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Guidelines for the clinical evaluation of dengue vaccines in endemic areas

Immunization, Vaccines and Biologicals

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Abbreviations and acronyms

AE	adverse events
CMI	cell mediated immunity
DB RCT	double blind randomized controlled trial
DF	dengue fever
DHF	dengue haemorrhagic fever
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
DSS	dengue shock syndrome
DV	dengue virus
ELISA	enzyme-linked immunosorbent assay
EIA	enzyme immuno assay
FDA	Food and Drug Administration (US)
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMO	genetically modified organism
GMP	Good Manufacturing Practice
HI	haemagglutination inhibition
HIV	human immunodeficiency virus
ICH	International Conference for Harmonization
IgM	immunoglobulin M
IgG	immunoglobulin G
IRB	Institutional Review Board
NIP	National Immunization Programme
NRA	National Regulatory Authority
NS	non-structural
PAHO	Pan American Health Organization
PBMC	peripheral blood mononuclear cell

PCR	polymerase chain reaction
PDVI	Pediatric Dengue Vaccine Initiative
PRNT	plaque reduction neutralization test
QA	quality assurance
QC	quality control
RNA	ribonucleic acid
RT	reverse transcription
SAE	serious adverse event
SOP	standard operating procedures
VE	vaccine efficacy

1. Introduction

1.1 Purpose

This document is addressed to national health and regulatory authorities in dengue-endemic countries interested in using vaccines to control the disease. It is also written for vaccine developers and research scientists interested in the development and field evaluation of such vaccines. The guidelines, which were developed with contributions and comments from many individuals from a variety of countries and institutions (see Annex 1), are designed to help identify the basic technical information required to design dengue vaccine field trials. The purposes of the field trials are firstly, to obtain sufficient data on vaccine safety and efficacy to support vaccine licensure, and secondly, to establish that, in post-licensure field studies, the vaccine proves to be safe and provides long-term protection.

1.2 Justification

There is an urgent need to field test dengue vaccines to control the accelerating spread of dengue in the world (1,2). Basic and clinical research on dengue vaccines has also advanced rapidly, supported by the vaccine industry, governments, foundations, WHO, the Pediatric Dengue Vaccine Initiative (PDVI) and others. Candidate dengue vaccines in preclinical or clinical stages of development include live vaccines attenuated by passage in dog kidney cells or constructed as live flavivirus chimeras, recombinant subunit formulations, inactivated whole virus, and other vaccine constructs (3,4,5). As several promising live-attenuated vaccine candidates are currently in the later stages of clinical development, there is a need for guidelines focused on the design of pivotal efficacy trials that can inform national regulatory authorities (NRAs) and vaccine developers.

An international consensus exists that clinical development of dengue vaccines should not be forestalled by certain hypothetical safety concerns. The first concern is the possible enhancement of the clinical response to live-attenuated dengue vaccine viruses when administered to flavivirus-immune individuals. The second is that a sub-immunogenic vaccine, or a vaccine whose efficacy wanes over time, could leave a recipient with an "immune profile" which not only fails to protect, but increases the risk for experiencing severe dengue through complex immunopathological mechanisms following subsequent natural infection (6). The third concern is that intragenic recombination between live vaccines and virulent wild-type viruses might increase the virulence of an attenuated dengue virus vaccine and enhance spread of disease. This possibility is highly speculative and has been dismissed by a majority of experts in flavivirus biology (7).

Indeed, following preclinical tests, the safety, immunogenicity and protective efficacy of vaccine candidates will be clarified by rigorous field trials, which will be designed in part to test such hypothetical safety concerns. This document aims to provide guidance for these trials.

The document is also meant to stimulate scientifically meritorious studies that may not directly accelerate vaccine licensure, but which nevertheless may help explain mechanisms related to vaccine safety and protective efficacy.

Finally, this document is not intended to provide guidelines for the introduction of dengue vaccines into national immunization programmes. This will require changing public-health policy to incorporate a new vaccine into health-care practice, which will require more information than that provided by the Phase 1 to 4 trials described in the document.

In spite of knowledge accumulated recently, some issues remain undecided, and indeed controversial. These guidelines reflect some of these uncertainties but do not attempt to settle them. Trial sponsors and authorizing agencies are encouraged to discuss pending issues via an informed dialogue. The guidelines are a living document, and as such should be revised as science progresses.

1.3 Scope

A first edition of the WHO Guidelines for the evaluation of dengue vaccines in populations exposed to natural infection, was published in 2002 (8). The present document reiterates and updates many of the issues contained in the 2002 edition and addresses more fully some others, including:

a) the need for **vaccine trial end-points**, from mild self-limited dengue fever through different clinical expressions of severe dengue to classical dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) (see Glossary 1.6;

b) the importance of efforts to establish **immune correlates of protection** against dengue disease;

c) possible **dengue inhibitory or enhancing effects** afforded by prior infection by yellow fever, Japanese encephalitis and other flaviviruses, or by flavivirus vaccinations in endemic areas;

d) the use of **Phase 2 or 3 bridging studies, post Phase 3 follow-up safety studies and Phase 4 post-licensure trials** to better elucidate vaccine immunogenicity, protective efficacy, or safety (see Glossary 1.6).

This guideline emphasizes the protection of participants in dengue vaccine trials and the need for a strong regulatory infrastructure, including the presence of local institutional review boards (IRBs), data safety monitoring boards (DSMBs), internal quality control (QC) and external quality assurance (QA) boards, and NRAs.

For a generic guidance on clinical trial design and methods, the reader is referred to the 1999 European Agency for the Evaluation of Medicinal Products document, *Note for guidance on clinical evaluation of new vaccines* (9), and the 2004 WHO document, Guidelines on clinical evaluation of vaccines: regulatory expectations (10). In addition, a few basic terms on the types of clinical trials and what they can accomplish is included in the Glossary (1.6).

Recent WHO documents on the non-clinical evaluation of vaccines (11), and more specifically in the WHO Guidelines for Production and Quality Control of Candidate Tetravalent Live Dengue Vaccines (12), provide additional background guidance, and should be consulted. Information on vaccine guidelines may be obtained from the Immunization, Vaccines and Biologicals Department, World Health Organization, Geneva (13).

As implied above, this document emphasizes many unique aspects of dengue vaccine trials. These include strong recommendations for: 1) extended times for preparing the trial and for follow-up after the trial in order to collect accurate dengue incidence data across multiple transmission seasons; 2) extended follow-up times for tracking vaccine safety and long-term protection of vaccinated populations in endemic areas where multiple dengue types and other flaviviruses circulate and where flavivirus vaccines are widely used.

1.4 Regulatory requirements for clinical trials and registration (licensing) of a vaccine

1.4.1 Preclinical evaluation guidelines

The reader is referred to the 2005 *WHO Guidelines on Nonclinical Evaluation of Vaccines* (11), and for live vaccines to the 2006 WHO preclinical document, *WHO Guidelines for the Production and Quality Control of Candidate Tetravalent Live Dengue Vaccines* (12).

For other vaccine types there are general guidelines on the production and control of recombinant products, such as recombinant DNA-derived vaccines (13), and DNA-vaccines (14), and these should be consulted. Guidelines on other live attenuated vaccines produced in primary cell cultures or eggs may be useful, e.g. live attenuated Japanese encephalitis vaccine (15), or yellow fever vaccine (16). International guidelines on release of genetically modified organisms (GMOs) will need to be consulted for live attenuated vaccines based on GMOs (14).

Information about assuring the quality of biologicals in general, and on procedures for approving (licensing) products can be found in WHO Guidelines for National Authorities on Quality Assurance for Biological Products (17), as well as WHO's Good Manufacturing Practices for Biological Products (18). The WHO document on Regulation and Licensing of Biological Products in Countries with Newly Developing Regulatory Authorities (19) also contains much useful information, including reference to authorization of clinical trials. All WHO guidelines can be downloaded from the Internet at http://www.who.int/immunization_standards/publications_media/en/ (accessed 2008).

1.4.2 Clinical evaluation guidelines

The World Health Organization, the International Conference for Harmonization (ICH) and The Food and Drug Administration (FDA) guidelines for Good Clinical Practice (GCP) for trials on pharmaceutical products (20,21,22) provide international ethical and scientific quality standards for designing, conducting, recording, and reporting biomedical research on human subjects. Documentation of compliance with GCP is required for all submissions approved by competent regulatory authorities.

Compliance with these guidelines is intended to assure that the rights, safety, and well-being of participants are protected, and that the data obtained from a clinical trial are credible. The guidelines should be applied during all stages of product development both before and after product registration and marketing, and are applicable, in whole or in part, to biomedical research in general. WHO will also consider requests for assistance from countries, and may be able to convene expert panels in GCP to help evaluate proposals (10).

1.5 Potential uses of vaccines for dengue control

In order to design meaningful field trials, some assumptions need to be made about the way vaccines might be used for dengue control in different epidemiological settings. Among other considerations, vaccination strategies depend on the intensity and duration of protection provided by the vaccine. As a rule, the product profile of a dengue vaccine should meet the most pressing public-health needs of disease-endemic countries, bearing in mind that, depending on the country, dengue may be found principally in children, or in individuals of all ages. Vaccination schedules should be discussed with national and international public-health authorities. Specific, distinct vaccine requirements may apply to WHO vaccine prequalification (23). Some plausible dengue vaccine applications are listed below. The list is not intended to be exhaustive.

a) **Routine immunizations:** Adding a dengue vaccine to routine immunization campaigns may be warranted in areas where significant DV transmission occurs and where the burden of disease on the local population is well established. The inclusion of dengue vaccinations in any national immunization programme (NIP) raises important issues that need to be addressed, such as possible immunologic interference between the dengue vaccine and the other NIP vaccines, and the optimal timing of dengue vaccination. In certain countries, safety and efficacy issues related to vaccination of HIV-positive children should be considered.

b) **Catch-up campaigns:** In conjunction with introduction into infant or childhood routine immunization, catch-up campaigns to immunize people who have completed their NIP vaccinations can target children, adolescents, and adults. This population may be partially flavivirus-immune, so that vaccine immunogenicity and safety in such a setting needs to be assured.

c) **Management of dengue epidemics:** Catch-up vaccination campaigns as part of a time-limited effort to interrupt transmission of DV, or as an emergency approach for managing a dengue epidemic, may include immunizing adolescents and adults in addition to children. Administering a vaccine to these age groups may require conducting specialized studies beforehand, such as determining the vaccine's ability to quickly stimulate an immune response, how long it will protect, and whether it can be safely administered to people with compromised immune systems and pregnant women.

d) **Travellers:** A dengue vaccine could provide protection of non-immune, temporary visitors to endemic areas (e.g. travellers, seasonal labourers, or military personnel). Among travellers, demand for a dengue vaccine is likely to be brisk in the absence of an effective preventive or therapeutic drug. The optimal vaccine should therefore rapidly induce high-grade protection. Determining when and how often to administer the vaccine may be complicated by the need to vaccinate travellers against other flaviviruses, such as Japanese encephalitis or yellow fever.

1.6 Glossary of clinical trial and dengue-related terms

The complex issues surrounding the design and implementation of dengue vaccine trials require that researchers and regulators become familiar with terms associated with dengue and clinical trials, and in particular those associated with dengue vaccine trials. A sample of terms and their definitions are listed below (10,20).

Adverse event: Any untoward medical occurrence in a clinical trial subject to which a vaccine has been administered; it does not necessarily have a causal relationship with the vaccine/vaccination.

Adverse reaction: A response to a vaccine that is noxious and unintended, and that occurs at doses tested in humans for prophylaxis, or during subsequent clinical use, following licensure. The term adverse reaction is usually reserved for cases where there is clear evidence that an adverse reaction is caused by a drug or a vaccine.

Audit: A systematic and independent examination of trial-related activities and documents to determine whether the evaluated trial-related activities were conducted, and the data were recorded, analysed, and accurately reported according to the protocol, sponsor's standard operating procedures (SOPs), GCP, and the regulatory requirements applicable.

Blinding: A procedure in which one or more parties to the trial are kept unaware of the treatment assignment(s). Single blinding usually refers to the subject(s) being unaware of the treatment assigned to them, and double blinding usually refers to the subject(s), investigator(s), and in some cases, data analyst(s), being unaware of the treatment assignment.

Booster vaccination: Vaccination given at a certain time interval after primary vaccination in order to improve immune responses and induce long-term protection.

Bridging studies: Studies intended to support the extrapolation of efficacy, safety and immunogenicity data from one formulation, population or dose regimen to another.

Case–control study: An observational study in which the exposure to a particular risk factor (the vaccine in the case of vaccine studies), is determined retrospectively, and the effect of this exposure is compared between individuals (the cases) who experience an event (the disease in vaccine studies), and individuals who do not (the controls).

Case definition: A set of diagnostic criteria that must be fulfilled to confirm a case of a particular disease. Case definitions can be based on clinical criteria, laboratory criteria, or combinations of the two.

Clinical trials: A prospective biomedical or behavioural research study of human subjects designed to answer specific questions about biomedical or behavioural interventions (drugs, vaccines, treatments, devices), or new ways of using these interventions. Clinical trials are used to determine whether new biomedical or behavioural interventions are safe, efficacious, and effective. Biomedical clinical trials of vaccines may proceed through four phases.

- **Phase 1:** Clinical trials of new vaccines are tested in a small group of closely monitored people (e.g. 20–80) for the first time to evaluate safety, side-effects and a safe dosage range. Preliminary information about vaccine immunogenicity is often obtained.
- **Phase 2:** Clinical trials study vaccines in a larger group of people (several hundred), and are often randomized and well controlled. Phase 2 vaccine trials are intended to demonstrate the immunogenicity and safety profile of a candidate vaccine. Ultimately, Phase 2 studies should define the optimal dose, initial schedule and safety profile of a candidate vaccine in the target population, before the Phase 3 trials can begin. Phase 2b trials are Phase 2 trials that have been expanded or extended in order to detect any preliminary evidence of efficacy in the target population.
- **Phase 3** studies are large-scale clinical trials designed to provide data on vaccine efficacy and safety by comparing the experimental vaccine to control or placebo vaccine under field conditions. Phase 3 studies are also designed to monitor adverse effects and to collect information that will allow the intervention to be used safely. Phase 3 trials are often designed to obtain data to support licensure by NRAs.
- **Phase 4** studies are conducted after the intervention has been licensed and marketed. These studies are designed to monitor effectiveness of the approved intervention in the general population, and to collect information about any adverse effects associated with its widespread use.

Cohort study: A retrospective or prospective study in which the development of a disease or infection, or any other relevant event, is observed over time in a defined group of subjects.

Control: A comparator in a vaccine trial that does not include the antigen under study. The control may be an inert placebo (e.g. saline solution or the vehicle of the vaccine) or an antigenically different vaccine (control vaccine).

Data Safety Monitoring Board: A group of individuals with pertinent expertise that reviews on a regular basis accumulating data from one or more ongoing clinical trials. The DSMB advises the sponsor regarding the continued safety of trial subjects and those yet to be recruited to the trial, as well as the continuing validity and scientific merit of the trial.

Dengue: For the purposes of this document, it refers to the entire spectrum of disease caused by dengue viruses and must be virologically confirmed. It includes clinically undifferentiated febrile illness, dengue fever, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (see below).

- **Severe dengue** are clinically severe dengue syndromes, including DHF and DSS, as well as all episodes of organ dysfunction after infection ("complicated dengue") (see 2.2).
- **Mild dengue** are those clinical episodes defined by exclusion as "non-severe." The terms "mild" and "severe" describe the clinical intensity of dengue and are defined by clinical course. The clinical (and laboratory) criteria defining mild and severe dengue need to be developed prior to beginning a dengue vaccine trial (see 2.2).
- **Dengue fever (DF):** The most common form of clinically apparent dengue virus infection. DF is characterized by fever of two to seven days duration, headache, gastrointestinal symptoms, muscle, joint, and bone pain, and a rash.
- **Dengue haemorrhagic fever:** The severest form of dengue. DHF is characterized by the signs and symptoms of DF followed by haemorrhage and/or increased vascular permeability which may lead to vascular collapse, DSS and death.

Effectiveness trial: A field trial, typically conducted post-licensure, which measures the performance of a vaccine under the pragmatic conditions of a public-health programme. An effectiveness trial can capture direct and indirect effects (e.g. herd immunity), and can address outcomes of public-health concerns such as serious but rare vaccine-associated adverse effects.

Efficacy trial: A field trial, typically designed to support vaccine licensure, which measures the performance of a vaccine under idealized conditions. Such trials typically seek to measure direct vaccine protection in restricted populations using idealized schedules, and do not depend on the pattern and extent of vaccine coverage of a population. For this reason, an efficacy trial demonstrating protection may not always be able to accurately predict the level of protection that will be achieved in public-health practice.

Experimental study: A study in which the conditions are under the direct control of the investigator. Such studies may include random allocation of subjects to treatment or control groups and blinding of subject and investigator to the placement status (i.e. whether an individual is in the treatment or control group).

Flavivirus: A virus in the genus of the family Flaviviridae, which comprises more than 68 principally arthropod transmitted or zoonotic viruses, of which 30 are known to cause human disease. In addition to the four dengue viruses, this virus family includes Japanese encephalitis, yellow fever, West Nile, tick-borne encephalitis, and other viruses that cause central nervous system infection, haemorrhagic fever, or acute febrile illnesses with joint pain and a rash. They share serologically cross-reacting antigens.

Good Clinical Practice (GCP): A standard for clinical studies that encompasses the design, conduct, monitoring, terminations, audit, analyses, reporting and documentation of the studies. It ensures that they are scientifically and ethically sound, and that the clinical properties of the pharmaceutical product under investigation (diagnostic, therapeutic or prophylactic) are properly documented.

Good Laboratory Practice (GLP): A collection of detailed standards that mandate specific operating procedures covering operating procedures for basic research, data acquisition and reporting. Also included are laboratory design and utilization requirements, which are enforced by regulatory agencies.

Good Manufacturing Practice (GMP): That part of the pharmaceutical quality assurance process which ensures that products are consistently produced and meet the quality standards appropriate to their intended use and as required by the marketing authorization. In these guidelines, good manufacturing practice refers to the current good manufacturing practice guidelines published by WHO [18]

Immune and immunity to dengue: They indicate resistance to developing overt clinical disease. Resistance may result from innate resistance to infection, from a previous dengue virus infection, or from vaccination.

Immune response: Describes the host's humoral (antibody-mediated) or cellular (immune-cell-mediated) immune responses as the result of exposure to viral proteins (antigens) via infection or vaccination.

Immunogenicity: Describes the ability of the vaccine to stimulate immune responses, such as antibody-mediated, cell-mediated and immunological memory. Although indicative that an immune response has occurred, such responses do not necessarily correlate with resistance to infection or disease.

Infection rate: The proportion of the population exposed to an infectious agent who become infected with that agent. The attack rate is the proportion of persons exposed to an infectious agent who become ill.

Incidence: The number of persons who fall ill with a certain disease during a defined time period.

Informed consent: A subject's voluntary confirmation of his or her willingness to participate in a particular trial, and the documentation thereof. This consent should be sought after giving the subject appropriate information about the trial, including an explanation of its status as research, its objectives, potential benefits, risks and inconveniences, other treatment that may be available, and also of the subject's rights and responsibilities in accordance with a variety of international standards.

Institutional Review Board (IRB): An IRB is charged with protecting the rights and welfare of people involved in research and in assuring that the benefits of the research exceeds the risks.

Investigator: A person responsible for the clinical trial, including the integrity of trial data, and for the rights, health and welfare of the subjects in the trial. The investigator should have qualifications and competence to conduct the trial in accordance with local laws and regulations.

Non-inferiority trial: A trial with the primary objective of showing that the response to the vaccine under investigation is not clinically inferior to the control vaccine (active or placebo).

Observational studies: Observational studies focus on events, exposures and diseases occurring in the population during their everyday life, and not subject to experimental interventions.

Outbreak: The occurrence of two or more linked cases of a communicable disease.

Post-marketing surveillance: A system for monitoring adverse events following licensure. Post-marketing surveillance can be passive or active and its objectives include, but are not limited to, the identification of rare adverse reactions not detected during pre-licensure studies and the identification of risk factors or pre-existing conditions that may promote reactions.

Potency: The quantitative measure of the specific ability or capacity of the product to achieve a defined biological effect.

Prevalence: The number of persons who have a particular disease at a specific time.

Primary vaccination: First vaccination, or series of vaccinations given within a predefined period, generally with an interval of less than six months between doses, to induce an immune response or clinical protection.

Protocol: A document that states the background, rationale and objectives of the clinical trial and describes its designs, methodology and organization, including statistical considerations, and the conditions under which it is to be performed and managed.

Randomization: In its simplest form, randomization is a process by which individuals are assigned to an experimental or control treatment. Thus, randomization avoids systematic bias in the assignment of treatment. It also promotes balance with respect to known and unknown prognostic factors that could affect the outcome of interest. While it does not guarantee that treatment groups will be exactly equal with respect to these factors, it does guarantee that any imbalance that occurs arose purely by chance. The process of randomization guarantees the validity of statistical analyses of treatment effect, and (with adequate sample size) allows the detection, or ruling out, of small or moderate treatment differences.

Reactogenicity: Reactions, either local or systemic, considered to be caused by the vaccination.

Serious adverse event: An event occurring in connection with the clinical trial that results in death, admission to hospital, prolongation of a hospital stay, persistent disability or incapacity, or is otherwise life-threatening. Seriousness, not severity, serves as a guide for defining regulatory reporting obligations. A "serious adverse event" (SAE) is a formal term defined by ICH regulations.

Severe adverse event: The term severe is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe allergic reaction); the event itself, however, may be of relatively minor medical significance (such as severe photophobia). This is not the same as serious, which is based on a patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or their functioning.

Seroconversion: Predefined increase in antibody titre, considered to correlate with the transition from seronegative to seropositive, providing information on the immunogenicity of a vaccine. If there are pre-existing antibodies, seroconversion is defined by a transition from a predefined low level to a significantly higher defined level, such as a fourfold increase for an individual in antibody titre.

Serosurveillance: The surveillance (see below) of an infectious disease by measuring disease-specific antibodies in a population or subpopulation.

Sponsor: An individual, company, institution or organization that takes responsibility for the initiation, management and/or financing of a clinical trial. When an investigator initiates and takes full responsibility for a trial, the investigator has then also assumed the role of the sponsor.

Surrogate of protection: A testable parameter that signals efficacy because of supporting data that arose directly from testing of the vaccine in humans, especially in a double blind, controlled, large-scale, Phase 3 efficacy trial.

Surveillance: This is the continuous collection, collation and analysis of data, with or without subsequent action.

Survey: An investigation in which information is systematically collected. It is usually carried out in a sample of a predefined population group for a defined time period. A survey is not a continuous investigation and may be repeated after a period of time. If repeated regularly, surveys can form the basis of a surveillance system.

Vaccine (protective) efficacy: The reduction in the chance or odds of developing clinical disease after vaccination relative to the chance or odds when unvaccinated. Vaccine efficacy measures direct protection (i.e. protection induced by vaccination).

Vaccine effectiveness: The protection rate conferred by vaccination in a specified population. Vaccine effectiveness measures both direct and indirect protection (i.e. protection of non-vaccinated persons by the vaccinated population). Vaccine effectiveness is also determined by vaccination coverage, correlation of vaccine strains with circulating strains, and incidence of disease due to strains not included in the vaccine following introduction of the vaccine in that population.

Vaccine failure: The onset of infection or disease, biologically confirmed, in a subject who is supposed to be protected following completion of age-appropriate immunization as recommended by the manufacturer.

1.7 Product information needed to conduct Phase 1–3 clinical trials

As previously stated, this document focuses on Phase 1–4 trials, with emphasis on Phase 3. Before conducting Phase 3 trials, the following types of information should be carefully considered.

Product information

The vaccine must:

- be adequately characterized, including information about manufacture, formulation, and quality control;
- be manufactured with preliminary evidence of consistency of production and stability;
- be able to be stored under acceptable conditions;
- have a specified route of administration and dosage.

Information from preclinical research

Live attenuated and inactivated or subunit vaccines have both shared and distinct product characterization and preclinical requirements (11). Listed below are several such requirements for live attenuated vaccines.

- Having knowledge of the degree of attenuation and the stability of the attenuated phenotype.
- Having laboratory markers of attenuation (if available), or in vivo markers, e.g. reduced viremia in primates that can distinguish the attenuated vaccine from fully virulent wild-type strains.
- Having documentation on the vaccine's genetic stability profile.
- Having shown a reduction in the ability of the live attenuated vaccine viruses to infect mosquitoes and their inability to be transmitted by mosquitoes.
- Knowing the safety and immunogenicity profile in laboratory animals. This may include conducting protection studies in non-human primates. For example, the candidate vaccine may need monkey neurovirulence testing, depending on the nature of the live virus candidate (e.g. a chimeric vaccine that may have potential neurovirulence).

2. Methodological considerations

2.1 Clinical definitions of dengue fever and dengue haemorrhagic fever

Dengue is an acute febrile disease caused by any one of four related flaviviruses known as dengue types 1, 2, 3, and 4. Dengue fever is the most commonly diagnosed form of dengue virus infection and is characterized by the sudden onset of fever lasting between two to seven days, accompanied by severe headache, gastrointestinal symptoms, muscle, joint and bone pain, and a rash (24,25). The classic form is self-limited and usually results in complete recovery.

The most severe form of dengue infection is dengue haemorrhagic fever. Although it represents a small proportion of all dengue illnesses, it is the only form of severe dengue that has been defined by a standardized criteria (25).

DHF is characterized in its early stages by the signs and symptoms of DF described above, followed by haemorrhaging and/or increased vascular permeability in its latter stages. This increased vascular permeability may lead to vascular collapse (also known as dengue shock syndrome) and death.

Main clinical/laboratory elements

WHO and the Pan American Health Organization (PAHO) guidelines (25,26) require that "all of the following four elements be present" for the clinical case definition of DHF to be met.

1) A fever or recent history of acute fever.
2) A tendency to haemorrhage, as evidenced by at least one of:
   - a positive tourniquet test (to assess fragility of capillary walls);
   - evidence of petechiae, ecchymoses, or purpura;
   - evidence of bleeding from mucosa, gastrointestinal tract, injection sites, or other sites.
3) A low platelet count (thrombocytopenia) of 100 000 mm3 or less,
4) Evidence of blood plasma leakage due to increased capillary permeability as manifested by at least one of:
5) a haematocrit on presentation that is equal or greater than 20% above average for that age, sex, and population;
6) other commonly associated signs of plasma leakage, such as pleural effusion, ascites, and hypoproteinemia.

In addition, the clinical definition of DSS in patients with DHF requires that there be evidence of circulatory failure manifested by the following signs and symptoms.

- A rapid and weak pulse.
- A narrow difference between systolic and diastolic pulse blood pressure (20 mm Hg or less).
- Low blood pressure (hypotension) for age.
- Cold, clammy skin and restlessness.

It should be noted that these criteria have been modified and adapted to different WHO regions and countries (26,27). For example, a more simplified case definition of DHF utilized in the Kingdom of Thailand includes an acute febrile illness of 2–7 days duration with at least two clinical signs or symptoms of dengue, plus at least one of the four clinical/laboratory elements of DHF listed above (27).

2.2 Classification of severe dengue syndromes

A number of severe dengue syndromes have been reported, some of which are common but poorly-defined, and others that fulfill some, but not all, of the four laboratory and clinical criteria for DHF (28–31).

For example, cases of dengue infection have been described that are accompanied by hypotension or shock in the absence of thrombocytopenia (as defined by WHO criteria) and show no signs of haemorrhage, including a negative tourniquet test (28,31).

As a result, when WHO criteria for DHF are strictly followed, some severe cases, including fatal cases, will not be called DHF. In order to confirm WHO criteria, it may be necessary to use a rigorous protocol requiring multiple daily clinical laboratory tests. This requirement, however, may impede comparisons of dengue severity from one study to another and across regions (28).

It remains to be determined if DHF is a syndrome distinct from other severe dengue syndromes or if it is one end of a continuum of clinical and laboratory changes with a common pathogenic etiology. If the latter, a number of patients satisfy some, but not all, criteria for the diagnosis of DHF. They may be severely ill and at risk of death by haemorrhage or shock caused by the same pathogenic mechanism causing DHF. These cases should be better defined and included in any assessment of the protective efficacy of dengue vaccines (28,29,30).

WHO dengue case-classifications are currently being revisited. The purpose is to develop more serviceable clinical classifications of dengue for early diagnosis, triage, and management of patients. These classifications could also be used to measure the effect of vaccine on dengue morbidity and mortality.

2.3 Selection of end-points

Each field trial requires a single primary efficacy end-point, namely the end-point used to calculate sample size and estimate vaccine efficacy (VE) (32). A variety of dengue disease definitions could serve as end-points (see 2.2). This would include undifferentiated febrile illness, dengue fever, severe dengue syndromes including DHF, and DHF alone (1.6, 2.2).

This document develops the argument that laboratory-confirmed dengue infection in patients showing clinical symptoms is the only practical primary end-point irrespective of dengue severity (see 2.3.1, 2.3.2 for discussion and rationale). In the context of a dengue vaccine trial, laboratory-confirmation implies direct detection of a dengue virus by culture, antigen assay or viral ribonucleic acid (RNA). Whatever dengue criteria are selected, they must be defined in the clinical trial protocol so they can be measured during the trial (2.3.1). Secondary end-points may be descriptive and not reach a frequency to generate statistically significant data (2.3.2).

2.3.1 Primary efficacy end-points

The most definitive option is to confirm the presence of dengue virus in a patient showing signs and/or symptoms of dengue disease, irrespective of severity. Such an end-point requires an active surveillance system that captures all febrile illness to avoid missing detection of mild dengue. Each protocol should specify the clinical signs and symptoms used to define cases of dengue and, thereby, triggering the need to obtain clinical specimens to confirm the presence of virus. This design is justified for the following reasons.

1) Depending on the country and locale, the incidence of mild dengue cases in the community and admitted to the hospital may be far more common than severe cases. Surveillance must be established to track febrile illness and to detect dengue virus circulating in febrile patients.

2) During a clinical trial, the number of severe cases is likely to be lower for several reasons. Firstly, trial participants will be closely followed and treated before they can develop severe symptoms. Secondly, the repeated clinical and laboratory tests necessary to establish the diagnosis of DHF may not be obtained at the appropriate times.

3) The statistical scoring for a decrease in the number of severe cases will extend the duration and cost of the trials, a major consideration that may undermine the practicality of conducting a Phase 3 trial.

4) Because the impact of all dengue illness is significantly larger than the impact of severe dengue alone, using severe dengue as a measure to compare to other diseases will seriously underestimate the human and economic burden imposed by dengue (33,34). Such data provide additional justification for targeting all dengue illness, rather than severe dengue alone, in vaccine efficacy trials.

A second option for a primary end-point, which may be far less practical for reasons summarized above, is to study virologically confirmed, severe dengue as determined through hospital-based surveillance. This option can be expected to extend the duration and cost of the Phase 3 trial significantly, unless it is done in the setting of a large and prolonged epidemic, which cannot be predicted with accuracy from one season to the next.

In any case, a virologic diagnosis must confirm primary end-points for every trial. It is usually possible to make a virologic diagnosis either by virus isolation, by reverse transcription/polymerase chain reaction (RT-PCR), or by a surrogate DV antigen marker such as NS1 (35–39). The standardization of viral diagnostic methods is encouraged. One caveat however, is that viral diagnostic methods, especially virus isolation and PCR-based assays, are more sensitive during the first five days of infection.

This requirement for early viral diagnosis has implications for the type of surveillance used in the trial. Hospital-based studies with enrolment criteria of less than 72 hours of fever (39) and a school-based study where home visits were made within a day or two of school absence (40) had high rates of isolation/identification for serologically confirmed disease.

In summary, it is feasible to define dengue as fever of at least two days duration in a person in whom dengue viremia has been proven by virological diagnosis. Such a case definition could be used as the basis for the primary efficacy analysis. The protective efficacy against virologically confirmed dengue can be established as a composite of the serotypes encountered during the trial. Serotype-specific protective efficacy should be studied as a secondary end-point as noted below (2.3.2).

2.3.2 Secondary efficacy end-points

Such end-points may be descriptive and not reach a level to generate statistically significant data. A judicious choice of secondary end-points, such as efficacy by age group and gender, can be evaluated concurrently with the primary end-point to increase the value of the trial in assessing the benefits afforded by the vaccine candidate.

Other secondary end-points could include:

- efficacy against each of the four distinct virus types;
- efficacy after the first of two or more doses of vaccine;
- effect on duration of hospitalization for dengue;
- severity of laboratory-confirmed dengue cases;
- vaccine efficacy against "possible" or "probable" dengue infection.

If used as a secondary end-point, the definition of "severe dengue" must be rigorous (see 1.6, 2.2, 2.3.1, 2.3.2). It could range from classical DHF at the extreme end, to severely ill patients who nevertheless lack all four criteria for DHF (fever, haemorrhagic manifestation, thrombocytopenia, or evidence of increased capillary permeability).

Vaccine efficacy against "probable" or "possible" dengue infection could be a secondary end-point, if serology is used as the basis for dengue diagnosis in patients in whom virologic diagnosis has been unsuccessful. The subjectivity of such terms and the lack of precision of serological data should be recognized (41).

Any sub-analysis of efficacy should be stratified according to pre-vaccination flavivirus serological status, as might be determined in a sub-cohort of vaccinees and controls who are followed for immunogenicity. It is possible that a dengue vaccine may alter the clinical course of subsequent wild-type dengue infection, reducing viremia and making the virus difficult to detect, yet be accompanied by abbreviated signs and symptoms. Thus, serological secondary end-points may help to describe the efficacy of vaccines in protecting against clinical disease, assuming that serological assays are equally sensitive in detecting dengue infection in vaccine and control groups.

Although there is extensive experience in diagnosing acute primary or secondary dengue infection using a single serum sample or paired (acute and convalescent) sera, almost all serological tests are fraught with inaccuracies or technical problems. For example, to appropriately utilize serologic data it is necessary to obtain pre-illness samples shortly before infection to compare with acute and convalescent serum samples. Unfortunately, this is usually impractical for the entire vaccinated cohort.

Serological assays can be obtained commercially or developed in-house. Enzyme immunoassay, immunofluorescence, haemagglutination-inhibition, and neutralization tests of acute and convalescent sera showing four-fold or greater rise in antibody titre, provide presumptive evidence of a dengue virus infection.

DV infection can also be diagnosed using the immunoglobulin M (IgM) capture enzyme immuno assay (EIA); IgM levels rise during the week after acute primary infection and remain detectable for approximately six weeks. Evidence of IgM seroconversion can be sought in convalescent sera.

Thus, the finding of dengue-specific IgM in serum is presumptive evidence of a recent flavivirus infection. However, the IgM response may be blunted if that individual had been vaccinated earlier with a tetravalent vaccine or had been infected previously by a flavivirus.

By using the ratio of the combination of IgM and IgG enzyme-linked immunosorbent assay (ELISA), it is also usually possible to classify the patient's infection as primary or secondary (42). The haemagglutination inhibition (HI) test, if antibody titres are high and broadly cross-reactive, is also indicative of secondary infection. However, interpreting serological reactions is complicated by cross-reactions found among flaviviruses in all of the above serological tests. In those instances where cross reaction with other flaviviruses does not occur, a four-fold or greater rise in dengue neutralizing antibodies makes it possible to attribute recent infection to a dengue virus presumptively, but not definitively.

A good network of home-visitors can be used to stimulate the collection of second (convalescent) samples. The term “clinically suspected” should be defined as a possible secondary end-point based on serological results.

2.4 Case detection and diagnosis

2.4.1 Detection and investigation of cases once clinical trials have been initiated

Adequate surveillance, such as periodic home visits or telephone calls, must be conducted to identify febrile illness in study participants sufficiently early to allow detection of viremia. Procedures should be in place to identify study participants presenting at any hospitals serving the catchment populations. A case evaluation algorithm should be implemented and adhered to in a consistent manner.

It is essential that clinical coverage be adequate for this surveillance system to be effective. As with the surveillance for other outcome measures, the examining physicians should be aware that the patient is participating in a dengue vaccine trial, but unaware if the patient received the dengue vaccine or control vaccine. Study design should include the provision to physicians and nursing staff of standardized clinical record sheets. A centralized laboratory should be used if possible for any clinical laboratory and dengue diagnostic testing.

Case detection and diagnosis of severe dengue

In some cases severe dengue will evolve from mild dengue disease that has been detected by the presence of viremia or antigenemia. Because documentation of severe dengue is vital, every virologically confirmed case of severe dengue should be followed to resolution and classified to outcome. However in no case should proper treatment of severe dengue be delayed. DHF/DSS should be evaluated using objective measures referenced in the 1994 PAHO (26), 1997 WHO (25), or 1999 South East Asian regional WHO guidelines (27). Diagnostic criteria need to be developed for "severe dengue" without fulfilling the strict WHO criteria for DHF (2.1) (28,29,30).

Despite the use of an active surveillance system for laboratory-confirmed dengue, there needs to be a protocol to identify and document cases that elude the viral diagnostic process. Such cases will not be confirmed but will be scored as suspected cases. Nonetheless, they should be carefully documented for inclusion in the analysis of the clinical trial as a secondary end-point. Hospital- based surveillance may be necessary to capture many such cases (see 2.3.1).

2.4.2 Immune assays and immune correlates of protection

Immune assays

In all dengue clinical trials, immunogenicity should be measured using assays which are as close as possible to the vaccine's postulated mechanisms of protection. Ultimately, an appropriate immunological assay may serve as a correlate or surrogate marker for clinical protection. Currently, neutralizing antibody is considered to provide the most relevant immune mechanism protecting against dengue. Hence the DV neutralization test is considered the most likely assay to correlate with protection against dengue. However, neutralizing antibodies are not yet proven correlates or surrogate markers of protection, and these markers await their validation through clinical trials (see below).

WHO has prepared guidelines for the plaque reduction neutralization test (PRNT), which aim at harmonizing methodologies and increasing comparability across studies. Test procedures should be carefully standardized, following the WHO guidance document on PRNT (43). At present, other high throughput neutralization assays are in development, which will require validation against the PRNT. These novel assays, such as the micro-neutralization assay, will facilitate the serological testing needed for large-scale vaccine trials. Reference virus strains and cell substrates are available from WHO. Control sera for assay validation are currently being produced (37,44).

Immune correlates of vaccine protection

It is important to determine the immune response to vaccination during the course of the clinical trial. For large-scale trials, a randomly selected subset of the study population may be used to assess immunogenicity.

In order to assess correlates of vaccine protection later, more samples of sera and peripheral blood mononuclear cell (PBMC) would need to be obtained from larger cohorts. Correlation between immune response and occurrence of cases may identify immunological correlates of protection. This may allow the protection afforded by the vaccine to be generalized to other populations achieving an equivalent immune response.

There are two primary study design options for analyzing the correlation between immune response and disease outcome. Both involve collection of serum and PBMCs before vaccination and at one or more intervals after vaccination. The samples would be stored initially without testing. The subjects whose samples will be tested will be chosen after unblinding of vaccination status. The specific study designs are as follows:

a) A cohort study, in which one or more measures of immune response are related to disease risk in all immunized subjects (or in a large subset of immunized subjects). This design is potentially the more informative, if there are enough cases among immunized subjects to allow quantitative estimation of disease risk as a function of immune response(s). However, it is also more difficult logistically and requires more resources than the case-control study design below.

b) A case-control study, in which immune responses are compared in immunized subjects who developed disease and a subset of immunized subjects who did not develop disease. This design enables establishment of an association between disease and measure(s) of immune response, but it does not allow quantitative estimation of disease risk as a function of immune response.

As discussed above, neutralizing antibodies are the most likely primary correlate of protection, and it appears unlikely that cell mediated immunity (CMI) will provide a primary correlate. However, specific CMI assays may be suitable for characterizing important vaccine features, such as immunological memory and durability of protection (37).

CMI data (e.g. a cytokine response) may also help to corroborate the safety profile of a vaccine, and to study the potential for causing disease through an adverse immune response to the vaccine itself or by increasing risk for more severe dengue during subsequent natural infection. Investigators are encouraged to pursue research opportunities, including following the duration of CMI and serological responses in relation to vaccination outcome.

2.4.3 Ascertainment of causes and investigation of deaths

Deaths can be counted and causes of death can be determined by analysing medical records and/or post-mortem clinical case review (e.g. verbal autopsy) (45,46). Virologic studies of tissues should also be conducted whenever possible. For example, post-mortem immunohistochemistry of liver samples obtained via needle puncture can establish the diagnosis of dengue infection. All efforts should be made to encourage parents of study participants to agree to post-mortem examination.

2.5 Study populations

2.5.1 Epidemiological criteria

It is necessary to conduct vaccine trials in geographic settings where there is sufficient incidence of dengue to make VE relatively easy to measure, and where there is a political commitment to introducing a vaccine to control dengue. Although potentially eligible study sites are likely to be many and varied, investments need to be made in order to measure the necessary epidemiological background information.

Extensive background information is required before selecting sites for Phase 3 trials. As previously mentioned, the most important consideration is the expected incidence of the primary efficacy end-point (see 1.4, 2.3.1), including a description of the types of dengue virus that are causing disease, preferably over a number of years. Collecting other dengue-related data (e.g. prevalence of each serotype, rates of infection, specific mortality, etc.) will also be critical. The collection of these data is likely to require a sophisticated active or enhanced passive surveillance system.

Analysis of the dengue data by season will identify when transmission is occurring. This result may affect the timing of vaccinations and patient surveys. Analysis by age, gender, and possibly occupation, may help determine whether human-vector contacts are mostly occurring in schools, work places, homes, or in other sites. This will allow vaccinations to be targeted to the most at-risk environments.

It is also necessary to document or improve the quality of health services to ensure they are adequate to support not only the success of the trial but also the research subjects who potentially may be placed at risk from candidate vaccines. In agreement with medical and ethical guidelines, participants should have access to appropriate medical care in the event they ultimately develop dengue (10,47).

2.5.2 Operational criteria

The potential trial sites should include the following desirable characteristics.

- The site should be endemic for one or more DV types. If the end-point is protection against all dengue illness independent of severity and DV type, then as few as one type needs be transmitted for between one and three seasons in a test site. It is unrealistic to expect all four DV types to be transmitted actively in a single season, so observations will need to continue for several additional years depending on the incidence of dengue and the number of DV types being transmitted at the site. Alternatively, or in addition, investigators could select geographically diverse field sites to maximize their ability to assess the efficacy of the vaccine against all four types, including DV strains that appear to be more virulent (e.g. strains in South-East Asia) (48).

- Background data on the epidemiology of dengue should be available. This will require the presence of a good community-based or laboratory-based surveillance system that is capable of monitoring the DV types that are circulating, as well as the incidence of mild and severe dengue. Such surveillance is critical for selecting a site for vaccine efficacy trials.
- It is especially important to document all species of flaviviruses in circulation at the trial site. Some may cause subclinical infections in study participants and may confound interpretation of serological results. Theoretically, infection by such viruses might also modulate the course of dengue illness.
- There must be informed and firm commitment from the NRA and local authorities to conduct the trial. In addition, NRAs should be competent to assess clinical trial protocols. This will assure competent oversight, increase the likelihood that the results of the trial are used to plan future dengue control strategies, and will also help to gain the support and confidence of community participants and health professionals.
- There must be informed and firm commitment from the study population to the trial and its associated investigations. This commitment should be obtained after they are given a balanced representation of the risks and benefits of participating in the trial. "Overselling" the vaccine by giving potential participants a false sense of security should be avoided to prevent backlash that might cause delay, or cause people to abandon other critical public-health measures, such as controlling mosquitoes. They should also be made aware that the conduct of a trial will inconvenience them. For example, participants should know that they may be asked to repeatedly donate blood samples for the trial, and that, according to national regulations, they may be unable to donate blood to local blood banks for weeks to months after having received an unlicensed vaccine,.
- There must be sufficient medical infrastructure, such as doctors, nurses, outpatient clinics and hospitals, x-ray and other diagnostic equipment, and clinical laboratory facilities, to assure adequate medical care and identification of adverse events.
- It is desirable to have maximal involvement of in-country qualified investigators and field and laboratory teams, and that they should give the trial a high priority.
- There must be reasonable expectation of social and political stability at the national and local levels for the duration of the trial.
- There must be a low expected emigration rate for the duration of the trial to minimize the attrition rate.

Adherence to Good Clinical Practice guidelines

All procedures should satisfy the WHO, FDA and or ICH Guidelines for GCP (20,21,22), the WHO Operational Guidelines for Ethics Committees that Review Biomedical Research (49), and guidance provided by local Institutional Review Boards (IRBs).

GCP requires that there be critical review of the components of GCP during the preparatory phase of a trial, and that these components should be utilized and improved on an ongoing basis. The critical components of GCP include:

- standardization of all field, clinical, and laboratory procedures, including establishment of Standard Operating Procedures (SOPs) and normal values;
- validation of Good Laboratory Practice (GLP) assays to support regulatory requirements and quality-control monitoring in the trial;
- reproduction of measurements made within and between field workers and at different times;
- comparison of procedures and methods with other studies;
- determination of the sensitivity and specificity of diagnostic methods;
- determination of the level of precision required for all measurements to be taken in the trial (this may not necessarily be the most precise measurement attainable);
- minimizing the numbers of specimens (e.g. of blood) to be collected;
- arrangement for the collection, transportation and preservation of specimens;
- development of procedures for recording and validating data and for the analysis of results.

Preparation and execution of dengue vaccine trials will be similar to other vaccine efficacy trials, including ensuring GCP and GLP compliance. However, dengue vaccine trials engender some unique methodological aspects. These include: 1) the need for extended preparation and follow-up timelines needed to collect accurate disease incidence data across multiple transmission seasons; 2) the need to extend safety and efficacy follow-up in the vaccine cohort.

Community education

The purposes of the trial and trial methods should be discussed with local community representatives. Those eligible for enrolment in the trial should be properly educated about possible benefits and adverse effects of vaccination, including the potential of acquiring severe dengue. It should be made clear that participation in the study is voluntary, and that those refusing to participate will still receive their routine vaccinations and will not suffer discrimination.

Arrangements for informing the community about trial results should be agreed upon in advance, including the provision that participants may remain blinded for an extended period of time after demonstration of vaccine efficacy. The community should be aware that even if the vaccine is protective for individuals, the community may not benefit unless a high proportion of the target population is immunized. The intention to vaccinate the control group after demonstration of vaccine efficacy and safety must be determined in consultation with the local and national regulatory authorities.

2.6 Vaccination protocol

2.6.1 Exclusions

The criteria for exclusion should be clearly spelled out in each trial. As far as possible, the trial should not exclude any persons who would be likely to receive vaccination if the vaccine were eventually introduced into a national immunization programme. It would be prudent, however, to exclude from an initial trial those likely to respond adversely to vaccination, or liable to develop episodes of illness which might be difficult to distinguish from adverse reactions to the vaccine. In addition to those who refuse to participate in the trial, it is strongly recommended to exclude individuals suffering from any severe acute illness at the time of vaccination. It is also advisable to exclude pregnant and nursing women, persons with known sensitivity to any vaccine component, and those suffering from any severe chronic disease. Persons seropositive for HIV should be considered for exclusion, and special studies may be needed to address vaccine safety and efficacy in those populations.

2.6.2 Season of vaccination

The seasonal transmission of dengue should be taken into account during trial design. Because a licensed vaccine will probably entail year-round administration, the vaccine should be analysed for safety and efficacy between the doses needed for primary immunization. Local programmes for the control of mosquito vectors should be maintained.

2.6.3 Impact on dengue virus transmission and herd immunity

Study design should take into account the potential that a highly efficacious vaccine could decrease dengue virus transmission in the community. This could reduce the overall risk of acquiring dengue infection and disease in the control group, and thereby reduce the apparent efficacy of an otherwise protective vaccine.

2.6.4 Choice of a control intervention

To maintain the double-blind protocol (see Glossary 1.6), the choice of the experimental control will depend on numerous factors, including a comparable route of administration, appearance, volume of the preparation, and dosage. Discussion with local IRBs and regulatory authorities will determine whether the control intervention will be a placebo injection or an active vaccine.

2.7 Data Safety Monitoring Board

A DSMB should be appointed for the trial (50,51). This should be an independent group with access to a statistician and with the ability to analyse for the possibility of treatment harm during the course of the trial. The DSMB should be empowered by its charter to recommend that the trial sponsor halt enrolment or stop the trial completely if necessary. The DSMB should be established before the trial begins, and it should meet regularly after the trial has started.

2.7.1 Provision for stopping the trial

Provision should be made to temporarily suspend or permanently stop vaccination using carefully crafted rules established before the trial begins. A randomized, controlled, Phase 3 trial should not be stopped unless there is evidence that the vaccine caused harm. Harm is usually defined by an unacceptable number of severe or serious adverse events, or unexpected adverse events (AEs) of any severity based on local or systemic clinical reactions or abnormal laboratory values. The participant treatment codes may then be broken by the DSMB to assess if there is a causal association with the vaccine. If associated, the results are reported to the sponsor and NRA and they may elect to stop the trial completely.

The study blind can be broken on a case-by-case basis by the DSMB. In addition, the analytic plan may include one or more interim analyses, which may also provide for stopping the trial under specified conditions. An interim analysis may be appropriate if an excessive number of cases of DHF or death occur during the course of the study. If these are occurring predominantly in the control arm, the DSMB could recommend that the vaccine be provided to the control arm in a crossover fashion; before doing so, the NRA should be confident that the safety and efficacy data supporting licensure are available. Alternatively, the DSMB may conclude that severe cases are occurring predominantly in the vaccinated group. If this is the case, the sponsor and NRAs may halt or stop vaccinations and institute precautionary measures to ensure that all previously vaccinated individuals are instructed to seek medical care immediately at the onset of symptoms.

2.8 Data management

Source records should be maintained in accordance with GCP. An experienced data manager should supervise the recording, keeping in mind that the trial is blinded and the data should be maintained so that the trial team does not have access to the code. Newly validated remote data entry systems may be ideal for rapid data entry shared with central data managers and multiple clinical and laboratory trial sites. Electronic data sets should be compliant with regulatory requirements (including quality assurance (QA) and quality control (QC) activities), and designed to be interactive so that laboratory data can be analysed with demographic and clinical data.

2.9 Statistical considerations

Appropriate methods for the statistical analysis of randomized controlled trials of dengue vaccines are essentially the same as for randomized controlled trials of other drugs and vaccines. However, issues specific to dengue vaccines arise because:

- with a tetravalent vaccine, efficacy may be shown for one or more of the four serotype components, but rarely for all;
- a large number of subsidiary questions might be addressed in a dengue vaccine trial. Examples include the vaccine effect on incidence and severity of haemorrhage or capillary leakage (analysed separately), DHF severity grade, organ failure, duration of hospitalization, viremia, and other possible secondary end-points (see 2.3.2).

The investigators should prepare a plan specifying how the primary analysis for efficacy and safety will be conducted prior to breaking the participant randomization code. This addresses the issue of multiple outcome variables by denoting one primary end-point only in such a way that it is clear that the results themselves have not influenced the choice of this measure. The analytical plan should also detail the inclusion criteria, the case definition to be used, and the methods of data analysis.

The first analysis should be the comparison of dengue vaccine recipients with control vaccine, or placebo recipients with respect to their baseline characteristics. Variables included in the analysis should include age, gender, area of residence, and preferably, the initial dengue immune status.

In trials with an adequate sample size, random allocation is likely to achieve comparable groups. For this reason, the primary analysis of protective efficacy should not require any adjustments for baseline differences. In assessing comparability, more emphasis should be given to the size of any differences than to statistical significance, since it is the former that affects the degree of confounding.

It is recommended that the investigators specify in the analytical plan the magnitude of differences or the extent of confounding that will be considered important. If important imbalances are detected, the results should be adjusted for these differences by stratification or by using regression methods. These adjustments will not be primary, but they can be examined as secondary or supplemental end-points and are likely to be very important in the case of dengue.

The primary analysis for vaccine efficacy should be limited to only those volunteers who are fully vaccinated and followed serologically and clinically for the required time defined in the protocol. However, a secondary analysis should also be performed on all persons entered into the trial, whether or not they received all of their vaccinations or completed follow-up (i.e. intent to treat), to assess the robustness of the trial's conclusions. Vaccine efficacy is then calculated using the standard formula:

$$VE\ (\%) = 100 \times (I - r1/ro)$$

where r1 = incidence rate in dengue vaccine group;
ro = incidence rate in control vaccine/placebo group.

Special statistical methods are required to analyse the relatively rare occurrence of multiple dengue episodes in any trial participant.

One way to demonstrate the effects of the vaccine against each dengue type or severe disease could be to perform analyses of pooled clinical data from different trials (i.e. meta analyses). Trials should be designed with this possibility in mind. An important requirement to pool clinical results is the use of standard case definitions for end-points.

In a dengue vaccine trial there are likely to be many supplementary questions of interest, particularly if the vaccine shows a substantial efficacy. A range of data analysis techniques may be appropriate to explore effects on secondary and other trial end-points. In reporting the results of these exploratory analyses, particularly those based on sub-group analyses, statistical correction will be needed for repeated comparisons. A precautionary approach demands that substantial weight be attached to exploratory results only if they are vaccine-associated AEs.

2.10 Vaccine safety

In discussing vaccine safety and AEs associated with their administration in particular, it must first be noted that the terms severe and serious are not synonymous and must be explicitly defined during the dengue trial design. The term severe is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe allergic reaction); the event itself, however, may be of relatively minor medical significance (such as severe photophobia).

This is not the same as serious, which is based on a patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Importantly, seriousness, not severity, serves as a guide for defining regulatory reporting obligations. A "serious adverse event" (SAE) is a formal term defined by ICH regulations (20).

Safety must be assessed in all vaccine trials by recording clinical or laboratory AEs, of which there are several categories (see 1.6) including:

- signs and symptoms solicited after vaccination to characterize vaccine reactogenicity. These are usually grouped into injection site or generalized (systemic) signs and symptoms. Relevant solicited signs and symptoms may be defined by experience obtained in Phases 1 and 2. During Phase 3, safety data should be generated that is adequate to support prescribing information about expected AEs when combined with data from Phase 1 and Phase 2 studies.
- AEs not specifically solicited but that are "spontaneously" reported by the volunteer.
- AEs are further classified as non-serious or serious. Serious AEs are defined by ICH guidelines (20) (see 1.6). Generally, non-serious AEs, whether solicited or non-solicited, are recorded for a defined period after each vaccination (e.g. 21 days) to identify vaccine-associated risks. Serious AEs, both vaccine-associated and not associated, are usually collected during the entirety of a vaccine trial and offer NRAs evidence upon which to infer the level of medically-significant risk associated with vaccination.

For novel vaccines, it is customary that a minimum of 3000 to 5000 vaccinated persons from a combination of Phase 2 and Phase 3 trials be enrolled for safety analysis before licensure. This sample will be adequate to detect, with a chance of at least 95%, a rare AE occurring at a rate of 1 in 1000 vaccinees (52). Larger safety databases may be required depending on several factors such as type of vaccine, or potential safety concerns that emerge during pre-license clinical testing.

Safety data collected on several trials of a candidate vaccine may be pooled to provide a more powerful integrated summary of safety analysis. Pooling is most appropriate when trial designs are similar; prospectively designed, pooled analyses are preferred.

The design of a vaccine's safety evaluation should include adequate characterization of the relative risk of dengue after vaccination. Specifically, trials will need to address the risk that persons acquire dengue or develop severe dengue as a direct consequence of having been vaccinated. While confirmation of dengue cases requires virological diagnosis, it is recognized that this might be impractical for prolonged follow-up of large cohorts. However, without virological confirmation, only presumptive dengue can be diagnosed based on clinical, serological or epidemiological analysis. The need for ongoing, enhanced DV surveillance of febrile illness should be addressed by the NRA. The safety evaluation can be done outside a trial designed to measure efficacy, if there is blinding to prevent misclassification bias.

Long-term follow-up of randomized double-blind trial cohorts (even after the Phase 3 vaccine efficacy trial has been concluded) is a powerful way to confirm or reject such a risk. Unblinding a cohort during long-term follow-up may result in substantial bias and is not advised. Long-term follow-up should be planned in advance and presented to volunteers during the informed consent process.

To reiterate and expand, AEs should be assessed in all phases of vaccine trials, and the assessment should preferentially be based on a comparison between vaccinated and control subjects. Most information describing the quality of the most common immediate side effects will have been collected during the Phase 1 and Phase 2 studies. Adequate safety monitoring for solicited and unsolicited AEs and SAEs must also be incorporated into Phase 3 clinical trials in order to obtain sufficient data to determine the probability of common and more rare AEs and SAEs. In practice, given the long duration of follow-up, this may have to be limited to a subcohort of participants if the trial is large. It may not be clinically possible to differentiate dengue resulting from natural infection and dengue caused by the vaccine virus. Where possible, laboratory studies should be undertaken to determine whether or not the vaccine is responsible for disease in a study subject who develops clinical signs within 21 days of vaccination.

Studies should be designed to detect increased risk of severe dengue in vaccine recipients throughout the duration of the Phase 3 clinical trial and beyond — for example by regularly reporting all deaths and/or all severe cases of dengue, to the DSMB. The storage of adequate samples of sera and PBMCs for a retrospective search will also provide important information about the immune mechanisms of protection or of sensitization conferred by dengue vaccines. WHO may provide additional guidance on this topic.

A suggested schedule to monitor the safety of a dengue vaccine includes the following.

- **Pre-licensure short term, Phases 1 to 3:** Monitoring days 1–21 of clinical reactions after vaccination and wild dengue exposures in endemic country trial sites.
- **Pre-licensure long term, Phases 2 and 3:** Monitoring of SAEs for six months or more after the last vaccination, and the relative risk of dengue disease versus control for three to five years in all vaccinees in endemic trial sites. A Phase 3 trial can be stopped after one year to assess efficacy, and then be continued for 2–4 more years to assess elements of long-term safety, even beyond licensure. The safety monitoring plan is an important component of the clinical protocol and must be finalized before starting the Phase 3 trial.
- **Post licensure:** The safety schedule should be designed to extend the certainty of conclusions drawn from the dataset or to identify safety signals related to rare events. The schedule includes extended follow-up of the participants enrolled in Phase 3 and Phase 4 trials, as well as national/regional epidemiological surveillance for presumptive dengue after licensure.

3. Ethical considerations

Clinical trials of dengue vaccines are subject to the same ethical constraints as trials of any new vaccine. The decision to embark on a large-scale clinical trial should be based on evidence from preclinical cellular and animal studies and Phase 1 and 2 clinical trials (see Glossary 1.6) suggesting that the vaccine is beneficial.

The design and implementation of a trial should conform to both national regulations and international ethical standards (49,53). These standards are outlined in WHO, ICH, and FDA Guidelines for GCP (10,20,22). Detailed information is also available in ethical guidance documents issued by WHO and others (47,53). In countries where national regulations or requirements do not exist, or where they require supplementation, relevant government officials may designate or adopt, in part or in whole, the WHO or ICH GCP standards to conduct the clinical trials.

Prior to implementation, the study design must be reviewed by a properly constituted local and/or national ethics committee, which must include representatives of the groups to be vaccinated, responsible health authorities, and technical experts.

There must also be informed consent from those who participate in the trial, and a written statement of how informed consent will be obtained, or if written consent will not be obtained from research participants. There must be no coercion to participate and no difference between standard community health- care services offered to acceptors and non-acceptors. Special considerations apply for consenting children and obtaining parental consent.

Health services for treatment of possible vaccine-related reactions and dengue infection, and adequate referral and follow-up capability, must be available. A DSMB should be designated, given the authority to break the trial code for any subject, and empowered to recommend that vaccinations be stopped in the event of unacceptable AEs associated with the vaccine (see 2.7.1).

Only those candidate vaccines that have acceptable levels of safety and are produced according to internationally recognized good manufacturing practices, such as those recommended by WHO (11,12), should be studied in a clinical trial. Before embarking on a Phase 3 trial, an assessment of the safety of a candidate vaccine will have been made in both flavivirus naive and primed individuals in Phase 2 trials, and perhaps in Phase 1.

Ethical considerations may affect the selection of trial communities, end-points or study design. Phase 3 trials of dengue vaccines should be carried out only in communities that are likely to benefit from the results of the trial. It is important to ensure that economically and socially deprived communities, which are sometimes those at greatest risk of dengue, are not exploited in conducting research that will be of no benefit to them. All participants should receive benefit from the research after they have taken the risk to participate. For example, cases of disease (even mild or incipient) detected as a trial's end-point, must be treated. Until a vaccine's safety and efficacy have been demonstrated in the target group, there should be no ethical objection to a double-blind randomized controlled (DB-RCT) design for Phase 3 trials. Other designs are not desirable until further data are available from the results of DB-RCT's, because they are more likely to give ambiguous results.

4. Clinical trials: Phases 1 to 4

4.1 Clinical phases

To reiterate, Phase 1 to 4 trials are defined in terms of objectives and registration status (10). Phase 1 trials typically involve the testing of single (monovalent) and combination (multivalent) vaccines in a small number of people who are not at immediate risk of becoming infected by dengue or other flaviviruses, or being vaccinated against other flaviviruses. Phase 2 trials are designed: 1) to determine the dosage of vaccine that is sufficiently immunogenic without causing significant adverse consequences; 2) to accumulate additional safety data beyond Phase 1, specifically the delineation of common AEs associated with vaccination. It is only in a controlled, randomized Phase 3 trial that true treatment effects can be distinguished, and efficacy can be evaluated with statistical certainty. Phase 4, often called post-marketing trials, are ongoing epidemiologic trials designed to monitor any adverse effects a treatment might entail after a prolonged period of time, or to study vaccine effectiveness (see Glossary 1.6).

4.2 Target groups

For effective dengue control, the ultimate target group for vaccination may range from a limited subgroup (e.g. infants) to the entire population. Although in endemic areas of south-east Asia approximately 5% of all hospitalized severe dengue occurs in infants younger than 9–12 months of age, Phase 3 trials in such infants may be difficult because of the possible adverse effect of maternal dengue antibodies on vaccine safety or immunogenicity (54).

In addition, for ethical reasons a new vaccine should be first tested in those expected to be more tolerant of possible reactogenicity, such as healthy adults (excluding pregnant women). Although dengue symptoms may be less severe in infants and younger children than in adults, they may not tolerate symptoms as well as adults, should symptoms occur. This concern, plus the concern of increased vulnerability among infants and young children, justifies initial Phase 1 and Phase 2 trials in adults and older children. Young children and infants may be tested later through age de-escalation studies, or possibly through bridging studies, but only if they could potentially benefit from immunization against dengue.

Before conducting a Phase 3 efficacy field trial in a population or population subgroup, it may be prudent to conduct Phase 1 and 2 safety and immunogenicity trials in a representative population. This will allow a preliminary assessment of safety and reactogenicity in a new study population and help those conducting the trial to refine study procedures.

Thus, vaccine evaluation will involve a sequence of trials done in phases and in different target populations, and may vary among vaccines. Pharmaceutical consistency, which compares three consistency lots produced according to the final manufacturing process, should be demonstrated before moving into Phase 3 trials.

4.3 Clinical trial design

4.3.1 Phase 1 trials

Safety and tolerability

The Phase 1 trials should provide information on the frequency and severity of local reactions at the site of injection after each inoculation of vaccine, as well as information on the frequency of general systemic effects such as fever. Both solicited and unsolicited AEs should be documented. Viremia should be monitored in the case of live vaccines, and clinical laboratory determinations should be monitored for all vaccines.

Phase 1 dengue vaccine trials, when conducted sequentially from adults to children and finally in infants, are never large enough to establish the frequency of local and systemic AEs with any degree of statistical certainty. Rather, a Phase 1 trial usually suggests only whether severe local and systemic AEs are common after vaccination and whether the vaccine stimulates an immune response. This information can be used in making decisions about the advisability of future studies.

Typically, each monovalent component of current live tetravalent vaccines have been tested separately in Phase I in order to establish the safety and immunogenicity of each prior to combining vaccines in a tetravalent mixture. Ideally, Phase I monovalent vaccine trials should be conducted in dengue-naïve subjects residing in countries where dengue does not occur. This is done to avoid putting vaccinees at risk for severe dengue, because such individuals will likely develop immunity against only one serotype as a consequence of their participation in the trial. In addition, it is desirable to test live vaccines initially in non-immunes in order to reveal major safety issues that might not occur in previously immune subjects. When tetravalent vaccines are under test, it would be acceptable to conduct trials in endemic areas.

A composite dengue reactogenicity score can help to interpret the safety profile of different vaccines in different age groups. The reactogenicity score is not a substitute for a more descriptive classification by intensity, duration, and seriousness, but it does provide a simple measurement (i.e. a single number). It proved to be relatively reliable for selecting formulations in a previous live attenuated dengue vaccine trial (55). It also takes into account the number of events experienced by each subject and the duration and severity of each event. Although several dengue illness scores have been published and are reasonably correlated (55,56,57), an internationally accepted standard system of calculating the illness score would be beneficial.

Immunogenicity

The assays used to measure vaccine-induced immunity in Phase 1 trials, as well as Phase 2 and 3 trials, have been discussed (2.4.2).

Populations and target groups

It is not required that a Phase 1 trial of a tetravalent vaccine be conducted in an endemic country. However, as discussed (4.2), it might be prudent to do so. Before the efficacy field trial in the final target group (e.g. children), a sequence of Phase 2 trials in the at-risk population may be required (4.3.2), for example, age de-escalation from adults into children and infants, with appropriate rules for proceeding from one trial to the next.

4.3.2 Phase 2 trials

Phase 2 trials are designed to extend the information on immunogenicity, safety, and dose-response (10). Where applicable, the number, intervals, and dose of vaccine inoculations should be refined and expanded in Phase 2 studies. In contrast to Phase 1 trials, Phase 2 trials are conducted in an at-risk population. They thus extend the information on effects of prior dengue and heterologous flavivirus infection. If the Phase 2 trial is being used to determine the optimal dose of vaccine to be used in a larger Phase 3 trial, the Phase 2 trial must be rigorously designed and have a large enough sample size to provide adequate statistical power so that the findings provide conclusive information for the Phase 3 design.

The duration of immune responses should be measured after primary and possible booster vaccination. Furthermore, if possible, Phase 2 vaccinations in endemic areas should be followed in at least a subset of vaccinees for several years for safety and to determine their protective ability to be extended, or boosted, by periodic revaccination or by natural infection.

In addition, Phase 2b trials that expand or extend Phase 2 trials in high-risk populations may be able to give some preliminary indications of vaccine efficacy in the target population.

Information from experimental dengue challenge in volunteers

Experimental challenge of vaccinated volunteers subsequently inoculated with fully virulent or partially attenuated DV are optional. Although there are ethical constraints and limitations of experimental challenge of DV-vaccinated individuals, such trials can yield additional information on vaccine safety, immunogenicity, DV viremia after challenge, some efficacy information, and selection of correlates of protection.

4.3.3 Phase 3 trials

The basic study design for a Phase 3 trial is a double-blind, vaccine or placebo-controlled, randomized control trial (DB-RCT) within individuals in the same community. As explained below, comparison between two communities, one vaccinated and one not, is not recommended.

Phase 3 studies must be performed in endemic areas where a large proportion of individuals are partially immune to one or more of the four dengue types. Protection can be measured only if vaccinated and control subjects are equally at risk to mild and severe dengue. A reasonable short-term objective for the first dengue vaccine efficacy (VE) trials is to demonstrate efficacy against laboratory-confirmed dengue illness resulting from at least one of the four serotypes (see 2.3).

The ultimate long-term objective for a tetravalent vaccine, and a major challenge, will be to demonstrate protective efficacy against each of the four dengue virus serotypes in the absence of any long-term safety concerns. A Phase 3 trial must have sufficient power simultaneously to measure protective efficacy whilst not interrupting dengue virus transmission significantly. Reduction of transmission in the control group would lead to failure to demonstrate a difference between vaccinated and control subjects. To prevent this, the trial could focus on a cohort of individuals at high risk of dengue. Such an age-specific, high-risk cohort can be defined by analysis of hospital and/or out-patient records for a previous period of several years (58). This design will allow randomization, blinding, use of a control vaccine, and the probability of measuring protective efficacy within a single dengue transmission seasonal cycle, and yet will not significantly reduce dengue virus transmission (see 1.5).

Furthermore, it is well established that the incidence of endemic classic DF and severe dengue, which includes DHF, varies greatly among different locales and also over time (59,60,61). Each locale establishes an independent cycle of DV transmission and of mild and severe dengue. These cycles can be determined by studying reported dengue hospitalizations over the past ten to 20-year period (some sites have 1–5 years of surveillance). This information can be used to suggest locales and years where there was a "higher risk" of mild or severe dengue. Such "at risk" locales can be evaluated for their suitability as Phase 3 vaccine efficacy study sites.

Before and during the course of a dengue vaccine trial, surveillance should be designed to detect febrile illness as early as possible to allow for laboratory confirmation of dengue. This can be approached in a variety of ways, including active surveillance for febrile illness at home, school or work, passive surveillance, and enhanced passive surveillance. A subset of the cohort will require detailed immunological characterization of prior dengue immune status and immunity to other flaviviruses that may be endemic in the area of the trial.

Within a community, various randomization schemes will have their own strengths and weaknesses. Such schemes include randomization by the individual, by households, or by geographic areas within a community. The scheme chosen should best maintain equal risk of DV infection between vaccine and control groups.

Scientifically, a design that compares VE between communities (one DV vaccinated and one control vaccinated) is not sufficiently rigorous for the reasons listed below.

1) Significant local geographic focality exists in DV transmission. For example, considerable variance has been shown between Thai schools and neighbourhoods separated by only a few miles (61).

2) If one community is highly vaccinated, the two communities are not equivalent because high coverage may significantly reduce dengue transmission, which may reduce morbidity and risk of sequential infections leading to severe dengue.

3) The risk of accidental bias is smaller with comparisons between individuals, because the units of randomization are more numerous, and easier to stratify (or match) by exposure. Hence, the comparison between dengue-vaccinated or control individuals residing in one community or location is recommended. This model can be extended to multiple locations if subjects are randomized within each location. Indirect vaccine effects (e.g. herd immunity) could be studied by surveillance for dengue after the vaccine is licensed and introduced into a community (see 6.2).

Mosquito abatement projects conducted during a trial may reduce virus transmission, but they nevertheless should not be put off or postponed. Such projects should be anticipated to take place during any clinical trial in an endemic country, and the statistical assumptions needed to calculate the size of the study cohort must consider the estimated reduction of dengue cases when DV transmission is reduced.

Efforts should be made to validate a surrogate of protection during the Phase 3 trial. A surrogate would allow alternative designs, such as a non-inferiority trial in bridging studies of additional candidate vaccines (see 5.).

5. Bridging studies

Bridging studies are done to extend conclusions regarding vaccine attributes such as protective efficacy or immunogenicity from one population to another, or from an existing manufacturing process to a new process. The end-point is the result of a serological (or CMI) assay, which can be demonstrated to be non-inferior between groups. The immune assay must be specific for each serotype included in a vaccine. If an immune correlate of protection is identified, a bridging study hypothesis could be confirmation of "no clinically meaningful difference in the seroprotection rate between groups". Lacking an immune correlate of protection, a bridging study hypothesis could be confirmation of a geometric mean titre ratio less than an agreed limit (e.g. less than 2.0) for each dengue serotype. However, the immune threshold for protection e.g. a specific antibody titre, may not be the same for all dengue serotypes.

This guideline emphasizes the importance of first achieving licensure for any one vaccine and confirming at least one immune assay which predicts protection against dengue illness. At present the best candidate assay for use as a correlate of protection from dengue is the in vitro virus neutralization assay. An immune correlate of protection would then justify head-to-head trials for immunogenicity of candidate vaccine(s) with licensed vaccine(s) when both vaccines are based on the same technology using non-inferiority trial design that efficiently allows for the recruitment of a minimal number of volunteers. A larger number of volunteers may be required for comparisons of safety, as discussed below. Bridging studies may also support any changes in manufacturing processes for dengue vaccines, new formulations, new dosing schedules, and new target populations based on age, genetic, or environmental characteristics (10).

Safety data obtained in the course of a bridging study can be added to safety data obtained in larger field studies, but will not be adequate per se to support licensure, because the numbers of subjects in a bridging study are typically insufficient to permit statistically powerful conclusions regarding safety.

6. Basic specifications of a Phase 4 field trial

6.1 Need for Phase 4 trials

Following licensure, when a vaccine is in use, monitoring of its effectiveness and safety is referred to as postmarketing surveillance or postmarketing studies (Phase 4 studies) (10,62). The types of studies used to evaluate new vaccines once they have been introduced into practice, include assessments of the duration of vaccine-induced immunity, vaccine safety, protection against clinical disease, and levels of vaccine coverage of the target populations.

Since the early 1990s, Phase 4 trials have successfully assessed the introduction of new vaccines into childhood immunization programmes and for their delivery at the community, provincial, and national levels (10,62). The question of whether an early, population-based Phase 4 trial will need to be planned immediately after licensure and/or even as a condition of licensure, should be addressed by the NRA. This is an important issue that cannot be answered unequivocally at this time. However, there is no doubt such a trial will be needed to provide robust assessments of vaccine safety, particularly after use in flavivirus immune populations and in populations in which other flaviviruses circulate.

Phase 4 studies will also provide estimates of the long-term effectiveness of immunization against multiple DV serotypes in large populations, and will help to establish the need for booster immunizations. Finally, Phase 4 may provide additional data on possible interference between the newly-licensed dengue vaccine and existing vaccines that are routinely administered to the same population of infants and children.

6.2 Study design

There are a number of study designs, several of which may be applicable to Phase 4 dengue vaccine trials, depending on the cohort characteristics, dengue prevalence, local culture, economic environment and resources available.

It may not be possible to simply monitor the incidence of mild or severe dengue before and after vaccine is introduced, because dengue is cyclic. If historic controls are being utilized, a “before and after” design may not take into account if temporal changes in dengue illness reflect VE, changes in disease epidemiology independent of the vaccine, changes in the intensity or accuracy of disease surveillance, changes in interventions such as mosquito control which modify disease occurrence, or changes in diagnostic definitions or reporting of disease.

For these reasons, more appropriate designs would utilize concurrent controls rather than historic controls. Concurrent studies allow evaluation in a relatively short period of time compared to historic controls.

Community or cluster-controlled studies can provide an important benefit of providing information on herd immunity and other indirect effects. In the stepped wedge design, the vaccine is introduced in phases, group-by-group, until the entire target population is covered. The groups form the unit of randomization (10,62).

6.3 Other post licensure studies and surveillance

After a vaccine is licensed in a country, it is necessary to continue dengue surveillance in order to ensure the proper performance of the vaccine and to possibly adjust immunization strategies. Special groups, especially HIV-positive individuals, can be studied during this period.

7. Moving towards vaccination programmes

Depending on the outcome of cost-effectiveness and operational analysis, it may be desirable that dengue vaccines be taken up by the national immunization programmes post-licensure for routine administration in dengue-endemic areas. As stated before, it is beyond the scope of this document to discuss requirements and special studies that might be needed for that purpose. In particular, if used as infant vaccine, dengue vaccination will need to be carried out on a schedule compatible with other vaccines (10,63). Therefore, interference between dengue vaccine and other vaccines likely to be given in the same time period must be ruled out. In addition, vaccine presentation, packaging and stability requirements should be compatible with large-scale use. Other WHO guidelines provide information on requirements for programmatic use of paediatric vaccines and elements of decision-making for public-health authorities (64).

Annex 1: Contributors

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Annex 2:

The guidelines represent a consensus document, and it should not be assumed that all the persons listed above agree with all the recommendations. Given the rapid development in the field, this version of the document should expire by 31 December 2012.

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The World Health Organization has provided technical support to its Member States in the field of vaccine-preventable diseases since 1975. The office carrying out this function at WHO headquarters is the Department of Immunization, Vaccines and Biologicals (IVB).

IVB's mission is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. The Department covers a range of activities including research and development, standard-setting, vaccine regulation and quality, vaccine supply and immunization financing, and immunization system strengthening.

These activities are carried out by three technical units: the Initiative for Vaccine Research; the Quality, Safety and Standards team; and the Expanded Programme on Immunization.

The Initiative for Vaccine Research guides, facilitates and provides a vision for worldwide vaccine and immunization technology research and development efforts. It focuses on current and emerging diseases of global public health importance, including pandemic influenza. Its main activities cover: i) research and development of key candidate vaccines; ii) implementation research to promote evidence-based decision-making on the early introduction of new vaccines; and iii) promotion of the development, evaluation and future availability of HIV, tuberculosis and malaria vaccines.

The Quality, Safety and Standards team focuses on supporting the use of vaccines, other biological products and immunization-related equipment that meet current international norms and standards of quality and safety. Activities cover: i) setting norms and standards and establishing reference preparation materials; ii) ensuring the use of quality vaccines and immunization equipment through prequalification activities and strengthening national regulatory authorities; and iii) monitoring, assessing and responding to immunization safety issues of global concern.

The Expanded Programme on Immunization focuses on maximizing access to high quality immunization services, accelerating disease control and linking to other health interventions that can be delivered during immunization contacts. Activities cover: i) immunization systems strengthening, including expansion of immunization services beyond the infant age group; ii) accelerated control of measles and maternal and neonatal tetanus; iii) introduction of new and underutilized vaccines; iv) vaccine supply and immunization financing; and v) disease surveillance and immunization coverage monitoring for tracking global progress.

The Director's Office directs the work of these units through oversight of immunization programme policy, planning, coordination and management. It also mobilizes resources and carries out communication, advocacy and media-related work.

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