defined as the percent of subjects with rubella antibody titers ≥ 10 IU/mL in all studies.

9.1.3 **Study Design**:

Studies 009, 011 and 012 were partially blinded, randomized, controlled, multi-center studies. Study 013 was an open label, randomized controlled multi-center study. Children enrolled in these studies were 12 to 23 months of age and in good health. Parents and guardians provided signed, informed consent for children who met the pre-specified inclusion/exclusion criteria prior to randomization to vaccine groups and prior to assignment of allocation numbers from computerized lists generated at Merck which were provided to the study sites. Children were immunized with the study vaccine(s) on the day of randomization and then followed for 42 days after each dose. Serum samples were obtained prior to vaccination and 6 weeks after. Parents or guardians filled out vaccination report cards for 42 days after each live virus vaccination. In study 013, children were followed for an additional 14 days after receipt of inactivated vaccines. Parents and guardians were required to note local and systemic adverse reactions and record temperatures for 42 days and note any exposures to measles, mumps, rubella or varicella (chickenpox and/or shingles). They were to contact study personnel in the event of any serious adverse reactions. Study personnel also evaluated children with measles like rash and varicella lesions. Blood samples were obtained for RT-PCR if measles like rashes were observed and pox lesions were cultured and/or tested by --- for varicella genome after additional informed consent was obtained for these studies.

9.1.4 Efficacy Findings:

Immunogenicity of a Single Dose of ProQuad™

A total of 5446 subjects 12 to 23 months of age were immunized with ProQuad containing a varicella dose ≥3.97 log 10 PFU. In study 009, 011 and 012 other vaccines were not given. In study 013, Tripedia and Comvax were given concomitantly to some children immunized with ProQuad. The combined immunogenicity results for 12- to 23-month-old subjects 6 weeks following administration of a primary dose of ProQuad™ containing a varicella virus dose ≥3.97 $\log_{10}$ PFU are presented in Table 1a, 1b and Table 2. The immunogenicity data from the recipients of M-M-R™II and VARIVAX™ at 6 weeks following the concomitant administration of the 2 vaccines at separate injection sites are also presented. Evaluation of the response to a primary dose of vaccine was used to determine the equivalence of the immune response to all 4 antigens between recipients of ProQuad™ and recipients of M-M-R™II and VARIVAX™. The results demonstrate that 1 dose of ProQuad™ containing a varicella virus dose ≥3.97 $\log_{10}$ PFU in 12- to 23-month-old initially seronegative subjects is highly immunogenic with response rates 6 weeks post-vaccination of 91.2% for varicella, 97.4% for measles, 98.8% for mumps (vaccine strain ELISA), 95.8% for mumps (wild-type mumps virus ELISA), and 98.5% for rubella. Protocols 009, 011, and 012 demonstrated the similarity of ProQuad™ to M-M-R™II and VARIVAX™ using pre-planned objectives, hypotheses, and statistical methods. The immune responses for each of the 4 antigens from each individual study in 12- to 23-month-old subjects are summarized in Table 9.1.1a and 9.1.1b. Information on the overall immunogenicity results is presented in Table 9.1.2.

Table 9.1.1a Individual Study Immunogenicity Results (Seroresponse Rates) 6 Weeks following the Primary Dose in 12 to 23 month Old Subjects (Per-Protocol Population) (Protocols 009, 011, 012 and 013)

			MEASLES	MUMPS	RUBELLA	VARICELLA
Study	**Group**	**N**	**Observed Response Rate (95% CI)**	**Observed Response Rate (95% CI)**	**Observed Response Rate (95% CI)**	**Observed Response Rate (95% CI)**
009	ProQuad™	323	95.7% (92.8%, 97.7%)	99.0% (97.1%, 99.8%)	95.1% (92.0%, 97.2%)	91.0% (87.1%, 94.1%)
	M-M-R™II + VARIVAX™	157	100.0% (97.6%, 100%)	98.7% (95.3%, 99.8%)	92.8% (87.5%, 96.4%)	92.4% (86.8%, 96.2%)
011	ProQuad™ (Middle Dose)	393	98.3% (96.3%, 99.4%)	99.1% (97.5%, 99.8%)	98.9% (97.1%, 99.7%)	80.8% (76.0%, 85.0%)
	ProQuad™ (High Dose)	381	99.1% (97.4%, 99.8%)	98.2% (96.1%, 99.3%)	97.9% (95.8%, 99.2%)	88.6% (84.5%, 91.9%)
	M-M-R™II + VARIVAX™	390	98.9% (97.2%, 99.7%)	99.7% (98.4%, 100%)	98.6% (96.8%, 99.5%)	93.1% (89.8%, 95.6%)
012	ProQuad™ (Lot 1)	985	97.9% (96.7%, 98.7%)	96.4% (94.9%, 97.5%)	99.0% (98.0%, 99.5%)	91.8% (89.6%, 93.6%)
	ProQuad™ (Lot 2)	968	97.3% (96.0%, 98.3%)	96.7% (95.3%, 97.8%)	98.3% (97.2%, 99.1%)	94.3% (92.5%, 95.9%)
	ProQuad™ (Lot 3)	962	96.1% (94.6%, 97.3%)	94.9% (93.2%, 96.3%)	99.0% (98.1%, 99.6%)	94.5% (92.6%, 96.0%)
	M-M-R™II + VARIVAX™	1012	97.7% (96.4%, 98.6%)	97.9% (96.8%, 98.8%)	99.2% (98.3%, 99.7%)	95.0% (93.2%, 96.4%)
013	ProQuad™ Concomitant	949	97.2% (95.8%, 98.2%)	95.4% (93.8%, 96.8%)	98.6% (97.5%, 99.2%)	89.7% (87.3%, 91.8%)
	ProQuad™ Nonconcomitant	485	98.8% (97.2%, 99.6%)	95.2% (92.7%, 97.0%)	99.3% (97.9%, 99.9%)	90.9% (87.5%, 93.6%)
	M-M-R™II + VARIVAX™	479	97.9% (94.0%, 99.6%)	98.6% (95.1%, 99.8%)	100.0% (97.5%, 100%)	93.5% (88.1%, 97.0%)

Table 9.1.1b Individual Study Immunogenicity Results (GMTs) 6 Weeks Following the Primary Dose In 12 to 23 month Old Subjects (Per-Protocol Population) (Protocols 009, 011, 012 and 013)

Study	Group	N	MEASLES Observed GMT (95% CI)	MUMPS Observed GMT (95% CI)	RUBELLA Observed GMT (95% CI)	VARICELLA Observed GMT (95% CI)
009	ProQuad™	323	2797.1 (2506.8, 3121.1)	94.5 (83.1, 107.4)	83.0 (74.9, 92.0)	12.7 (11.5, 13.9)
	M-M-R™II + VARIVAX™	157	2004.2 (1770.3, 2268.9)	68.1 (57.0, 81.4)	79.6 (67.6, 93.7)	13.0 (11.4, 14.7)
011	ProQuad™ (Middle Dose)	393	3014.7 (2728.2, 3331.3)	106.3 (94.4, 119.8)	96.1 (86.9, 106.3)	10.5 (9.4, 11.7)
	ProQuad™ (High Dose)	381	3090.2 (2804.9, 3404.5)	114.7 (101.3, 130.0)	90.1 (81.8, 99.3)	11.9 (10.8, 13.1)
	M-M-R™II + VARIVAX™	390	2478.0 (2256.9, 2720.7)	97.4 (87.5, 108.5)	100.5 (91.3, 110.8)	16.5 (15.1, 18.1)
012	ProQuad™ (Lot 1)	985	3065.8 (2874.3, 3270.0)	100.5 (94.3, 107.2)	88.7 (84.0, 93.6)	16.0 (14.9, 17.2)
	ProQuad™ (Lot 2)	968	3015.1 (2818.3, 3225.7)	102.3 (96.0, 109.0)	89.7 (84.6, 95.2)	18.4 (17.2, 19.7)
	ProQuad™ (Lot 3)	962	2875.2 (2678.6, 3086.3)	85.6 (79.9, 91.8)	89.2 (84.4, 94.3)	19.7 (18.4, 21.0)
	M-M-R™II + VARIVAX™	1012	2138.3 (2007.2, 2277.9)	89.7 (84.7, 94.9)	103.6 (98.2, 109.4)	17.6 (16.6, 18.7)
013	ProQuad™ Concomitant	949	3560.7 (3328.2, 3809.4)	89.4 (83.5, 95.7)	98.7 (92.8, 105.0)	13.8 (12.8, 14.8)
	ProQuad™ Nonconcomitant	485	3601.8 (3296.3, 3935.7)	84.1 (76.2, 92.8)	99.9 (91.8, 108.7)	15.4 (13.8, 17.0)
	M-M-R™II + VARIVAX™	479	2582.1 (2224.6, 2997.0)	98.1 (85.7, 112.3)	126.3 (111.9, 142.6)	15.8 (13.8, 18.0)

Table 9.1.2 Summary of Combined Immunogenicity Results 6 weeks Following the Administration of a Primary Dose of ProQuad (varicella potency ≥3.97 log 10 PFU/dose) or MMRII and VARIVAX in the Per Protocol Population (Protocols 009, 011,012 and 013 Combined)

Group	Antigen	N†	n	Observed Response Rate (95% CI)	Observed GMT (95% CI)
ProQuad™	Varicella	5446	4381	91.2 % (90.3%, 92.0%)	15.5 (15.0, 15.9)
	Measles	5446	4733	97.4% (96.9%, 97.9%)	3124.9 (3038.9, 3213.3)
	Mumps (OD cutoff)‡	1097	973	98.8% (97.9%, 99.4%)	105.3 (98.0, 113.1)
	Mumps (wild-type ELISA)‡	4349	3735	95.8% (95.1%, 96.4%)	93.1 (90.2, 96.0)
	Rubella	5446	4773	98.5% (98.1%, 98.8%)	91.8 (89.6, 94.1)
M-M-R™II + VARIVAX™	Varicella	2038	1417	94.1% (92.8%, 95.3%)	16.6 (15.9, 17.4)
	Measles	2038	1516	98.2% (97.4%, 98.8%)	2239.6 (2138.3, 2345.6)
	Mumps (OD cutoff)‡	547	501	99.4% (98.3%, 99.9%)	87.5 (79.7, 96.0)
	Mumps (wild-type ELISA)‡	1491	1017	98.0% (97.0%, 98.8%)	90.8 (86.2, 95.7)
	Rubella	2038	1528	98.5% (97.7%, 99.0%)	102.2 (97.8, 106.7)

† Includes ProQuad™ + Placebo followed by ProQuad™ (Visit 1) (Protocol 009), ProQuad™ Middle and High Doses (Visit 1) (Protocol 011), ProQuad™ (Lot 1, Lot 2, Lot 3) (Protocol 012), both the Concomitant and Nonconcomitant groups (Protocol 013).
‡ The mumps antibody response was assessed by a vaccine-strain ELISA in Protocols 009 and 011 and by a wild-type ELISA in Protocols 012 and 013. In the former assay, the serostatus was based on the OD cutoff of the assay. In the latter assay, 10 mumps ELISA units was used as the serostatus cutoff.
The response rate for varicella is the percent of subjects with a baseline VZV antibody titer <1.25 gpELISA units/mL who had a post-vaccination VZV antibody titer ≥5 gpELISA units/mL.
The response rate for measles is the percent of subjects with a baseline antibody titer <255 mIU/mL who had a post-vaccination measles antibody titer ≥255 mIU/mL.
The response rate for mumps (OD cutoff) is the percent of subjects with a baseline mumps antibody titer below the OD cutoff who had a post-vaccination mumps antibody titer above the OD cutoff.
The response rate for mumps (wild-type ELISA) is the percent of subjects with a baseline mumps antibody titer <10 ELISA units who had a post-vaccination mumps antibody titer ≥10 ELISA units.
The response rate for rubella is the percent of subjects with a baseline rubella antibody titer <10 IU/mL who had a post-vaccination rubella antibody titer ≥10 IU/mL. N = Number of subjects vaccinated with a primary dose of ProQuad™ containing a varicella virus dose ≥3.97 log10 PFU.
n = Number of per-protocol subjects with evaluable serology.
CI = Confidence interval.
GMT = Geometric mean titer.
gpELISA = Glycoprotein enzyme-linked immunosorbent assay.
ELISA = Enzyme-linked immunosorbent assay.
PFU = Plaque-forming units.
OD = Optical density.
VZV = Varicella-zoster virus.

Immunogenicity of a Second Dose of ProQuad™

A subset of 1035, 12 to 23 month old children received a second dose of ProQuad containing a varicella dose ≥3.97 log 10 PFU at least 3 months after the first dose.

In Protocols 009 and 011, a total of 1097 recipients of ProQuad™ received a second dose of ProQuad™ 3 months after the first dose (at ~15 months of age). The combined immunogenicity results for these subjects 6 weeks following administration of a first and second dose of ProQuad™ (containing a varicella virus dose ≥3.97 log10 PFU) for each of the 4 antigens are presented in Table 9.1.3. Detailed summaries can be found in Table 9.1.3 and the individual case study reports (CSRs) for Protocols 009 and 011. For measles, mumps, and rubella, the response rates remained above 98% and the GMTs increased 1.7-fold to 2.4-fold in subjects who received a second dose of ProQuad™ ~3 months following the primary dose. The varicella responses increased significantly from 86.6% after 1 dose to 99.4% after 2 doses, with up to an approximate 41-fold increase in GMTs after 2 doses. These data are summarized in Table 9.1.3 below.

Table 9.1.3 Summary of Immune Responses to a First and Second Dose of ProQuad™ in Subjects who Received ProQuad™ with a Varicella Virus Dose ≥3.97log$_{10}$ PFU in Protocols 009 and 011 (per protocol analysis).

Antigen	Time Point	Serostatus Cutoff/ Response Criteria	N	n	Observed Response Rate (95% CI)		Observed GMT (95% CI)
Measles	Post Dose 1	≥120 mIU/mL	1097	915	98.1%	(97.0%, 98.9%)	2956.8 (2786.3, 3137.7)
		≥255 mIU/mL	1097	943	97.8%	(96.6%, 98.6%)	2966.0 (2793.4, 3149.2)
	Post Dose 2	≥120 mIU/mL	1097	915	99.5%	(98.7%, 99.8%)	5958.0 (5518.9, 6432.1)
		≥255 mIU/mL	1097	943	99.4%	(98.6%, 99.8%)	5919.3 (5486.2, 6386.6)
Mumps	Post Dose 1	≥OD Cutoff (ELISA antibody units)	1097	920	98.7%	(97.7%, 99.3%)	106.7 (99.1, 114.8)
	Post Dose 2	≥OD Cutoff (ELISA antibody units)	1097	920	99.9%	(99.4%, 100%)	253.1 (237.9, 269.2)
Rubella	Post Dose 1	≥10 IU/mL	1097	937	97.7%	(96.5%, 98.5%)	91.1 (85.9, 96.6)
	Post Dose 2		1097	937	98.3%	(97.2%, 99.0%)	158.8 (149.1, 169.2)
Varicella	Post Dose 1	<1.25 to ≥5 gpELISA units	1097	864	86.6%	(84.1%, 88.8%)	11.6 (10.9,12.3)
		≥OD Cutoff (gpELISA units)	1097	695	87.2%	(84.5%, 89.6%)	11.6 (10.9,12.4)
	Post Dose 2	<1.25 to ≥5 gpELISA units	1097	864	99.4%	(98.7%, 99.8%)	477.5 (437.8, 520.7)
		≥OD Cutoff (gpELISA units)	1097	695	99.4%	(98.5%, 99.8%)	478.7 (434.8, 527.1)

Includes the following treatment groups: ProQuad™ + Placebo followed by ProQuad™ (Visit 1) (Protocol 009) and ProQuad™ (Middle and High Dose) (Protocol 011). Samples from Protocols 009 and 011 were assayed in the legacy format Measles ELISA, which reported antibody titers in Measles ELISA units. To convert titers from ELISA units to mIU/mL, titers for these 2 protocols were divided by 0.1025. The lowest measurable titer post-vaccination is 207.5mIU/mL. The response rate for measles in the legacy format is the percent of subjects with a negative baseline measles antibody titer, as defined by the optical density (OD) cutoff, with a post-vaccination measles antibody titer ≥207.5 mIU/mL. Samples from Protocols 009 and 011 were assayed in the legacy format Rubella ELISA, which reported antibody titers in Rubella ELISA units. To convert titers from ELISA units to IU/mL, titers for these 2 protocols were divided by 1.28. ProQuad™ (Middle Dose) = ProQuad™ containing a varicella virus dose of 3.97 log10PFU. ProQuad™ (High Dose) = ProQuad™ containing a varicella virus dose of 4.25 log10PFU. ELISA = Enzyme-linked immunosorbent assay. gpELISA = Glycoprotein enzyme-linked immunosorbent assay. N = Number vaccinated at baseline. n = Number of subjects who were per-protocol Post dose 1 and Post dose 2 and satisfied the given pre-vaccination serostatus cutoff. CI = Confidence interval. GMT = Geometric mean titer. PFU = Plaque-forming units.

Antibody Persistence after ProQuad™ Immunization

Persistence of measles, mumps, rubella, and VZV antibodies 1 year post-vaccination was evaluated in Protocol 012, the Consistency Lots Study, in all subjects who received ProQuad™ and compared to antibody persistence in subjects immunized with M-M-RII™ and VARIVAX™. Samples to assess antibody persistence samples were obtained on over 2200 subjects. The measles, mumps, rubella, and VZV antibody results for these subjects are shown in Table 9.1.4 below. The results indicate that among subjects who had initially seroconverted 6 weeks post-vaccination, antibody to each of the 4 antigens persisted for at least 1 year, the longest period tested to date, in >96% of the subjects. The persistence of antibody in recipients of ProQuad™ was generally comparable to the persistence of antibody in subjects who received M-M-R™ II and VARIVAX™. (See Table 9.1.4)

Table 9.1.4 Persistence of Measles, Mumps, Rubella and VZV Antibody in Initially Seronegative Healthy Subjects 1Year Post Vaccination in Subjects Who Responded at 6 Weeks Post Vaccination (Protocol 012).

Antigen	Parameter	ProQuad™ (Combined Lots)	M-M-R™II + VARIVAX™
Measles†‡	Antibody persistence rate (95% CI)	98.9% (1722/1741) (98.3%, 99.3%)	98.7% (597/605) (97.4%, 99.4%)
	1-Year GMT (95% CI)	3652.9 (3493.0, 3820.2)	2222.9 (2071.0, 2386.0)
Mumps†§	Antibody persistence rate (95% CI)	96.7% (1676/1733) (95.8%, 97.5%)	96.2% (587/610) (94.4%, 97.6%)
	1-Year GMT (95% CI)	105.0 (99.5, 110.8)	84.1 (77.0, 91.9)
Rubella†§	Antibody persistence rate (95% CI)	99.6% (1796/1804) (99.1%, 99.8%)	99.5% (620/623) (98.6%, 99.9%)
	1-Year GMT (95% CI)	126.2 (121.2, 131.4)	159.0 (148.5, 170.2)
Varicella‖	Antibody persistence rate (95% CI) 1-Year GMT (95% CI)	97.5% (1512/1550) (96.7%, 98.3%) 42.7 (40.3, 45.4)	97.5% (537/551) (95.8%, 98.6%) 37.6 (34.0, 41.7)

9.1.5 Efficacy Conclusions for Indication #1

The immunogenicity data from the Phase II and III clinical trials of ProQuad™ support the following conclusions:

1. A single dose of ProQuad™ is highly immunogenic. The immune response to ProQuad™ is similar (non-inferior) to that obtained following administration of the component vaccines given by concomitant administration of M-M-R™II and VARIVAX™ at separate injection sites.

2. The minimum clinically acceptable dose of varicella virus in ProQuad™ is 3.97 $\log_{10}$ PFU. The immune response in terms of percent of subjects with a VZV antibody titer ≥5 gpELISA units/mL with this dose is comparable to the response obtained with VARIVAX™ (at end-expiry). The minimum clinically acceptable doses for the measles, mumps, and rubella components of ProQuad™ will be the same as those for M-M-R™II because the same doses of these 3 components in ProQuad™ resulted in similar measles, mumps, and rubella immune responses between recipients of ProQuad™ and recipients of M-M-R™II.

3. Manufacturing consistency was confirmed in clinical studies through the evaluation of 3 consistency lots of ProQuad™, all of which were shown to be similar to each other, as well as to the concomitant administration (at separate injection sites) of M-M-R™II and VARIVAX™ in terms of the immune responses to each of the 4 antigens in ProQuad™.

4. ProQuad™ may be administered concomitantly with COMVAX™ with no negative effect on the immune responses for either product.

9.2 Indication # 2: ProQuad™ may be used in children 12 months to 12 years of age if a second dose of measles, mumps, and rubella vaccine is to be administered.

9.2.1 Methods

In Protocol 014, 399 subjects 4 to 6 years old received ProQuad as their second dose of measles containing vaccine or a second dose of routinely recommended M-M-R™II and VARIVAX™. Current childhood immunization guidelines in the United States and many other countries recommend a second dose of a measles-containing vaccine (preferably M-M-R™II) prior to school entry (usually at 4 to 6 years of age) to ensure immunity to measles. Subjects in Protocol 014 had previously received a primary dose of M-M-R™II and VARIVAX™.

9.2.2 General Discussion of Efficacy Endpoints:

Immunogenicity endpoints were the same as those described above under Indication 1. Pre and post-vaccination antibody responses were evaluated using ELISAs specific for each vaccine component as described above.

9.2.3 Study Design

Study 014 was a randomized, double-blinded, multi-center study. Children were randomized 2:1:1 to receive ProQuad™ plus placebo given concomitantly but in separate arms, MMRII ™ plus placebo given concomitantly but in separate arms or MMRII ™ plus VARIVAX ™ given concomitantly but in separate arms. For additional details regarding the study design see the review of Study 014 in Section 8.

9.2.4 Efficacy Findings:

Non-inferiority was established for all assessments as measured by comparison of GMTs, adjusted for pre-dose titer and stratified by primary vaccination history, between subjects who received ProQuad™ and subjects who received MMR™II alone or concomitantly with VARIVAX™ at separate injection sites. Protocol 014 demonstrated that ProQuad™ could be administered in place of this second dose of M-M-R™II (alone or concomitantly with VARIVAX™) in 4- to 6-year-old children. These data are summarized in Table 9.2.1.

Table 9.2.1 Summary of Immune Response to a First and Second Dose of ProQuad in Subject Who Received ProQuad with a Varicella Virus Dose ≥3.97 log_{10} PFU in Protocols 009 and 011 (Per Protocol Analysis)

Antigen	Time Point	Serostatus Cutoff/ Response Criteria	N	n	Observed Response Rate (95% CI)		Observed GMT (95% CI)
Measles	Post Dose 1	≥120 mIU/mL	1097	915	98.1%	(97.0%, 98.9%)	2956.8 (2786.3, 3137.7)
		≥255 mIU/mL	1097	943	97.8%	(96.6%, 98.6%)	2966.0 (2793.4, 3149.2)
	Post Dose 2	≥120 mIU/mL	1097	915	99.5%	(98.7%, 99.8%)	5958.0 (5518.9, 6432.1)
		≥255 mIU/mL	1097	943	99.4%	(98.6%, 99.8%)	5919.3 (5486.2, 6386.6)
Mumps	Post Dose 1	≥OD Cutoff (ELISA antibody units)	1097	920	98.7%	(97.7%, 99.3%)	106.7 (99.1, 114.8)
	Post Dose 2	≥OD Cutoff (ELISA antibody units)	1097	920	99.9%	(99.4%, 100%)	253.1 (237.9, 269.2)
Rubella	Post Dose 1	≥ 10 IU/mL	1097	937	97.7%	(96.5%, 98.5%)	91.1 (85.9, 96.6)
	Post Dose 2	≥ 10 IU/mL	1097	937	98.3%	(97.2%, 99.0%)	158.8 (149.1, 169.2)
Varicella	Post Dose 1	<1.25 to ≥5 gpELISA units)	1097	864	86.6%	(84.1%, 88.8%)	11.6 (10.9,12.3)
		≥OD Cutoff (gpELISA units)	1097	695	87.2%	(84.5%, 89.6%)	11.6 (10.9, 12.4)
	Post Dose 2	<1.25 to ≥5 gpELISA units)	1097	864	99.4%	(98.7%, 99.8%)	477.5 (437.8, 520.7)
		≥OD Cutoff (gpELISA units)	1097	695	99.4%	(98.5%, 99.8%)	478.7 (434.8, 527.1)

Includes the following treatment groups: ProQuad™ + Placebo followed by ProQuad™ (Visit 1) (Protocol 009) and ProQuad™ (Middle and High Dose) (Protocol 011).
Samples from Protocols 009 and 011 were assayed in the legacy format Measles ELISA, which reported antibody titers in Measles ELISA units. To convert titers from ELISA units to mIU/mL, titers for these 2 protocols were divided by 0.1025. The lowest measurable titer post-vaccination is 207.5mIU/mL. The response rate for measles in the legacy format is the percent of subjects with a negative baseline measles antibody titer, as defined by the optical density (OD) cutoff, with a post-vaccination measles antibody titer ≥207.5 mIU/mL.
Samples from Protocols 009 and 011 were assayed in the legacy format Rubella ELISA, which reported antibody titers in Rubella ELISA units. To convert titers from ELISA units to IU/mL, titers for these 2 protocols were divided by 1.28.
ProQuad™ (Middle Dose) = ProQuad™ containing a varicella virus dose of 3.97 log10PFU.
ProQuad™ (High Dose) = ProQuad™ containing a varicella virus dose of 4.25 log10PFU.
ELISA = Enzyme-linked immunosorbent assay. gpELISA = Glycoprotein enzyme-linked immunosorbent assay.
N = Number vaccinated at baseline.
n = Number of subjects who were per-protocol Post dose 1 and Post dose 2 and satisfied the given pre-vaccination serostatus cutoff.
CI = Confidence interval. GMT = Geometric mean titer. PFU = Plaque-forming units.

9.2.5 Efficacy Conclusions for Indication #2

1. ProQuad™ may be used to provide a second dose of measles, mumps, or rubella vaccine to children. Administration of ProQuad™ following either a primary dose of ProQuad™ or following receipt of the primary doses of MMR™II and VARIVAX™ results in a significant increase in the VZV antibody response.

10 Overview of Safety across Trials

10.1 Safety Database

This section summarizes the safety data for ProQuad from the 4 randomized trials performed in 12 to 23 month old children (Studies 009, 011, 012 and 013) given ProQuad as a primary dose and the one study in 4 to 6 year old children (Study 014) given ProQuad to children previously immunized with MMRII and VARIVAX.

The number of subjects vaccinated by treatment group and dose was previously described (see Table 1.1). Over 93% of the 12 to 23 month old subjects and over 97% of the 4 to 6 year old subjects completed their respective studies. A subject was considered to have completed the study if he/she received all scheduled vaccinations, completed all safety follow-ups, and provided blood samples as defined in the protocol.

A total of 8670 subjects participated in the 5 clinical trials conducted in support of licensure of ProQuad. A total of 6232 subjects received ProQuad and 2438 received one of the controls. Of the 6232 immunized with ProQuad, 5832 were 12 months to 23 months old and of these, 5446 received ProQuad formulated to contain varicella at a dose ≥3.97 $\log_{10}$ PFU/dose and, of these, 4497 received ProQuad alone without other vaccines.

The data for the 4497 children immunized with ProQuad were compared with the data obtained from the 2038 children immunized with MMRII and VARIVAX. Safety follow-up was obtained for ~98% in both groups. Comparisons between the two groups were made by observation of the estimated risk differences and associated 95% confidence intervals. If a specific adverse experience was not observed in a safety database of 4497 subjects provided 97.5% confidence then the true incidence rate was <0.08%. With this safety database, there was an 80% probability of detecting an adverse event occurring with an incidence rate of 1 of every 2794 subjects (0.04%).

In Study 012, subsets of ~1800 children were followed for antibody persistence and to assess clinical efficacy against measles, mumps, rubella, and varicella disease.

In Studies 009 and 011, 1471 children were given a second dose of ProQuad and followed for an additional 42 days to monitor safety.

An additional 399 children who had been previously immunized with MMRII and VARIVAX were given ProQuad at 4 to 6 years of age in Study 014.

10.2 Safety Assessment Methods:

In Study 009 (for Dose 1) and in Study 012 the parents/guardians, children and study personnel performing follow-up for safety were blinded to group assignment. In Study 011, the participants and study personnel were blinded to varicella dose only but otherwise knew if vaccinees received ProQuad or a control vaccine. Study 013 was an open label study.

Parents and guardians filled out the Vaccination Report Cards for 42 days after each vaccination. They were required to note local and systemic adverse reactions and record temperatures daily for 42 days. They were to contact study personnel immediately if any serious adverse reactions were noted. Study personnel evaluated all

children with measles-like rashes or varicella-like rashes and obtained additional informed consent prior to collecting samples for testing. Active surveillance for cases of measles, mumps, rubella, or varicella in the community was not performed however, parents were requested to report any known exposures or documentation/diagnosis of these diseases that occurred both prior to the post-vaccination blood sample and, in study 12, for the year after vaccination.

10.3 Significant/Potentially Significant Events

10.3.1 Deaths

There were no deaths during the 42 day follow-up period after vaccination. One death was reported 56 days after primary immunization in a child (AN 02123 in Study 012) with croup and asthma who died of cardiac arrest. This death was not thought to be due to prior vaccination with ProQuad.

10.3.2 Other Significant/Potentially Significant Events

Sixty-four subjects of 4424 providing safety follow-up reported serious adverse reactions after ProQuad™ vaccination. Of these, 7 events were assessed to be vaccine related. These vaccine-related serious adverse events included fever, febrile seizure, cough, and bronchiolitis. All subjects recovered and none discontinued the study due to the experiences reported.

10.3.3 Dropouts

Dropout rates during ProQuad Clinical Trials were lower than expected. Approximately 98% of vaccinated individuals in the ProQuad group, as well as in each control group, completed the safety assessment and follow-up period. Dropouts, when they occurred, were distributed evenly across study groups. These data are summarized in Table 10.3.1 below.

Table 10.3.1 The Number and Percentage of Dropouts by Study.

Study	Total Enrolled		Drop-outs		Completed Study	
	ProQuad	Control	ProQuad	Control	ProQuad	Control
009	323	157	20 (6.2%)	4 (2.5%)	303 (93.8%)	153 (97.5%)
011*	774	390	85 (11%)	20 (5.1%)	689 (89%)	370 (94.9%)
012	2915	1012	123 (4.2%)	47 (4.6%)	2792 (95.8%)	965 (95.4%)
013	1434	479	97 (6.8%)	37 (7.7%)	1337 (93.2%)	442 (92.3%)
Subtotal	**5446**	**2038**	**325 (6%)**	**108 (5.3%)**	**5121 (94%)**	**1930 (94.7%)**
014	401	400	9 (2.2%)	11 (2.8%)	392 (97.8%)	389 (97.2%)
Total	**5847**	**2438**	**334 (5.7%)**	**119 (4.9%)**	**5513 (94.3%)**	**2319 (95.1%)**

* Medium and High dose groups only.

10.4 Other Safety Findings

10.4.1 ADR Incidence Tables

A. Clinical Adverse Reactions in 12 to 23 months old children following primary immunization with ProQuad with a varicella potency ≥3.97 log10 PFU/dose.

The percent of subjects 12 to 23 months old with one or more clinical adverse experiences following primary immunization was comparable between recipients of ProQuad and those vaccinated with MMRII and VARIVAX (81.5% and 79.6%, respectively). (See Table 10.4.1)

Table 10.4.1 Summary of Clinical Adverse Reactions Days 0 to 42 Post vaccination in 12 to 23 month Old Children Who Received ProQuad With Varicella Virus Potency ≥3.97 log10 PFU/dose or MMRII and VARIVAX

	ProQuad™ With a Varicella Virus Potency ≥3.97 log10 PFU/dose (N=4497)		**M-M-R II™+ VARIVAX™ (N=2038)**		**Estimated Risk Difference (ProQuad™—[M-M-R II™+ VARIVAX™]) (95% CI)**	
	n	%	n	%		
Number of subjects	4497		2038			
Subjects without follow-up	73		41			
Subjects with follow-up	4424		1997			
Number (%) of subjects:						
with no adverse experience	820	18.5%	408	20.4%		
with one or more adverse experiences	3604	81.5%	1589	79.6%	1.9%	(-0.2%, 4.0%)
injection-site adverse experiences	1395	31.5%	690	34.6%	-3.0%	(-5.5%, -0.5%)
systemic adverse experiences	3366	76.1%	1444	72.3%	3.8%	(1.5%, 6.1%)
measles-like rashes	142	3.2%	43	2.2%	1.1%	(0.2%, 1.8%)
varicella-like rashes	105	2.4%	49	2.5%	-0.1%	(-1.0%, 0.7%)
rubella-like rashes	14	0.3%	4	0.2%	0.1%	(-0.2%, 0.4%)
elevated temperatures ≥102°f (≥38.9°c) oral equivalent or abnormal	1635	37.3%	624	31.6%	5.7%	(3.2%, 8.2%)
with vaccine-related adverse experiences	2320	52.4%	1007	50.4%	2.0%	(-0.6%, 4.7%)
injection-site adverse experiences	1386	31.3%	686	34.4%	-3.0%	(-5.5%, -0.5%)
systemic adverse experiences	1461	33.0%	561	28.1%	4.9%	(2.5%, 7.3%)
serious adverse experiences	31	0.7%	14	0.7%	-0.0%	(-0.5%, 0.4%)
serious vaccine-related adverse experiences	6	0.1%	1	0.1%	0.1%	(-0.2%, 0.3%)

†Determined by the investigator to be possibly, probably, or definitely related to the vaccine.
Percentages are calculated based on the number of subjects with follow-up after each visit.
CI = Confidence interval; N = Number of subjects vaccinated.

Local Injection Site Adverse Reactions:

The rate of injection site reactions was significantly lower among ProQuad recipients than those immunized with MMRII and VARIVAX (31.3% vs. 34.6%, respectively). The rate of pain/tenderness/soreness at the injection site was significantly lower among recipients of ProQuad compared with the rate at either injection site for MMRII or for VARIVAX (22.0% vs. 26.8%, respectively, data not shown). In contrast, the rate of rash at the injection site was significantly higher after ProQuad immunization when compared with the rate at either injection site for recipients of MMRII and VARIVAX (2.4% vs. 1.6%, respectively, data not shown). Erythema occurred more frequently at the ProQuad site than at the VARIVAX site (14.5% vs. 12.4%, respectively). Rash at the injection site also occurred at the ProQuad site at a rate that was higher than the rate at the MMRII site or at the VARIVAX site (2.4% vs. 0.5% vs. 1.4%, respectively). However, the rate of rash reported at the ProQuad site is within the range reported for rashes seen after VARIVAX administration (1.3% to 4.0%) in children in the same age group evaluated in previous studies. These data are summarized in Table 10.4.2.

Table 10.4.2 Comparison of Injection site reactions after ProQuad vs. MMRII and VARIVAX

	ProQuad with Varicella Virus Potency ≥3.97 Log10PFU/0.5-mL Dose (N=4497)			M-M-R II + VARIVAX.						Estimated Risk Difference (ProQuad minus MMRII Injection Sites) (95% CI)	Estimated Risk Difference. (ProQuad.Minus VARIVAX. Injection Sites) (95% CI)
				M-M-R II. (N=2038)			VARIVAX (N=2038)				
	N	%									
Number of subjects	4497			2038			2038				
Subjects with no follow-up	73			41			41				
Subjects with follow-up	4424			1997			1997				
Number (%) of subjects with one or more injection-site adverse experiences	1385	31.3%		599	30.0%		607	30.4%		1.3% (-1.1%, 3.7%)	0.9% (-1.5%, 3.3%)
Ecchymosis	72	1.6%	68	22	1.1%	22	32	1.6%	31	0.5% (-0.1%, 1.1%)	0.0% (-0.7%, 0.7%)
Erythema	642	14.5%	639	255	12.8%	254	248	12.4%	246	1.7% (-0.1%, 3.5%)	2.1% (0.3%, 3.8%)
Pain/Tenderness/Soreness	975	22.0%	972	484	24.2%	483	471	23.6%	469	-2.2% (-4.5%, 0.0%)	-1.5% (-3.8%, 0.7%)
Rash	107	2.4%	102	9	0.5%	8	27	1.4%	27	2.0% (1.4%, 2.5%)	1.1% (0.3%, 1.7%)
Swelling	371	8.4%	370	148	7.4%	147	153	7.7%	153	1.0% (-0.5%, 2.3%)	0.7% (-0.7%, 2.1%)

Based on adverse experiences of any relatedness, not just those that are vaccine related (VR).

Percentages are calculated based on the number of subjects with follow-up after each visit.

Although a subject may have had 2 or more injection-site adverse experiences, the subject is counted only once in the overall total.

VR = Vaccine related. Numbers in this column refer to subjects with adverse experience that were determined by the investigator to be possibly, probably, or definitely related to the vaccine.

CI = Confidence interval.

N = Number of subjects vaccinated.

PFU = Plaque-forming units.

Systemic Adverse Reactions:

The number of subjects reporting systemic adverse reactions was higher after ProQuad than after MMRII and VARIVAX administration (76.1% vs. 72.3%, respectively). The most frequently reported AEs after ProQuad and after MMRII and VARIVAX immunization (>10%) were fever, upper respiratory tract infection, otitis media, and irritability. The only systemic clinical adverse experiences that were reported at a higher rate after ProQuad were fever (37.2% vs. 31.5%, respectively), URI (23.5% vs. 20.7%, respectively) and measles-like rash ((3.2 vs. 2.2%, respectively). Most URIs were not thought to be due to vaccination, as they were randomly distributed over 42 days after immunization, did not coincide with episodes of measles-like rashes and the difference was only noted when the data across studies were combined and this risk difference was small (2.8% [95%CI 0.6%, 4.9%]). Fever occurred significantly more frequently after ProQuad than after MMRII and VARIVAX immunization however the fevers were short

(average 1.7 days) and most (61%) were judged to be mild. Measles like rashes occurred significantly more frequently after ProQuad than after MMRII and VARIVAX (3.0% vs. 2.1% respectively) but varicella like rashes occurred at similar rates (2.4% vs. 2.5%, respectively, (Table 10.4.3).

Table 10.4.3 Comparison of Systemic AEs by Body System after ProQuad vs. MMRII + VARIVAX

	ProQuad. With Varicella Virus Potency ≥3.97 Log_{10}PFU/0.5 mL (N=4497)		M-M-R II + VARIVAX. (N=2038)		Estimated Risk Difference. Difference (95% CI)
	n	%	N	%	
Number of subjects	4497		2038		
Subjects without follow-up	73		41		
Subjects with follow-up	4424		1997		
Subjects with one or more AEs	3366	76.1	1444	72.3	3.8% (1.5%, 6.1%)
Subjects with no adverse experience	1058	23.9	553	27.7	
Body as a Whole/Site Unspecified	**1799**	**40.7**	**693**	**34.7**	**6.0% (3.4%, 8.5%)**
Digestive System	**786**	**17.8**	**345**	**17.3**	**0.5% (-1.6%, 2.5%)**
Metabolic/Nutritional/Immune	**50**	**1.1**	**30**	**1.5**	**-0.4% (-1.1%, 0.2%)**
Nervous System and Psychiatric	**564**	**12.7**	**246**	**12.3**	**0.4% (-1.4%, 2.1%)**
Respiratory System	**1701**	**38.4**	**731**	**36.6**	**1.8% (-0.7%, 4.4%)**
Skin and Skin Appendage	**1328**	**30.0**	**579**	**29.0**	**1.0% (-1.4%, 3.4%)**
Special Senses	**755**	**17.1**	**319**	**16.0**	**1.1% (-0.9%, 3.0%)**

ProQuad minus [M-M-R II + VARIVAX.]. Based on adverse experiences of any relatedness, not just those that are vaccine related (VR).

One subject from Protocol 011 (Allocation Number [AN] 00681), 3 subjects from Protocol 012 (ANs, 07313, 01390, and 05651), and 2 subjects from Protocol 013 (ANs 11375 and 12443) who received ProQuad and 1 subject from Protocol 013 (AN 11837) who received M-M-R.II + VARIVAX were diagnosed with a measles-like/rubella-like rash during the 42-day follow-up period. One (1) subject from Protocol 012 (AN 05637) who received ProQuad was diagnosed with a rubella-like/measles-like rash during the 42-day follow-up period. All of these subjects were counted in both the measles-like rash and rubella-like rash categories for the purpose of analysis. The incidence rate of rubella-like rash was <1% in both treatment groups (0.3% versus 0.2%, respectively; therefore, rubella-like rash is not included in this table.

§One subject from Protocol 009 (AN 00113) who received ProQuad reported measles after vaccination, which was later confirmed to be a measles-like rash. This event is captured as a measles-like rash for the purpose of analysis.

Percentages are calculated based on the number of subjects with follow-up after vaccination Visit 1.

Although a subject may have had 2 or more systemic adverse experiences, the subject is counted only once within a category. The same subject may appear in different categories.

All body systems are listed in which at least one subject had a systemic adverse experience.

VR = Vaccine related. Numbers in this column refer to subjects with adverse experience that were determined by the investigator to be possibly, probably, or definitely related to the vaccine.

CI = Confidence interval.

N = Number of subjects vaccinated.

PFU = Plaque-forming units.

Fever:

The rate of fever ≥102 F oral equivalent or abnormal during the 42 days after ProQuad immunization was statistically higher when compared with the rate after MMRII and VARIVAX administration (37.3% vs. 31.6%, respectively). The rate of temperatures ≥ 104 F was also significantly higher after ProQuad vaccination when compared to the rate in controls (5.8% vs. 4.7%, respectively). These data and the analyses are summarized in Table 10.4.4 below.

The increase in the rate of fevers in children in this age group may lead to an increase in the rate of febrile seizures and this will be evaluated in a post-marketing study in 25,000 children, 12 months to 6 years of age immunized with ProQuad. (See Post Marketing Commitments, Section 13.2)

Table 10.4.4 Summary of Fevers Reported Days 0-42 and Days 5-12 after Immunization with ProQuad vs. MMRII and VARIVAX

	Days 0 to 42 Post-vaccination			Days 5 to 12 Post-vaccination		
	ProQuad. With Varicella Virus Potency ≥3.97 Log10 PFU/0.5-mL Dose (N= 4497)	M-M-R.II + VARIVAX (N= 2038)	Estimated Risk Difference (ProQuad. Minus [M-M-R.II + VARIVAX.]) (95% CI)	ProQuad. With Varicella Virus Potency ≥3.97 Log10 PFU/0.5-mL (N= 4497)	M-M-R.II + VARIVAX (N= 2038)	Estimated Risk Difference (ProQuad. Minus M-M-R.II + VARIVAX.) (95% CI)
	n (%)	n (%)		n (%)	n (%)	
Number of subjects	4497	2038		4497	2038	
Subjects with no follow-up	110	61		121	66	
Subjects with follow-up	4387	1977		4376	1972	
Maximum temperature (oral equivalent)						
<102°F (<38.9°C) or Normal	2750 (62.7%)	1352(68.4%)	5.7%	3399 (77.7%)	1680(85.2%)	7.5%
≥102°F (≥38.9°C) or Abnormal	1635 (37.3%)	624 (31.6%)	(3.2%, 8.2%)	975 (22.3%)	292 (14.8%)	(5.4%, 9.4%)
Missing method	2 (0.0%)	1 (0.1%)		2 (0.0%)	0 (0.0%)	
Maximum temperature (oral equivalent)						
<104°F (<40.0°C) or Normal						
≥104°F (≥40.0°C)	4130(94.1%)	1884(95.3%)	1.2%	4249 (97.1%)	1933(98.0%)	0.9%
Missing method	255 (5.8%)	92 (4.7%)	(-0.0%, 2.3%)	125(2.9%)	39 (2.0%)	(0.0%, 1.6%)
	2 (0.0%)	1 (0.1%)		2 (0.0%)	0 (0.0%)	

Including subjects who received VARIVAX. (Process Upgrade) in Protocol 011. One subject in Protocol 013 who had a reported maximum temperature ≥104°F Days 5 to 12 post-vaccination was inadvertently excluded from the elevated temperature analyses in the clinical study report. This subject is included in this summary. Percentages are calculated based on the number of subjects with follow-up after each visit. All temperatures have been converted to oral equivalent by adding 1°F to axillary temperature or subtracting 1°F from rectal temperatures. In the absence of a numeric temperature, the parent/legal guardian was instructed to report whether or not the subject felt warm to touch. A report of a child feeling warm to touch was entered as abnormal into the database. If the child did not feel warm to touch, the temperature was entered as normal. CI = Confidence Interval. PFU = Plaque-forming units.

Measles-Like Rashes:

The appearance of measles-like rashes and fever ≥102 F appeared to be temporally associated and the majority of these events began days 5 to 12 after immunization in each of the 4 clinical studies. This 5 to 12 day timeframe is the same time period in which most fevers and measles-like rashes occur following natural measles and receipt of MMRII. Interestingly, post-vaccination measles GMT antibody titers in ProQuad vaccinated children were higher than the post vaccination titers after MMRII and VARIVAX (3125 mIU/mL vs. 2240 mIU/mL, respectively). This led to the hypothesis that there was an increase in local measles virus replication (as evidenced by higher measles antibody titers, an increase in measles like rash rates and the increased rate of fever) in the presence of all 4 viruses in ProQuad. Results from statistical modeling and logistic regression analysis revealed that the rate of fever and level of post

vaccination measles antibody was positively associated with increasing varicella potency but titers reached a plateau and did not increase further over the range of potencies that will be used to release ProQuad.

Varicella-Like Rashes:

Varicella-like rashes occurred at similar rates when the frequency after ProQuad was compared to that seen in the controls.

Limitations of the Safety Data

Parents and guardians of children immunized in Studies 011 and 013 were not blinded to vaccine assignment. Thus, there is the possibility of reporting bias.

Fever rates included those with documented fevers as well as those who felt warm to touch and those who felt abnormally hot. The inclusion of children who "felt warm" could have overestimated the true rate of fever in these studies.

B. Clinical Adverse Reactions Days 0 to 42 Post-vaccination in 12 to 23 month Old Children Who Received ProQuad Dose 2 following ProQuad Dose 1 Given at Least 3 Months Later With Varicella Virus Potency ≥3.97 log10 PFU/dose.

As expected in an immune population, a second dose of ProQuad was less reactogenic than the first dose (77.6% vs. 70.1%, respectively). These data are summarized in Table 10.4.5 below.

Table 10.4.5 Summary of Clinical Adverse Reactions Days 0 to 42 Post-vaccination in 12 to 23 month Old Children Who Received ProQuad Dose 1 followed by ProQuad Dose 2 Given at Least 3 Months Later With Varicella Virus Potency ≥3.97 log10 PFU/dose.

	ProQuad™ With a Varicella Virus Potency ≥3.97 log10 PFU/dose Dose 1		**ProQuad™ With a Varicella Virus Potency ≥3.97 log10 PFU/dose Dose 2**		**Estimated Risk Difference (Dose 1 –Dose 2) (95% CI)**
	n	%	n	%	
Number of subjects	1018		1018		
Subjects without follow-up	0		1		
Subjects with follow-up	1018		1017		
Number (%) of subjects:					
with no adverse experience	228	22.4	304	29.9	-7.5
with one or more adverse experiences	790	77.6	713	70.1	7.5
Injection-site adverse experiences	224	22.0	164	16.1	5.9
systemic adverse experiences	745	73.2	666	65.5	7.7
Measles-like rashes	49	4.8	7	0.7	4.1
Varicella-like rashes	28	2.8	4	0.4	2.4
rubella-like rashes	3	0.3	0	0.0	0.3
elevated temperatures ≥102°f (≥38.9°c) oral equivalent or abnormal	377	37.1	269	26.5	10.6
with vaccine-related adverse experiences	508	49.9	311	30.6	19.3
Injection-site adverse experiences	222	21.8	164	16.1	5.7
systemic adverse experiences	383	37.6	176	17.3	20.3
serious adverse experiences	4	0.4	3	0.3	0.1
serious vaccine-related adverse experiences	1	0.1	0	0.0	0.1

Percentages are calculated based on the number of subjects with follow-up after each visit.
CI = Confidence interval; N = Number of subjects vaccinated.

Local Injection Site Adverse Reactions:

The overall rate of injection site AEs following the second injection of ProQuad was lower than the rate after the first injection (16.1% vs. 22.0%, respectively).

Systemic Adverse Reactions:

The overall rate of systemic adverse events after the second dose of ProQuad was also decreased in comparison with the rate after the first dose (65.5% vs. 73.2%, respectively).

Fever:

The rate of fever was decreased following the second dose of ProQuad when compared with the rate after the first injection (26.3% vs. 37.1%, respectively).

Measles-Like Rashes:

The rate of measles like rash was decreased following the second dose of ProQuad when compared to the rate after dose 1 (0.7% after dose 2 vs. 4.8% after dose 1).

Varicella-Like Rashes:

Varicella like rashes occurred at similar rates after ProQuad dose 1 (2.8%) and ProQuad dose 2 (2.4%) in this study.

Limitations of the Safety Data:

It is possible that some of the decrease in reporting adverse events was due to reporter fatigue associated with having to complete the vaccination report card for an additional 42day reporting period.

Groups were not blinded at the time of administration of the second dose of ProQuad and this could have led to reporting bias.

C. Clinical Adverse Reactions following ProQuad immunization in 4 to 6 year old children previously immunized with MMRII and VARIVAX.

In Study 014, 4 to 6 year old children who had been previously immunized with MMRII and VARIVAX were given a dose of ProQuad, MMRII and placebo or MMRII and VARIVAX. The percent of subjects with one or more clinical adverse experience were comparable between ProQuad and the control group given MMRII and placebo or MMRII and VARIVAX with rates of 77.6%, 78.0% and 75.6%, respectively). (See Table 10.5.6)

Table 10.5.6 Summary of Clinical Adverse Reactions following ProQuad administration to Children 4 to 6 years old who were Previously Immunized with MMRII and VARIVAX

	ProQuad™ + Placebo (Group 1) N=399		**M-M-R™II + Placebo (Group 2) N=205**		**M-M-R™II + VARIVAX™ (Group 3) N=199**	
	n	(%)	n	(%)	n	(%)
Number of subjects vaccinated	399		205		195	
Subjects without follow-up	2		0		2	
Subjects with follow-up	397		205		193	
Number (%) of subjects:						
With no adverse experience	89	(22.4)	45	(22.0)	47	(24.4)
With one or more adverse experiences	308	(77.6)	160	(78.0)	146	(75.6)
Injection-site adverse experiences	223	(**56.2)**	104	(50.7)	99	(51.3)
Systemic adverse experiences	217	(54.7)	123	(60.0)	114	(59.1)
Measles like rashes	1	(0.3)	0	(0.0)	1	(0.5)
Varicella like rashes	3	(0.8)	1	(0.5)	0	(0.0)
Elevated temp.≥102 F or oral equivalent	40	(**10.2)**	20	(9.9)	18	(9.4)
With vaccine-related† adverse experiences	231	(**58.2**)	110	(53.7)	105	(54.4)
Injection-site adverse experiences	223	(**56.2**)	103	(50.2)	99	(51.3)
Systemic adverse experiences	32	(8.1)	19	(9.3)	18	(9.3)
With serious adverse experiences	1	(**0.3**)	0	(0.0)	0	(0.0)
With serious vaccine-related adverse experiences	0	(0.0)	0	(0.0)	0	(0.0)
Who died	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued‡ due to an adverse experience	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a vaccine-related AE	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a serious adverse experience	0	(0.0)	0	(0.0)	0	(0.0)
Discontinued due to a serious vaccine-related AE	0	(0.0)	0	(0.0)	0	(0.0)

† Determined by the investigator to be possibly, probably, or definitely related to the vaccine. ‡ Discontinued = Subject discontinued from study. Percentages are calculated based on the number of subjects with follow-up after any visit. One (1) subject in this trial was incorrectly vaccinated. The subject (AN 25842) was randomized to Group 1 but inadvertently received 2 doses of diluent. This subject withdrew consent immediately after vaccination, and did not participate in the safety follow-up period. Therefore, there are no additional data from this subject; they are not included in this table. N = Number of subjects vaccinated. n = the number of subjects with one or more of the specified adverse experiences.

Local Injection Site Adverse Reactions:

The overall percent reporting injection site reactions was comparable between groups with 56.2% reporting an injection site reaction after ProQuad vs. 50.2% after MMRII and placebo or 51.3% after MMRII and VARIVAX. The only injection site AE that was consistently higher after ProQuad vaccination was erythema (24.4% for ProQuad, 15.6 and 14.5% for each MMRII group and 15.5% for VARIVAX). Most injection site reactions were mild, small in size, and not considered to be clinically important.

Systemic Adverse Reactions:

Systemic adverse reactions were reported in 54.7% of ProQuad recipients, 60.0% after MMRII with placebo and in 59.1% after MMRII with VARIVAX. There was no noticeable trend for higher rates of systemic adverse reactions for ProQuad when rates were compared to those reported in the two control groups.

Fever:

As expected, the overall rates of fever were lower in all vaccine groups in this study when compared with the rate after ProQuad dose 1. The rates of fever ≥102 F or abnormal was 10.2% after ProQuad, 9.9% after MMRII plus placebo and 9.4% after a second dose of MMRII and VARIVAX.

Measles-Like Rashes:

Measles-like rashes were reported infrequently in all groups: 0.3% after ProQuad, none after MMRII and placebo and 0.5% after MMRII and VARIVAX.

Varicella-Like Rashes:

Varicella-like rashes were reported infrequently in all groups: 0.8% after ProQuad, 0.5% after MMRII plus placebo and none after MMRII and VARIVAX.

Limitations of the Safety Data:

This was a double-blinded study and there were no apparent limitations to assessing the safety data from this study with the exception that fevers may have been overestimated because not all were documented using a thermometer.

10.4.2 Laboratory Findings, Vital Signs, ECGs:

There were no laboratory studies routinely performed during ProQuad™ clinical trials. There was no prospective evaluation of the prevalence or severity of post immunization thrombocytopenia following ProQuad compared to post immunization thrombocytopenia following MMRII alone or MMRII given concomitantly with VARIVAX.

Rates and duration of fevers are reported above.

10.4.3 Product-Demographic Interactions

When the Case Study Reports for each individual study were reviewed, the subjects in each group were comparable in terms of age, race, gender, and with regards to prior therapies or medications. Serological status at baseline was also comparable across groups.

10.4.4 Product-Disease Interactions

There was limited follow-up provided for children immunized during study 012. In the year after immunization there were no cases of measles, mumps, or rubella reported in children immunized with ProQuad™ or with MMRII™ and VARIVAX™. Fourteen of 2497 or 0.6% of individuals immunized with ProQuad™ reported chickenpox while 0.7% or 6/858 of those immunized with MMRII™ plus VARIVAX™ reported breakthrough cases. In both vaccine groups the cases of chickenpox were mild with fewer than 50 varicella lesions per case.

ProQuad was evaluated in healthy children; safety and immunogenicity were not studied in children with underlying diseases.

ProQuad™ should not be given to children with active untreated tuberculosis.

ProQuad is contraindicated in pregnant women and should not be given to varicella susceptible pregnant women in the postpartum period because of the possibility of contact transmission of vaccine virus to her varicella susceptible newborn.

ProQuad™ is contraindicated in children whose immune status is unknown or in those with a family history of congenital or hereditary immunodeficiency until the immune status of the vaccine recipient is known. ProQuad™ is contraindicated in those with primary or secondary immunodeficiency disorders, cellular immune deficiencies, hypogammaglobulinemic and dysgammaglobulinemic states, leukemia, lymphomas of any type, malignant neoplasms affecting the bone marrow or lymphatic system or blood dyscrasias. ProQuad™ is not approved for use in children with HIV infection. ProQuad™ is contraindicated in children on immunosuppressive medications including but not limited to high dose corticosteroids. Death as a direct consequence of disseminated vaccine virus infection has been reported in severely immunocompromised individuals inadvertently vaccinated with measles containing vaccine.

ProQuad™ is contraindicated in children with a history of anaphylactic reaction to neomycin, hypersensitivity to gelatin or to any component of the vaccine.

10.4.5 Product-Product Interactions

ProQuad™ should be given at least one month after receipt of measles containing vaccine and at least 3 months should elapse between doses of varicella containing vaccines and between receipt of a varicella containing vaccine and other live viral vaccines.

ProQuad™ should not be given at the same time as immunoglobulin or other blood products unless the benefit of the blood product or plasma derivative outweighs the benefits of immunization.

ProQuad™ may be given concomitantly but at separate sites with Comvax.

ProQuad was given concomitantly with TRIPEDIA, DTaP vaccine to children 12 to 15 months of age in Study 013. Antibody responses to diphtheria and tetanus antigens were similar when responses were compared between those immunized concomitantly with those given ProQuad and TRIPEDIA 6 weeks apart. In contrast, the immune responses to pertussis hemagglutinin and pertactin antigens were significantly lower in the group given the vaccines concomitantly. Therefore, it is not currently recommended to administer ProQuad concomitantly with DTaP vaccine. See the following sections for further discussion: Drug-Drug Interactions under section 3.1.5 of this document, Clinical Review, Dr. Karen Farizo, Appendix B, and Phase 4, Post-marketing studies, Section 13.2

10.4.6 Immunogenicity

ProQuad™ is immunogenic and elicits measles, mumps, rubella, and varicella virus specific antibody in 97.4%, 99.9%, 98.3% and 91.2%, respectively, of children 12 to 23 months of age following a single dose.

Following two doses of ProQuad™ given approximately 3 months apart, seropositivity rates were 99.4% for measles, 99.9% for mumps, 98.3% for rubella and 99.4% for varicella.

Comvax did not interfere with the immune response to the vaccine antigens in ProQuad™ when these vaccines were administered concomitantly.

ProQuad™ is not approved for concomitant administration with vaccines other than Comvax. (See Section, Post Marketing Studies).

10.4.7 Carcinogenicity

Studies of ProQuad carcinogenicity have not been done.

10.4.8 Withdrawal Phenomena/Abuse Potential:

There is no potential for ProQuad™ abuse or withdrawal symptoms following use.

10.4.9 Human Reproduction and Pregnancy Data

ProQuad is contraindicated in individuals who are pregnant because the possible effects on fetal development are unknown. Pregnancy should be avoided for 3 months after vaccination.

Animal reproduction studies have not been done with ProQuad

There are no studies of the attenuated measles vaccine strain during pregnancy.

Although mumps vaccine virus may infect the placenta and fetus, there is no evidence that it causes congenital malformations in humans.

In a 10-year survey involving over 700 pregnant women, who received rubella vaccine within 3 months before or after conception (189 of whom received Wistar RA27/3 rubella vaccine virus), none of the newborns had abnormalities compatible with congenital rubella infection.

Wild type varicella can cause congenital varicella infection but data from the first nine years of the Varicella Pregnancy Registry indicates that of 129 seronegative women and 423 women of unknown varicella serostatus who received varicella vaccine during pregnancy or within 3 months before pregnancy, none had newborns with abnormalities compatible with congenital varicella syndrome.

10.4.10 Assessment of Effect on Growth.

Only one or two doses of ProQuad™ are administered. Formal studies to assess the impact of immunization on growth have not been done with ProQuad™ or with any other vaccine.

10.4.11 Over-dosage Exposure

No one was inadvertently vaccinated with an overdose of ProQuad.

One child in Study 011, AN 01284, was inadvertently immunized with MMRII and ProQuad at the same time. This child did not report any adverse reactions after immunization.

10.4.12 Person-to-Person Transmission, Shedding

Person-to-person transmission and shedding is discussed above under the section on Pharmacology. Formal studies of shedding and transmission following ProQuad immunization have not been done.

There are no reports of transmission of infectious more attenuated Ender's Edmonston vaccine strain measles virus to susceptible contacts.

There are no reports of transmission of infectious Jeryl Lynn vaccine strain of mumps virus to susceptible contacts.

The majority of rubella susceptible individuals vaccinated with RA 27/3 vaccine excrete small amounts of live attenuated virus from the nose or throat for 7 to 28 days after vaccination. There is no confirmed evidence to indicate that virus is transmitted to susceptible persons who come in contact with the vaccinated individual. Transmission through close personal contact, while a theoretical possibility, is not thought to be a significant risk. Transmission of rubella vaccine virus to infants via breast milk has been documented.

Transmission of varicella vaccine virus may occur rarely between healthy vaccine recipients who develop a varicella like rash and contacts susceptible to varicella as well as high-risk individuals susceptible to varicella.

10.4.13 Post-marketing Exposure

ProQuad has not been licensed previously.

10.5 Safety Conclusions

10.5.1 ProQuad is generally well tolerated in children 12 to 23 months of age when given as a first dose of measles, mumps, rubella, and varicella vaccines.

10.5.2 The rate of fevers ≥102 F oral equivalent or abnormal is significantly higher in ProQuad recipients than in recipients of MMRII and VARIVAX (37.3% vs. 31.6%, respectively), however, they were generally mild and of short duration (average of 1.7 days).

10.5.3 The measles-like rash rate is significantly higher in ProQuad recipients than in recipients of MMRII and VARIVAX (3.2% vs. 2.2%, respectively).

10.5.4 The majority of fever and rashes following ProQuad occurred days 5 to 12 after vaccination the same as the majority of fever and rashes after measles vaccines

10.5.5 Administration of a second dose of ProQuad to children 3 months after the first dose was generally well tolerated.

10.5.6 A dose of ProQuad given to children 4 to 6 years of age who had previously received MMRII and VARIVAX was generally well tolerated.

10.5.7 The safety profile following concomitant administration of ProQuad and COMVAX is comparable to the safety profile following non-concomitant administration of these vaccines.

11 Additional Clinical Issues

11.1 **Special Populations**: ProQuad is not indicated for use in children with HIV infection. ProQuad is not indicated for use in pregnant women or in varicella susceptible women in the post-partum period.

11.2 **Pediatrics:** ProQuad is indicated for use in healthy children 12 months to 12 years of age. ProQuad is not indicated for use in children less than 12 months old; ProQuad is not indicated for use in those who are 13 years and older.

11.3 **Other:** ProQuad is not indicated for the prevention of zoster or shingles in adults. ProQuad is not indicated for use in the elderly.

12 Conclusions:

12.1 Immunogenicity and safety data from these studies indicate that ProQuad may be used for simultaneous vaccination against measles, mumps, rubella, and varicella in healthy children 12 months to 12 years of age.

12.2 Immunogenicity and safety data from these studies indicates that ProQuad may be used in healthy children 12 months to 12 years of age if a second dose of measles, mumps and rubella vaccine is to be administered.

13 Recommendations

13.1 Approval

13.1.1 ProQuad is approved for simultaneous vaccination against measles, mumps, rubella, and varicella in children 12 months to 12 years of age.

13.1.2 ProQuad is approved for use in children 12 months to 12 years of age if a second dose of measles, mump, or rubella vaccine is to be administered.

13.2 Phase 4 Post-Marketing Studies:

CBER agrees with Merck's commitment to conduct the following Phase 4 studies:

Study 020: Post-licensure evaluation of the short term safety of ProQuad in 25,000 children enrolled in a managed care organization to assess the frequency of occurrence of febrile seizures relative to the frequency of occurrence to a) control period before vaccination and b) age matched historical controls immunized with MMRII and VARIVAX.

Safety of a second dose of ProQuad™ administered at least 90 days after ProQuad™ Dose 1 in approximately 3000 children 12 to 23 months of age.

Long term monitoring for a period of 15 years for varicella epidemiology in the US.

If Merck seeks additional indications for concomitant administration of ProQuad with other vaccines then CBER agrees with their plan to conduct the following studies.

Study 019: An open, randomized, multi-center study of the safety, tolerability, and immunogenicity, of ProQuad, given concomitantly with a fourth dose of Prevnar in healthy children 12 months to 15 months of age.

Study 066: ProQuad plus VAQTA safety study.

Study 067: Safety and immunogenicity of concomitant vaccination with ProQuad, VAQTA and Prevnar.

13.3 Labeling:

The proposed package insert accurately summarizes the immunogenicity and safety data derived from the studies reviewed herein as well as the summary experience with MMRII and VARIVAX vaccines

13.4 Other

None.

14 Comments and questions for the applicant:

None.

Appendix A

Table of Clinical Studies

Study Number	Study Title	Primary Study Objectives
009	A Pilot Study to Compare the Safety, Tolerability, and Immunogenicity of Measles, Mumps, Rubella, and Varicella (MMRV) Vaccine and the Concomitant Administration of the Currently Licensed VARIVAX™ and M-M-R™ II in Healthy Children.	(1) To determine if 1 or 2 doses of ProQuad™ can elicit a similar immune response to varicella as the concomitant administration of 1 dose of the currently licensed VARIVAX™ and M-M-R™ II (2) To assess the safety and tolerability of ProQuad™ after 1 and 2 doses
011	A Dose Selection Study in Healthy Children Comparing Measles, Mumps, Rubella, and Varicella (ProQuad™) Vaccine to M-M-R™ II Given Concomitantly With Process Upgrade Varicella Vaccine (PUVV) in Separate Injections	(1) To select at least 1 dose level and regimen of ProQuad™ that has a similar immune response to varicella as the control group of M-M-R™ II and PUVV given concomitantly but in separate injections (2) To demonstrate that there is similar immunogenicity for measles, mumps, and rubella between at least 1 dose level and regimen of (3) ProQuad™ and the control group of M-M-R™II and PUVV given concomitantly but in separate injections To demonstrate that ProQuad™ is generally safe and well tolerated
012	Comparison of the Safety, Tolerability, and Immunogenicity of 3 Consistency Lots of Frozen Measles, Mumps, Rubella, and Varicella Vaccine (ProQuad™) in Healthy Children	(1) To demonstrate that the 3 consistency lots of ProQuad™ will elicit (2) similar immune responses to measles, mumps, rubella, and (3) varicella To determine whether the 3 consistency lots of (4) ProQuad™ combined will elicit an immune response similar to M-M-R™ II and VARIVAX™ given concomitantly, but at separate injection sites To demonstrate that each of the 3 consistency lots of ProQuad™ provides an acceptable immune response to measles, mumps, and rubella To demonstrate that the 3 consistency lots of ProQuad™ are well tolerated (5) To evaluate the persistence of antibodies to all 4 vaccine antigens 1 year post-vaccination
013	An Open, Randomized, Multi-center Study of the Safety, Tolerability, and Immunogenicity of ProQuad™ (Frozen) Given Concomitantly Versus Non-concomitantly With Other Pediatric Vaccines in Healthy Children 12 to 15 Months of Age	(1) To demonstrate that ProQuad™ can be administered (2) concomitantly with TRIPEDIA™ and COMVAX™ without impairing the immune response to measles, mumps, rubella, varicella, diphtheria, tetanus, pertussis toxin (PT), pertussis filamentous haemagglutinin (FHA), hepatitis B, or *Haemophilus influenzae* type b (Hib) To demonstrate that the concomitant administration of ProQuad™, TRIPEDIA™, and COMVAX™ provides an acceptable immune response to measles, mumps, rubella, and varicella (3) To show that ProQuad™ is generally well tolerated when administered concomitantly with TRIPEDIA™ and COMVAX™ at the same visit or separated by an interval of 6 weeks (4) To show that ProQuad™, whether administered concomitantly with TRIPEDIA™ and COMVAX™ at the same visit or separately by an interval of 6 weeks, is generally well tolerated compared with the concomitant administration of M-M-R™ II and VARIVAX™
014	Administration of Frozen Measles, Mumps, Rubella, and Varicella (ProQuad™) Vaccine to Healthy Children at 4 to 6 Years of Age	(1) To show that the antibody responses to measles, mumps, and rubella following a dose of ProQuad™ at 4 to 6 years of age will be similar to the antibody responses after the recommended second dose of M-M-R™II (2) To show that the antibody responses to measles, mumps, rubella, and varicella following a dose of ProQuad™ at 4 to 6 years will be similar to the antibody responses after a second dose of M-M-R™II and VARIVAX™ administered concomitantly at separate injection sites (3) To show that a dose of ProQuad™ at 4 to 6 years will be generally well tolerated (4) To summarize the following immunogenicity parameters by treatment group: seroconversion rates to measles, mumps, and rubella in subjects initially seronegative to the respective antigen; seropositivity rates to measles, mumps, and rubella in all subjects; the percent of subjects with post-vaccination varicella antibody titer ≥5 gpELISA units/mL in subjects initially seronegative to varicella, in subjects with pre-dose varicella titer <1.25 gpELISA units/mL, and in all subjects; for each of measles, mumps, rubella, and varicella, the percent of subjects achieving ≥4-foldrise in antibody titer

APPENDIX B

DEPARTMENT OF HEALTH & HUMAN SERVICES
FDA/CBER/OVRR/DVRPA

Memorandum

Date: March 4, 2005

From: Karen M. Farizo, M.D.
Medical Officer
Vaccines Clinical Trials Branch

Subject: BLA STN 125108\0: Merck & Co., Inc. Measles, Mumps, Rubella, and Varicella [Oka/Merck] Virus Vaccine, Live (ProQuad): Review of Pertussis Immunogenicity Data from Study P013

To: BLA STN# 125108

Through: Antonia Geber, M.D.
Team Leader
Vaccines Clinical Trials Branch

cc: Judy Beeler, M.D.
Herbert Smith, Ph.D.
Douglas Pratt, M.D.

1. Background

Based on the proposed package insert for ProQuad submitted in BLA 125108, the requested indication for ProQuad is simultaneous vaccination against measles, mumps, rubella, and varicella in individuals 12 months to 12 years of age. The requested indication also includes use of ProQuad for the second dose of MMRII.

In the ProQuad BLA, the sponsor submitted the results of a study (P013) designed, in part, to assess whether ProQuad can be administered in children 12-15 months of age, concomitantly with a DTaP vaccine, without impairing the immune responses to the acellular pertussis component of DTaP.

The clinical reviewer for the ProQuad BLA requested input regarding the following:

- ---
- ---
- ---

2. Review of pertussis immunogenicity data from study P013

2.1 Applicant's Protocol # and Protocol Title: P013 An Open, Randomized, Multi-center Study of the Safety, Tolerability, and Immunogenicity of Frozen MMRV Given Concomitantly Versus Non-concomitantly With Other Pediatric Vaccines in Healthy Children 12 to 15 Months of Age

2.1.1 Objectives

The following primary objective is the only study objective relevant to the evaluation of pertussis immunogenicity:

To demonstrate that ProQuad can be administered concomitantly with Tripedia and Comvax without impairing the immune response to measles, mumps, rubella, varicella, diphtheria, tetanus, pertussis toxin (PT), pertussis FHA, hepatitis B, and *Haemophilus influenzae* type B (Hib).

2.1.2 Design

This was an open, multi-center, randomized study designed to evaluate the concomitant administration of ProQuad, Tripedia, and Comvax (Hib conjugate and hepatitis B vaccine, Merck). The targeted enrollment was 1600 healthy children, 12 to 15 months of age. The study period was 27-Jun-2000 through 23-Oct-2001.

Subjects were assigned to 1 of 3 study groups in a 2:1:1 ratio, with a planned enrollment of 800 subjects in Group 1 and 400 subjects in each of Groups 2 and 3.

Group 1 (concomitant group) received ProQuad, Tripedia, and Comvax concomitantly at separate injection sites on Day 0.

Group 2 (nonconcomitant group) received ProQuad on Day 0, and on Day 42 received Tripedia and Comvax concomitantly at separate injection sites.

Group 3 (control) received M-M-R II (measles, mumps, and rubella vaccine) and Varivax (varicella virus vaccine) on Day 0 concomitantly at separate injection sites and on Day 42 received Tripedia and Comvax concomitantly at separate injection sites. (Group 3 was intended to serve as a control for the safety analyses only).

2.1.3 Study Population

Subjects enrolled in this study were healthy children, 12 to 15 months of age. With regard to pertussis vaccination history, subjects were eligible if they had received 3 doses of whole-cell DTP or DTaP made by any manufacturer.

2.1.4 Products mandated by the protocol and vaccination schedule

Table 1. Vaccines, Dosage Schedules, and Route of Administration by Treatment Group

Group	Vaccine	Dosage Schedule	Route
1	ProQuad Tripedia Comvax	Day 0 Day 0 Day 0	Subcutaneous Intramuscular Intramuscular
2	ProQuad Tripedia Comvax	Day 0 Day 42† Day 42†	Subcutaneous Intramuscular Intramuscular
3	Varivax M-M-R II Tripedia Comvax	Day 0 Day 0 Day 42† Day 42†	Subcutaneous Subcutaneous Intramuscular Intramuscular

†For Groups 2 and 3 only, a +14-day window was allowed for administration of Tripedia and Comvax

2.1.5 Immunogenicity Assessment (only pertussis immunogenicity addressed in this review)

Serum samples were obtained from each subject prior to the first vaccination and 6 weeks following each scheduled vaccination. Serum samples from Groups 1 and 2 were tested for antibody to PT and FHA at ---------- by an indirect, noncompetitive EIA. The anti-PT and anti-FHA primary endpoints and non-inferiority criteria are listed in Table 2.

Table 2. Anti-PT and anti-FHA primary endpoints and non-inferiority criteria

Antigen	Endpoint	Non-inferiority criterion
PT, FHA	% with ≥4-fold rise in titer	LL of two-sided 90% CI for difference (Concomitant Group 1 – Non-Concomitant Group 2) >-15

2.1.6 Relevant protocol amendments

Apparently, because of a delay in the processing of serum samples for antibodies to PT and FHA due to conflicting testing priorities at ----, the protocol was amended to specify that the primary analyses for PT and FHA would be conducted using all results available at the time of writing the Clinical Study Report. It was expected that at least 400 subjects from Group 1 and 200 subjects from Group 2 would have their results available for inclusion in the primary

analyses for PT and FHA. When all results are available for the complete set of subjects, a special report updating the analysis for these components will be issued.

2.1.7 Statistical considerations

2.1.7.1 Statistical power
Assuming that 400 subjects from Group 1 and 200 subjects from Group 2 would have available serology data for PT and FHA, the statistical power for each of the pertussis antigen primary analyses was >99%.

2.1.7.2 Study cohorts/data sets analyzed
The primary immunogenicity analyses were based on the per-protocol population that excluded subjects with significant protocol violations. The allowable day range for the 42-day post vaccination blood sampling was 27 to 84 days after the vaccine was administered. The analyses for anti-PT and anti-FHA were restricted to subjects who had valid baseline serology results.

Immunogenicity analyses were also conducted on two other subject populations. The “all subjects with serology” population included all subjects with valid serology endpoints regardless of any protocol violations. The “subjects with all serology” population, a subpopulation of the per-protocol population, included only those subjects in the per-protocol population who also had valid per-protocol serology results for all endpoints.

2.1.7.3 Adjustments for Covariates
The primary immunogenicity analyses of seroresponse rates were adjusted for study center.

2.1.7.4 Interim Analyses and Data Monitoring
No interim analyses were conducted. However, during data review and statistical program development, immunogenicity summaries were generated and differences in anti-PT and anti-FHA responses between groups were noted, which prompted further investigation at that time. This evaluation was conducted prior to file cleaning.

2.2 Results

2.2.1 Study population
Subjects were enrolled from 48 U.S. sites. Enrollment per site ranged from 1 to 253 subjects.

Table 3 presents an accounting of subjects enrolled in Study Group 1 (Concomitant) and Study Group 2 (Nonconcomitant). Table 4 presents demographic and other baseline characteristics by study group for Groups 1 and 2. Tables 3 and 4 include all enrolled subjects. Only a subset of these subjects had serology results available for the primary analyses of pertussis immunogenicity presented in subsequent sections.

Table 3. Accounting for subjects in the study

	Concomitant Group (N=949)		Nonconcomitant Group (N=485)	
	n	(%)	n	(%)
Entered:	949		485	
Male (age range in months)	507	(11 to 15)	262	(11 to 16)
Female (age range in months)	442	(12 to 15)	223	(12 to 15)
Vaccinated At:				
Vaccination Visit 1	949	(100)	485	(100)
Vaccination Visit 2	909	(95.8)	468	(96.5)
Completed	884	(93.2)	453	(93.4)
Discontinued:	65	(6.8)	32	(6.6)
Clinical adverse experience	0	(0.0)	0	(0.0)
Deviation from protocol	2	(0.2)	1	(0.2)
Refused further participation	18	(1.9)	9	(1.9)
Lost to follow-up	31	(3.3)	12	(2.5)
Clinical adverse experience - discontinued test vaccine	0	(0.0)	1	(0.2)
Missed one or more blood samplings	3	(0.3)	7	(1.4)
Incomplete safety follow-up	11	(1.2)	2	(0.4)

Concomitant Group = ProQuad + Tripedia + Comvax at Day 0.
Nonconcomitant Group = ProQuad at Day 0 followed by Tripedia and Comvax 6 weeks later.
Source: p013.pdf, page 91

Table 4. Demographic and other baseline characteristics for concomitant group and nonconcomitant group

	Concomitant Group (N=949)		Nonconcomitant Group (N=485)	
	n	(%)	n	(%)
Gender				
Male	507	(53.4)	262	(54.0)
Female	442	(46.6)	223	(46.0)
Age (Months)				
Mean	12.4		12.3	
SD	0.7		0.7	
Median	12.0		12.0	
Range	11 to 15		11 to 16	
Male	11 to 15		11 to 16	
Female	12 to 15		12 to 15	
Race/Ethnicity				
African American	101	(10.6)	52	(10.7)
Asian/Pacific	103	(10.9)	54	(11.1)
Caucasian	678	(71.4)	336	(69.3)
Hispanic	38	(4.0)	26	(5.4)
Native American	3	(0.3)	0	(0.0)
Other	26	(2.7)	17	(3.5)

Concomitant Group = ProQuad + Tripedia + Comvax at Day 0.
Nonconcomitant Group = ProQuad at Day 0 followed by Tripedia and Comvax 6 weeks later.
SD = Standard deviation; Source: p013.pdf, page 104

Table 5 presents a summary of subjects from Groups 1 and 2 who were excluded from the primary per-protocol pertussis immunogenicity analyses.

Table 5. Summary of subjects excluded from the primary per-protocol immunogenicity analyses of anti-PT and anti-FHA at 6 weeks post-vaccination

	Anti- PT		Anti-FHA	
	Concomitant Group	Nonconcomitant Group	Concomitant Group	Nonconcomitant Group
Subjects vaccinated with Tripedia at Visit 1:	949	-	949	-
Subjects vaccinated with Tripedia at Visit 2:	-	468	-	468
Subjects included in the analysis:	468	247	468	248
Subjects excluded from the analysis:	481	238	481	237
Younger than 12 months at the 1st vx	1	1	1	1
Randomized to wrong treatment group	3	8	3	8
Received compromised vaccines	21	12	21	12
Did not have correct dosage prior to the study	1	0	1	0
Missing or not evaluable baseline result	453	219	453	218
Missing or not evaluable post-vx result:‡	439	215	439	215
Sampling outside the specified day range	23	15	23	15
Refused to provide blood sample	15	12	15	12
Difficult to obtain blood sample	30	20	30	20
Sample quantity not sufficient	1	0	1	0
Sample hemolyzed	2	0	2	0
Sample was not taken	1	0	1	0
Discontinued due to adverse experience	0	1	0	1
Lost to follow-up	20	12	20	12
Refused further participation	13	9	13	9
Result was not available§	362	145	362	145

‡ Including randomized to the wrong treatment group.
§ Results were not available because the testing schedule only planned to finish ~50% of samples for inclusion in this Clinical Study Summary.
- = No vaccination with Tripedia scheduled.
A subject may be counted in more than one exclusion category.
Concomitant Group = ProQuad + Tripedia + Comvax at Day 0.
Nonconcomitant Group = ProQuad at Day 0 followed by Tripedia and Comvax 6 weeks later.
PT = Pertussis toxin.
FHA = Filamentous hemagglutinin.
Source: p013.pdf, page 97

2.2.2 Evaluation of antibody responses to PT and FHA, per-protocol analysis

Table 6 presents a summary of anti-PT and anti-FHA seroresponse rates and GMTs for the per-protocol population.

Table 6. Summary of anti-PT and anti-FHA seroresponse rates and GMTs, per-protocol analysis

Vaccine Component	Parameter	Concomitant Group (N=949)				Non-concomitant Group (N=485)			
		Pre-vaccination		Post-vaccination		Pre-vaccination		Post-vaccination	
		n	Observed Response (95% CI)	N	Observed Response (95% CI)	n	Observed Response (95% CI)	n	Observed Response (95% CI)
PT	% ≥4-fold rise	NA	NA	468	80.3% (376/468) (76.4%, 83.8%)	NA	NA	247	90.3% (223/247) (85.9%, 93.7%)
	GMT (Units/mL)	468	7.74 (7.06, 8.47)	468	60.5 (55.9, 65.4)	247	6.46 (5.63, 7.41)	247	71.2 (63.0, 80.4)
FHA	% ≥4-fold rise	NA	NA	468	69.7% (326/468) (65.3%, 73.8%)	NA	NA	248	87.5% (217/248) (82.7%, 91.3%)
	GMT (Units/mL)	468	12.5 (11.4, 13.7)	468	77.3 (71.3, 83.7)	248	10.7 (9.4, 12.2)	248	101 (91, 113)

Percentages were calculated as the number of subjects who met the criterion divided by the number of subjects contributing to the per-protocol analysis.
Only subjects who had valid antibody results at both pre-vaccination and 6 weeks post-vaccination are included in the calculation of percent with ≥ 4-fold rise.
Concomitant Group = ProQuad + Tripedia + Comvax at Day 0.
Nonconcomitant Group = ProQuad at Day 0 followed by Tripedia and Comvax 6 weeks later.
N = Number of subjects vaccinated in each treatment group.
n = Number of subjects contributing to the per-protocol analysis.
GMT = Geometric mean titer.
CI = Confidence interval.
NA = Not applicable.
PT = Pertussis toxin.
FHA = Filamentous hemagglutinin.
Source p013.pdf, page 116

Table 7 presents the evaluation of the primary non-inferiority analyses for anti-FHA and anti-PT seroresponse (≥4-fold increase) rates.

Table 7. Evaluation of non-inferiority of antibody responses for FHA and PT for the concomitant group compared with the nonconcomitant group (per-protocol population)

Vaccine Component	Parameter	Concomitant Group (N=949)		Nonconcomitant Group (N=485)		Estimated Difference†‡ (90% CI)	Non-inferiority Criterion
		n	Estimated Response†	n	Estimated Response†		
PT	% ≥4-fold rise in titer	468	80.5%	247	90.0%	-9.5 (-13.8 , -5.1)	LL>-15.0
FHA	% ≥4-fold rise in titer	468	69.6%	248	87.4%	-17.7 (-22.6 , -12.7)	LL>-15.0

† Responses and their differences are based on a statistical analysis model adjusting for study center.
‡ [ProQuad + Tripedia + Comvax] - [ProQuad Followed by Tripedia+ Comvax].
Concomitant Group = ProQuad + Tripedia + Comvax at Day 0.
Nonconcomitant Group = ProQuad at Day 0 followed by Tripedia and Comvax 6 weeks later.
N = Number of subjects vaccinated in each treatment group.
n = Number of subjects contributing to the per-protocol analysis.
CI = Confidence interval.
PT = Pertussis toxin.
FHA = Filamentous hemagglutinin.
LL = Lower Limit (of 2-sided 90% confidence interval).
Source p013.pdf, page 123

2.2.3 Evaluation of antibody responses to PT and FHA, "subjects with all serology" and "all subjects with serology"

The results of the anti-PT and anti-FHA GMTs and seroresponse (≥4-fold rise) rates for the "subjects with all serology" population and the "all subjects with serology" population, including the non-inferiority analyses for the concomitant group relative to the nonconcomitant group, were similar to the results of the per-protocol analyses presented above in Tables 6 and 7.

2.2.4 Post-hoc exploratory analyses of immune response to FHA

By design, subjects in the concomitant group received ProQuad, Tripedia, and Comvax on Day 0 when they were at least 12 months of age, while the nonconcomitant group did not receive Tripedia until 6 weeks later, at which time most subjects were at least ~13.5 months of age (12 months + 6 weeks). The mean age at time of vaccination with Tripedia was 12.7 months for the concomitant group and 14.2 months for the nonconcomitant group. Also inherent to the study design, there were fewer subjects in the concomitant group with at least 180 days (~6 months) between the third dose of DTP vaccine and receipt of Tripedia in this study. This dosing interval was analyzed because it is the minimum interval between the third and fourth dose of DTaP recommended by the ACIP.

The effects of age at receipt of Tripedia and of interval since the third dose of DTP vaccine on the immune responses to FHA were investigated in exploratory post-hoc analyses presented in Tables 8 and 9.

Table 8 provides a summary of immune responses to FHA by age at vaccination with Tripedia (<13.5 months of age or ≥13.5 months of age). The age cutoff of 13.5 months was selected because most subjects in the nonconcomitant group were at least this age at the time of administration of Tripedia. As demonstrated in Table 8, the observed treatment differences were diminished in subjects ≥13.5 months of age relative to younger subjects, suggesting that age at vaccination with Tripedia may have had an effect on the immune response to FHA.

Table 9 provides a summary of immune responses to FHA by the number of days elapsed since receipt of the third dose of DTP vaccine (<180 days or ≥180 days). The sponsor noted that subjects who had at least 180 days elapsed since the third dose of DTP vaccine had smaller differences in their post-vaccination FHA GMT between the two treatment groups than the subjects who did not, and suggested that the dosing interval might also influence the immune response to FHA. However, this analysis was based on only 9 subjects in the nonconcomitant group who received Tripedia <180 days since the third dose of DTP vaccine. Furthermore, among subjects with a dosing interval of ≥180 days, the anti-FHA seroresponse rate was still considerably lower in the concomitant group than the nonconcomitant group (76.4% vs. 88.7%, with non-overlapping confidence intervals).

To further explore the relationship between the FHA antibody response as measured by GMTs and various potential explanatory variables, several analysis of covariance models were constructed with the natural logarithm of the post-vaccination antibody titer as the dependent variable, and explanatory variables as potential independent variables. In each of these models, the statistical significance of each parameter in predicting post-vaccination GMTs was examined and the fold difference of GMTs (concomitant/nonconcomitant) was estimated along with its 90% CI. The results of these models, not detailed here, but presented in the BLA (p013.pdf, p. 150, Table 32), suggested that age at vaccination with Tripedia had an influence on the antibody responses, independent of days since previous DTP vaccination and pre-vaccination antibody titer.

The sponsor acknowledged that interpretation of these post-hoc analyses is limited by small sample sizes for some comparisons and the fact that treatment group, age at vaccination with Tripedia, and interval since previous dose of DTP vaccine are highly confounded.

Table 8. Antibody responses to FHA by age at vaccination with Tripedia, per-protocol population

Parameter	Age at Vaccination With Tripedia	Concomitant Group (N=949)				Nonconcomitant Group (N=485)			
		Pre-vaccination		Post-vaccination		Pre-vaccination		Post-vaccination	
		n	Observed Response (95% CI)	n	Observed Response (95% CI)	N	Observed Response (95% CI)	n	Observed Response (95% CI)
% ≥4-fold rise in titer	<13.5 Months	NA	NA	406	67.5% (62.7%, 72.0%)	NA	NA	20	90.0% (68.3%, 98.8%)
	≥13.5 Months	NA	NA	62	83.9% (72.3%, 92.0%)	NA	NA	228	87.3% (82.2%, 91.3%)
GMT (Units/mL)	< 13.5 Months	406	12.6 (11.4, 13.9)	406	73.9 (67.9, 80.3)	20	14.4 (10.6, 19.7)	20	108 (76.5, 153.5)
	≥13.5 Months	62	11.7 (9.0, 15.2)	62	104 (81.8, 132.3)	228	10.5 (9.1, 12.0)	228	101 (90.0, 112.9)

Percentages were calculated as the number of subjects who met the criterion divided by the number of subjects contributing to the per-protocol analysis.
Concomitant Group = ProQuad + Tripedia + Comvax at Day 0.
Nonconcomitant Group = ProQuad at Day 0 followed by Tripedia and Comvax 6 weeks later.
N = Number of subjects vaccinated in each treatment group.
n = Number of subjects contributing to the per-protocol analysis.
GMT = Geometric mean titer.
CI = Confidence interval.
NA = Not applicable.
Source: p013.pdf, page 142

Table 9. Antibody responses to FHA by days elapsed since the third dose of DTP vaccine, per-protocol population

Parameter	Days Elapsed Since Third Dose of DTP	Concomitant Group (N=949)				Nonconcomitant Group (N=485)			
		Pre-vaccination		Post-vaccination		Pre-vaccination		Post-vaccination	
		n	Observed Response (95% CI)	n	Observed Response (95% CI)	n	Observed Response (95% CI)	n	Observed Response (95% CI)
% ≥4-fold rise in titer	<180 Days	NA	NA	95	43.2% (33.0%, 53.7%)	NA	NA	9	55.6%
	≥180 Days	NA	NA	373	76.4% (71.8%, 80.6%)	NA	NA	239	88.7% (84.0%, 92.4%)
GMT (Units/mL)	<180 Days	95	17.2 (14.3, 20.8)	95	66.5 (56.3, 78.6)	9	27.6	9	121
	≥180 Days	373	11.5 (10.4, 12.8)	373	80.3 (73.3, 87.9)	239	10.4 (9.1, 11.8)	239	101 (90.3, 112.3)

Percentages were calculated as the number of subjects who met the criterion divided by the number of subjects contributing to the per-protocol analysis.
Concomitant Group = ProQuad + Tripedia + Comvax at Day 0.
Nonconcomitant Group = ProQuad at Day 0 followed by Tripedia and Comvax 6 weeks later.
N = Number of subjects vaccinated in each treatment group.
n = Number of subjects contributing to the per-protocol analysis.
GMT = Geometric mean titer.
CI = Confidence interval (not provided for n <10)
FHA = Filamentous hemagglutinin.
NA = Not applicable.
Source: p013.pdf, page 144

3. Reviewer Comments and Conclusions

3.1 Major findings

Study P013 was designed, in part, to evaluate the immune responses to the acellular pertussis component of Tripedia when administered to children 12-15 months of age concomitantly with ProQuad and Comvax or on a staggered schedule, with ProQuad administered at one visit, and Tripedia and Comvax administered 42 days later. The pertussis immunogenicity results demonstrate a tendency towards lower antibody responses (as measured by GMTs and proportion of subjects with a $\geq$4-fold rise in titer) for both PT and FHA when Tripedia and ProQuad were given concomitantly versus non-concomitantly. The magnitude of the differences between groups was greater for FHA than PT. Non-inferiority criteria (LL of 90% CI for difference of Concomitant – Nonconcomitant > -15%) were pre-specified for seroresponse (4-fold rise) rates for FHA and PT. These criteria are less stringent than that currently recommended by CBER (non-inferiority margin of 10%, based on LL of 95% CI) and used in other more recent studies with assays performed in other labs. For FHA, the pre-specified non-inferiority criterion was missed, with the lower limit of the 90% CI for the difference (Concomitant – Nonconcomitant) calculated as –22.6. For PT, the pre-specified non-inferiority criterion was met, with the lower limit of the 90% CI for the difference (Concomitant – Nonconcomitant) calculated as -13.8.

3.2 Data interpretation

The pertussis immunogenicity data from this study are difficult to interpret for a number of reasons. First, subjects who previously received 3 doses of whole-cell DTP or DTaP made by any manufacturer could have been enrolled. Given the numerous priming scenarios possible (e.g., all DTwP, mixed DTwP-DTaP, all Tripedia, all DTaP from a single different manufacturer, mixed DTaP), it is conceivable that potential differences in priming history between groups may have affected the results.

Second, inherent in the study design, treatment group, age at receipt of Tripedia, and interval since the third dose of DTP were highly confounded. These confounding factors make it difficult to reliably interpret the exploratory analyses of the effect of age and dosing interval on the immune responses to FHA. Furthermore, these exploratory analyses have the important limitation of being post-hoc and data driven. Reliability of the post-hoc analyses is also limited by the small sample sizes for some comparisons.

Third, it is not clear from the study report contained in the BLA whether information on the methodology and validation for the anti-PT and anti-FHA assays performed by ----------, have been submitted and reviewed by the appropriate product reviewers at CBER, and whether CBER has agreed that the assays are acceptable.

In addition to the difficulties with data interpretation described above, of note is that in Study P013, use of Tripedia was inconsistent with the approved schedule in which the fourth dose is recommended at 15 to 18 months of age, and interchanging Tripedia and DTaP vaccine from different manufacturers for successive doses of the vaccination series is not recommended.

In conclusion, the pertussis immunogenicity data from this study are not adequate to support concomitant immunization of ProQuad and Tripedia in any age group.

3.3 Addressing concomitant immunization with DTaP in the ProQuad package insert
In the draft of the ProQuad package insert submitted with the BLA, the sponsor has addressed concomitant immunization of ProQuad with DTaP in the Clinical Pharmacology section and in the Precautions section (see sections 3.3.1 and 3.3.2 below). A subsection on use with other vaccines in Dosage and Administration includes reference to the Precautions section (see section 3.3.3 below).

3.3.1 Clinical Pharmacology

The Clinical Pharmacology Section of the ProQuad package insert submitted with the BLA contains a section entitled "Studies With Other Vaccines" in which the sponsor describes Study P013 and summarizes the immunogenicity results, stratified by age <13.5 months and ≥13.5 months. This section also includes the statement that "No clinically significant differences in adverse experiences were reported between the 2 treatment groups".

Reviewer recommendations:

1. ----------

2. I have not reviewed the Adverse Reactions section of the ProQuad package insert, nor the safety evaluation methods or safety data from Study P013. I also do not know the relative contribution of this study to the overall safety evaluation of ProQuad. If the BLA review committee finds that insufficient information is available to reliably assess the safety of concomitant administration of ProQuad with Tripedia but no concerning safety signal is detected from the available data, one consideration for the package insert might be to provide the number of children who received these vaccines concomitantly, with no further comment on the safety evaluation. However, I defer to the BLA review committee regarding interpretation of the safety data from Study P013 and decisions about including these data in the ProQuad package insert.

3.3.2 Precautions

The Precautions section of the ProQuad package insert submitted with the BLA contains a subsection entitled "Drug Interactions, Use with Other Vaccines". This section contains the following statements:

"The fourth dose of diphtheria, tetanus, and acellular pertussis vaccine (DTaP) is indicated for children 15 months of age and older. Limited data suggest that ProQuad may be administered concomitantly (at separate injection sites) with DTaP in children 15 months of age and older (for children less than 15 months of age see CLINICAL PHARMACOLOGY)."

Reviewer recommendations:

1. The paragraph quoted above should be deleted.

2. ----------.

3.3.3 Dosage and Administration

Reviewer recommendations:

1. ----------

2. As recommended above for the Precautions section, this subsection should also indicate that data are not available to reliably assess the pertussis immune responses to DTaP when administered concomitantly with ProQuad.

3.4 Post-marketing evaluation of ProQuad administered concomitantly with DTaP

3.4.1 Safety evaluation of concomitant administration of ProQuad and DTaP

Not having reviewed the safety data in the ProQuad BLA, I defer to the BLA committee regarding the overall need for post-marketing safety evaluation of ProQuad. The following comments pertain specifically to the evaluation of the safety of concomitant administration of ProQuad with DTaP.

Assessment of local reactions has been of primary interest in the safety evaluation of consecutive doses of DTaP vaccines since available data demonstrate that the frequency and severity of local reactions increase with consecutive doses in the DTaP series, particularly with the fourth and fifth doses. It seems unlikely that administration of ProQuad with a fourth or fifth consecutive dose of DTaP would potentiate local reactions at the DTaP injection site relative to separate, concomitant administration of MMR, Varivax, and DTaP. In previous studies of DTaP vaccines, no other particular safety concerns with administration of the fourth and/or fifth doses in the series have been identified. Therefore, unless concerns arise with the review of the safety data in the ProQuad BLA, there does not appear to be a need to specifically assess the safety of concomitant administration with DTaP post-marketing.

3.4.2 Immunogenicity evaluation of concomitant administration of ProQuad and DTaP

My comments below pertain only to the evaluation of the effect of ProQuad on the pertussis immune responses following concomitantly administered DTaP.

If the sponsor desires to claim that ProQuad does not interfere with the pertussis immune responses to DTaP when these vaccines are administered concomitantly, an appropriately designed, controlled immunogenicity study would be needed. To obtain interpretable pertussis immunogenicity data, such a study should be conducted in subjects who received the same DTaP vaccine being evaluated at the fourth dose for all three previous doses. In addition, the fourth dose of DTaP should be administered in accordance with the package insert recommendations for age at vaccination (generally 15-20 months of age, with some differences in age range among licensed DTaP vaccines) and minimum interval since the third dose. If such a study is conducted, the assessment of the pertussis immune responses should include evaluation of both GMTs and seroresponse rates for each of the pertussis antigens contained in the DTaP vaccine evaluated. The methodology and validation data for the pertussis serology assays would need to be reviewed by CBER product reviewers.