U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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WORKSHOP ON IMPLEMENTATION OF NUCLEIC ACID TESTING

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TUESDAY
DECEMBER 14, 1999

The workshop took place in the Masur Auditorium, National Institutes of Health, Bethesda, Maryland at 8:00 a.m., Edward Tabor, M.D., Chair, presiding.

Present:

Edward Tabor, M.D., Chair
Michael Busch, M.D., Ph.D., Speaker
Michael Chudy, M.D., Speaker
Masaharu Nakajima, M.D., Ph.D., Speaker
John Saldanha, Ph.D., Speaker
Robin Biswas, M.D., Speaker
Andrew Dayton, M.D., Speaker
Andrew Conrad, Ph.D., Speaker
Don Baker, Ph.D., Speaker
Charles Heldebrant, Ph.D., Speaker
Alan Liss, Ph.D., Speaker
Brian Renoe, Ph.D., Speaker
Christina Giachetti, Ph.D., Speaker
Susan Stramer, Ph.D., Speaker
Sally Caglioti, MT(ASCP)SBB, Speaker
Richard Gammon, M.D., Speaker
Michael Strong, M.D., Speaker
Mary Elizabeth Jacobs, Ph.D., Speaker
Paul Mied, Ph.D., Speaker
Kay Gregory, MS, MT, (ASCP) SBB, Speaker
Chris Healey, Speaker
Mei-Ying Yu, Ph.D., Speaker
Thomas Lynch, Ph.D., Speaker
Aris Lazo, Ph.D., Speaker

ALSO PRESENT:

Thomas Weimer, Ph.D., Speaker
Gerold Zerlauth, Ph.D., Speaker
Andrew Conrad, Ph.D., Speaker
Sheryl McDonough, Ph.D., Speaker
James Gallarda, Ph.D., Speaker
Klaus-Heinrich Heerman, Ph.D., Speaker
Professor Jean-Pierre Allain, Ph.D., Speaker
Celso Bianco, M.D., Ph.D.
Roger Dodd, Ph.D., Speaker
Steve Kleinman, M.D., Speaker
Paul McCurdy, M.D., Speaker

I-N-D-E-X

P-R-O-C-E-E-D-I-N-G-S

(8:04 a.m.)

DR. TABOR: Welcome to the Workshop on Implementation of Nucleic Acid Testing. I'm Edward Tabor, Associate Director for Medical Affairs in the Office of Blood. Unfortunately, Dr. Indira Hewlett, who was going to be playing a major part in this workshop, is unable to be here because of illness. She's just returned from a trip to the Far East and I certainly hope she has a domestic, rather than a foreign illness. Anyway, we wish her well.

We are in the midst of a scientific revolution in blood and plasma safety, truly a revolution. This revolution is associated with the implementation of nucleic acid amplification testing, known by its abbreviation NAT to detect viruses and other pathogens in blood. It is a revolution of great significance because it moves the detection of potential viral and other contaminants of blood for transfusion into the realm of molecular biology and because, in the long run, it may permit us to eliminate some organisms for which efficient immunologic assays have not yet been developed. This technology also may permit the simultaneous detection of numerous, diverse infectious

agents in one highly sensitive assay system.

One of the most common forms of NAT, the polymerase chain reaction test was invented in the early 1980s and it came into relatively wide use in research laboratories for the detection of viral nucleic acids by 1988. A review of this technology at an FDA-sponsored workshop held in September 1994 concluded that practical application of it, for making blood and plasma derivatives safer, was not possible at that time. The test was expensive and time- consuming to run. It lacked automation and high throughput. Contamination of fresh samples by amplified gene sequences, known as amplicons, from previous test runs was a difficult problem.

The very concept of trying to test 25 million units of blood and plasma each year and getting the results back to the right place in time to interdict the use of infectious products was a daunting challenge. However, over the next several years, additional developments reduced the importance of these problems. Commercial manufacturing of semi-automated and automated test kits in large quantities reduced the time it took to run the tests. Special pipette tips, known as positive displacement

pipette tips, prevented carryover of contaminants in aerosol droplets adhering to the pipette beneath the disposable tips. The use of DUTP in place to TTP as a nucleotide in the amplification process in NAT screening for blood, permitted the inclusion of an enzyme urasyl n-glycosylase in each new sample mixture to destroy contaminating amplicons which, unlike native DNA, would contain hydrolyzable DUTP.

However, the most important innovation was a concept, rather than a gadget. Although screening tests for viral markers on pooled samples had been conducted previously in some countries outside the U.S., it was FDA's decision in 1996 to permit such screening on minipools under IND applications that accelerated the pace of the development and application of this technology. Enough was known about the assays that were proposed that there was general agreement that the blood and plasma supply would be safer as a result of this policy.

The philosopher Wittgenstein once said, "The aspects of things that are most important for us are hidden because of their simplicity." This applies to the remarkable, but simple innovation of combining minipool testing and matrix analysis to identify a

contaminated unit. The use of the minipool reduced the cost of the testing by necessitating fewer tests than would be needed if individual units were tested. The use of matrix analysis permitted rapid donor identification in order to remove the infected unit without discarding the entire minipool. Donor identification also permitted deferral of the donor from possible future donations and notification enabled the donor to seek early medical attention and to take precautions to prevent spread of infection to close contacts.

Subsequently, modifications in NAT methods by some manufacturers increased the sensitivity of the testing. Today, most tests that are currently under investigation can detect as few as 20 to 50 viral copies per mL with the high rate of reproducibility.

The ability of testing facilities to get the results back to the collecting facilities within a few days with the positive unit correctly identified so that the positive unit or its components would not be transfused into patients is being made possible by logistic innovations and computer technology. This aspect of NAT related technology is still under development, particularly with regard to platelets

because of their shelf life of only five days.

A major change in public perception of blood safety also contributed to this revolution. In the late 1970s, during the first five years after the introduction of sensitive tests for hepatitis B surface antigen, the elimination of most of the cases of post-transfusion hepatitis B was considered such a marvelous improvement in blood safety that the remaining rare cases were considered minimal. In fact, at that time there was no hope felt for eliminating, in the foreseeable future, what we now call window period cases and perhaps other cases in which the virus, for some reason, is present at levels below the limits of detection.

Of course, it was soon recognized that an even greater remaining problem was non-A, non-B hepatitis, now known to have been mostly due to hepatitis C virus and that problem was indeed vigorously attacked in laboratories around the world. But the epidemic of human immunodeficiency virus type 1 infection brought a new level of concern to the public and particularly to users of plasma derivatives. The public and their representatives in Congress began to expect a zero-risk goal for blood safety. This resulted

in efforts in research and in regulatory policy to eliminate even those few units containing residual virus that could be transmitted by blood or plasma infusion.

During the past two years the FDA has permitted the interim application of NAT testing of minipools under INDs. This has permitted the public to benefit from that screening of blood and plasma for HCV and HIV, while ensuring that proper studies are done to validate the assays and to validate the logistics of interdicting potentially infectious units and identifying and notifying infected donors.

By the beginning of December 1999 -- this month -- it was expected that approximately 99 percent of plasma and 80 percent of blood collected in the United States for transfusion or for further manufacture into plasma derivatives was being tested by NAT to detect HCV and HIV. The prevalence of testing was expected to reach 99 percent for both blood and plasma by the end of this month, the end of December 1999.

Increasingly, the results are being made available soon enough to identify infected units before the components are infused into a patient. Also, some companies have begun to develop mechanisms to permit individual unit testing under INDs and probably we will

be hearing more about this important advance today.

Well, what does NAT offer us in the future? The likelihood of individual unit testing within the next few years should eliminate nearly all transmission of HCV and HIV by blood transfusion. The risk of these infections from plasma derivatives is already zero when the inactivation and removal steps in manufacturing are carried out correctly. But there are other potential future benefits as well. One possible benefit is the possibility of the near elimination of the transmission of hepatitis B virus by blood transfusion when single unit HBV NAT is introduced. Another possible benefit, the elimination of the transmission of viruses that are not affected by currently available inactivation steps in the manufacture of plasma derivatives, viruses such as human Parvovirus B-19 and hepatitis A virus.

Another possible benefit, the simultaneous screening for multiple blood-borne pathogens in one sensitive assay. Another possible benefit, the elimination of bacterial infections as a complication of transfusion, particularly as a complication of the transfusion of platelets. Transfusion-transmitted bacterial infections are now a cause of 10 to 15 percent of transfusion-related fatalities. The use of NAT with

micro-array technology could permit rapid screening for a large number of bacterial species at one time. Another possible benefit, the identification of new agents, such as the causative agent of what we know as non-A to E hepatitis and their eventual elimination as causes of transfusion- transmitted disease.

I want to thank in advance all of the speakers who will be participating in today's workshop and also all of you who have come a long distance to attend the workshop. I know that the presentations that we will hear today will be a valuable resource for those working with this rapidly advancing technology.

The first session is titled Regulatory Perspectives and Issues and I'd like to introduce the first speaker, Dr. Michael Busch, who is well known to all of you, who will be speaking on Estimates of Residual Risk in Infectious Disease Transmission During the Window Period.

Dr. Busch?

DR. BUSCH: Thank you, Ed. I was asked to briefly review the current risk estimates and then focus on window period characteristics to estimate the yield of both minipool and single donation NAT screening. And then toward the end I'll present a little bit of data

with respect to yield, some recent work on cost-effectiveness, and also a little bit of discussion around some future studies that are in the planning stages to address some of the still- unresolved questions.

Just to begin, it's important to recognize how far we've come. This slide is from some work with Jim AuBuchon, just summarizing the risk of HIV, hepatitis B and hepatitis C over the last several decades and each of these points is actually an empirically derived or in the very recent data, modeled estimates of risk, based mostly on National Institutes of Health-funded studies. And what you can see is really profound reductions in risk. This is a logarithmic scale, from risk estimates in the one percent or so range for HIV and hepatitis C, down to risk estimates in the range of less than 1 in 100,000 for the hepatitis viruses and about one in a half a million for HIV and HTLV.

In the last few years, our group in the REDS study group, and particularly Steven Kleinman and myself, have endeavored to try to understand the sources of residual risk and we focused on estimates that relate

to the window period based on modeling, the incidence rate times the duration of the window period to estimate risk or project yield of new tests, understanding of potential contribution of viral variance that may be immunologically divergent and not tested by the -- not detected accurately by the tests that are typically based on a single strain prototype, concern over immunosilent infections or carriers who fail to form antibody and testing errors. A fair bit of work has gone on both in this country and overseas to really understand the prevalence of these various types of risk. From that analysis we've derived estimates for each of the major agents, for each of these different sources of risk as well as the sum total for all of these sources of risk. And fortunately, in this country, contributions from viral variants is extremely small, that these unusual variants are very rare and the tests, I think, the companies, with a lot of FDA encouragement, have been very proactive to maintain a global surveillance for new variants and modify and improve the tests to be able to detect these. So certainly in the States, viral variants contribute very little. Unusual seroconverters or so-called immunosilent carrier is very rare. We'll come back to

HCV a little bit later. There has been controversy and continues to be some data to suggest that there may be more frequent HCV infected persons who fail to seroconvert, but certainly it's, I think, been shown to be fairly rare. And in tests there, I think we'll hear a little bit from the Red Cross later about observing some test error with NAT testing that corroborates an estimate that's impressed, that estimates that around 1 in 200 to 300 test performed in blood banks routinely may be erroneously run for various reasons, but the contribution of test error to risk is very small because an error has to occur on a seropositive unit in order for that unit to be released. So what you see here is a fairly small number of estimated transmissions or donations that are infected and missed due to test error.

So walking through these, we see fairly small contributions to risk from all of the sources, other than the window period and it's really therefore, the window period estimates that are the critical sources of the risk and the critical target of nucleic acid screening.

And we were challenged, back at the conference Dr. Tabor alluded to, by Dr. Kessler back in

1994 to move forward, to bring the new technology of nucleic acid amplification to really eliminate the window period and it was this challenge that I think set the ball in motion, set the government to both fund and put a fair bit of regulatory direction in front of us to move into bringing forward nucleic acid testing.

The window period is again the major target of the screening. To understand the window period, one needs to really look at data from a number of different sources. As we'll see as I go through the data, we've done a lot of work on units from seroconverting plasma donors where these have been compiled into panels of serial specimens collected usually at two-times-a-week intervals from plasma donors and these have been extremely valuable to characterize the time course and the dynamics of markers during the window period.

There are also important data coming from cases with known exposure dates, be these transfusion-related transmissions or needle-stick accidents and I'll show you a little bit of data from each of those in the presentations in a few minutes. These are important because these are the types of situations where we can actually ascertain the time from exposure to seroconversion or to detectable viremia. This is

important in a concept, that I'll get to in a moment, of the eclipse phase.

We also have people coming in who have primary syndromes, be it the primary HIV syndrome, the flu-like syndrome or hepatitis. Now these situations actually are not very informative for the window period because when people present with clinical symptoms, they're usually well into the viremic window phase, usually at peak antigen phase or well into the hepatitis viremia. So very little information comes from the primary syndrome-type cases in terms of early window period characteristics.

Recipients of seroconverting donors, when one has a seroconverting donor and does look back, one can derive a relationship between the time interval between the seropositive and the prior seronegative donation and whether or not transmission occurred. And from that, one can derive an estimate for an infectious, transmissible window and this was done by Lyle Pederson about a decade ago now for HIV. These seroconverting donors, though, are so rare today and the frequency of look-back transmission is so rare that actually we've been unable to update these estimates of infectious window from sort of a human transmission perspective

over the last decade.

Cohort studies are quite valuable and actually I'll show you some data for hepatitis C where we've done some analysis recently of some cohorts for hepatitis C viremia from seroconverters in high-risk cohorts and I think, again, an important source of data, although usually the samples in the freezer from these cohort subjects are separated by three to six months, and so don't give us nearly as refined an understanding as these plasma panels.

And finally, animal model studies, which are quite important and I think we're coming back to these to understand many of the unanswered questions in terms of when infectivity occurs during the early window period.

The new concept that's kind of evolved over the last few years by combining data from exposure to seroconversion with the data from plasma panels, for example, is the concept of an early eclipse phase following exposure before one can detect any evidence of viremia in the blood by nucleic acid testing or infectivity studies.

So we talk now about an early window period that probably is not infectious and it cannot be closed

with any testing method simply because there is no virus in the blood, followed by the development of infectious viremia. And this is really the target of nucleic acid testing, to close the infectious viremic window period, recognizing that we're never going to completely close the theoretical exposure to viremia window phase.

For HIV, we do have some significant data in terms of time from exposure to seroconversion and this is from health care workers who acquired HIV from a needle-stick or other accident. And this is data from CDC, Glen Satten analysis, looking at a series of about 52 health care workers who became infected and had a reasonable sampling of testing after the exposure. And from this analysis, there was an estimate of around 40 days average time from exposure to seroconversion. And this was based on a mix of antibody assays in the late 1980s, early 1990s, so we probably estimate today and most data would support that there's probably about a 20- to 25-day period from exposure to antibody seroconversion.

But we've really gained an enormous insight into the dynamics and time course is from these plasma seroconversion panels. This is a representative panel showing the time, somewhat arbitrarily dated here from

the date of the first available specimen and then the viral load, in this case, quantitative PCR. We can see that these individuals are giving at usually twice a week intervals, quite regularly, and then these people are typically detected based on the antibody seroconversion in the past. And then fortunately, the companies have retained in the freezers and are now required to retain for several months the prior donations. So when a donor seroconverts, we can then go back and retrieve the earlier components and build panels of serial samples and then go back and characterize them for the development of antibody and other markers. So here you can see that the antibody comes up on approximately Day 28 or so. We can detect antigen for about four or five days prior to antibody and then RNA can be detected in this particular panel a couple days prior to the detection of antigen.

By compiling data from a large number of these panels, we can derive figures such as this, which is what's called a box and whisker plot, which divides the time course of viremia up into different stages: the RNA-only period, the p24 antigen EIA negative phase, and then on through the development of antibody reactivity by EIA and Western blots.

An analysis of these kind of data can yield two parameters. One is the duration of each of these stages. In this analysis, which is based on the RNA determinations by the Roche monitor assay which has about a 200-copy sensitivity, Glen Satten analyzed this data to yield an estimate of about three and a half days for the RNA-only phase; five days for the p24 antigen positive pre-antibody phase; and then progressive periods when the EIA is reactive, but the blot negative, the blot indeterminant and then going through an early incomplete Western blot pattern. What you can also determine then is for each of these stages the distribution of viral load during each stage and we can see that the RNA-only stage, in this case, had an average copy number of a couple thousand and low copy number, down to the limit of sensitivity of the assay, of approximately 100, whereas the p24 antigen phase, the viral load is typically much higher.

In a more recent analysis of data, actually from alpha and NGI, we've looked at the distribution of viral load in the RNA-only phase, versus the p24 antigen pre-seroconversion phase for a larger number of seroconversions. I think this is based on around 40 or 50 seroconverters and we have a fairly large number,

both of RNA-only samples and of p24 antigen positive samples. This RNA assay was also more sensitive, sensitivity down to the 100-copy or so range.

This distribution then lets us look at the -- a more formal analysis of the viral load distribution and in this analysis for the p24 antigen-positive phase, the median copy number is 140,000 and the 2.5 percent confidence limit, so in other words, you detect 97.5 percent of the antigenemic samples had copy numbers estimated at greater than 7,000 copies. So this is important because this is a parameter that's sort of been floated as a target goal that we, in introducing minipool NAT, one of the objectives is obviously to replace the antigen assay and in order to do that with high confidence the assay should have at least 7,000 copy sensitivity, the minipool NAT assay.

In contrast, if we want to completely close the RNA window we would basically have to have an assay that's extraordinarily sensitive because the RNA-only phase has samples that do have copy numbers down at 100 copies or less. The median copy number is about 2,000. So during the RNA-only phase, the viral load is substantially lower than can be confidentially detected uniformly with minipool NAT.

This is some data from Sue Stramer, directly, empirically kind of addressing the issue of can we comfortably eliminate the p24 antigen assay once we introduce minipool NAT and Sue took a large number of samples, 92 samples, from 25 seroconverting panels and ran those samples at the 1 to 128 dilution that is the dilution that Red Cross was operating at. And she was able to demonstrate 100-percent detection. So all of these samples that were antigen positive had very high copy number of RNA. And in addition, there were 21 samples that were detected as RNA-positive that were p24 antigen-negative and they were detected even at the 1 to 128 dilution. So data like this for HIV is very reassuring that we can not only replace the p24 antigen test, but interdict and identify a number of additional viremic specimens.

We've gone a little bit further in terms of modeling the antigen-RNA relationship and this slide summarizes a correlation analysis between the level of RNA and the S to CO range of the p24 antigen assay. To ask the question of what is the cut-off of the -- what's the RNA concentration at the cut-off of the p24 antigen assay. So this is a regression analysis of 146 samples from seroconversion panels. And what this tells us is

that the cut-off of the p24 antigen assay is just almost exactly at 10,000 genome equivalence per mL. So that would tell us that as long as our nucleic acid screening test in the context, for example, of minipool screening is as sensitive as 10,000 copies, it should detect every single seroconverter who is detected by antigen at the cut off-of the antigen assay. So again, another parameter that's useful as we try to set a target objective for minipool screening.

Now as we try to project back, that's kind of empiric analysis of hard numbers. As we've tried to project back to the RNA load prior to the detection of RNA, we've used a parameter called the doubling time, so we've taken these panels and we've looked at particularly the early ramp-up phase prior to any antibody during which the RNA levels are increasing and derive the slope and using that slope we can estimate the doubling time or the rate at which the virus is increasing in the concentration in plasma or time. And for HIV we've recently updated this analysis and the doubling time is estimated at approximately 30 hours, so the concentration of virus in the plasma doubles every 30 hours. But in addition, using that slope, we can back-estimate a theoretical concentration of virus at

earlier time points for each seroconverter. And using this projection approach here, you can see that in this population of seroconverting plasma donors, we would estimate that there would be a single copy of RNA per mL, about 20 days before the peak viremia. And this is quite consistent with the number I alluded to earlier of approximately a 20 day period from exposure to detectable virus. So this suggests that perhaps there might be infectivity existing prior to RNA for a period of five or ten days.

But more recently, in collaboration with Harvey Alter and J.P. Allain, we've begun to do studies in chimpanzees that are directly asking the question of when does infectivity occur during the eclipse phase. And this paper was published a few months ago in Transfusion and what was done in this sort of pilot seminal experiment that is now being expanded was to take a chimpanzee and infect that animal with HIV and draw blood from that animal on actually twice weekly bases through the early seroconversion window period. And that first animal detected detectable viremia about five weeks following inoculation and then finally seroconverted out at Week 8. Now the blood and plasma in the cells from each of these sampling time points was

frozen away and then after the characterization of the window period virology, we went back and took material from bleeds extending back several bleeds prior to detectable viremia and used that material to inoculate a second chimpanzee to ask the question of when did infectivity occur relative to the ability to detect virus by amplification methods or culture. And what was discovered was that the sample collected two weeks before viremia was not infectious, this second chimp did not become infected. Likewise, the sample collected a week before detectable viremia, a full month into the eclipse phase was, again, not infectious in a secondary passage attempt. And finally, the first viremic sample was shown to transmit. So again, in a single chimp experiment this study suggests that this eclipse phase is not infectious and even though we can theoretically project back that there may be a very low copy number in the body that we're not able to detect any infectivity until the time when we can detect the viral RNA. I'll close with -- this is really the kind of experiment that we're really very interested in expanding, both for HIV and hepatitis C.

Moving on to hepatitis C, it's a very different window period. With HIV we have this very

rapid ramp-up phase and then quick seroconversion within a week or so. With HCV, as illustrated in this plasma panel, we similarly have a very rapid ramp-up phase, but then there's a very prolonged viremic pre-seroconversion period, averaging in different studies in the range of 50 to 60 days of high-titer viremia prior to antibody seroconversion. And this slide actually again from Sue Stramer shows that this viremia is so high-titer that actually you can't even tell the difference in terms of an S-to-CO ratio or a quantitative analysis of the samples, be they tested undiluted or at 128 dilution, very consistent detection because the viral load is so high during this prolonged window phase.

This is a summary of Sue's data. I think in this case with NGI, looking at the viral load distributions particularly focusing out here, during the RNA-only phase prior to antibody and the lower confidence bound on the viral load distribution during the 60-day or so viremic pre-seroconversion window is around 10,000 copies. So very, very high copy number and very amenable, as we'll talk later, to minipool testing.

And Sue again formally looked at the question of how many of the window phase samples could

be detected at a high dilution as she addressed the question of the value of minipool testing. In this particular example from 22 panels looking at two different lots, I think this is Gen-Probe's assay. She determined that at 1 to 128 dilution she could pick up 93 to 94 percent of the samples that could be detected undiluted. So a very high proportion of the window phase samples can be detected at a 1 to 128 dilution and I think the majority of those samples that were missed could be detected at the current 1 to 16 dilution factor.

There were a few surprising observations that are still in need of further study. For example, in a couple of these samples that went negative at the 1 to 128 dilution, so they were reactive at the NEAT, but went negative at the 1 to 128, the viral load in these samples was really quite high, 100,000; 90,000. These samples should have been readily detectable at a 1 to 128 dilution given the sensitivity of the assays. This has led to some concern that there may be some aggregation phenomenon going on, complexing or for some reason that these samples are not diluting out as predicted based on the viral load, suggesting that perhaps minipool testing may have some surprises in

terms of matrix effects or failure of seropositives -- I'm sorry, viremic samples to be fully represented when they're sampled in the context of pipetting to create the pool. So just one area of continued concern in need of further study.

In addition to looking at these plasma seroconverting panels, we've also done similar analysis of the cohort, the transfusion transmitted viruses study from the late 1980s. In this population, we have a fairly large number, about 100 HCV seroconverting recipients. The advantage of these samples is that the date of transfusion is known, so you actually know the date that these recipients were infected by transfusion of a known seropositive and we actually have the samples so we can go back and characterize the viral load and the virus itself in the transmitting unit. That lets us then really benchmark the time course to detectable viremia relative to the day of exposure, i.e., the day of transfusion.

And this is just a representative panel from this cohort showing a very similar pattern of a very rapid detection of high level virus, persistent high level plateau viremia prior to the development of ALT and full antibody seroconversion. When we look at

a large number of these panels we see that we can detect, this is the serial case number, over on this axis and this is time course following the transfusion. We can detect viral RNA typically with one to two weeks. The vast majority of cases, in fact, the very first specimen collected following transfusion is already viremic and often at peak viremia. And then downstream, about 50 to 60 days later, ALT comes up in an antibody seroconversion.

In a formal survival analysis of this data has yielded time estimates for the time from transfusion or exposure to first detected RNA and that's averaging around 12 days following exposure, we can detect virus RNA. And then about 50 days after exposure, ALT elevation occurs on average and seroconversion occurs 70 to 80 days. So this gives us again a very consistent finding relative to the plasma seroconversion panels of around a 50-day viremic

pre-antibody period.

In TTVS like the plasma donor panels, if we look at the viral load during the window period, it averages 10^5 to 10^6 copy numbers, so very high viral load, very amenable to minipool screening.

So for HCV this just summarizes, we believe

that on average about 10 to 12 days following exposure there's a rapid ramp-up of viremia. Recently, we've begun to characterize the doubling time with some of the newer panels that are being identified and it averages, probably, in the range of two to four hours. So unlike HIV which is about a 30-hour, relatively slower ramp-up with HCV, an extremely rapid, early ramp-up phase, followed by this plateau, a very high-titer viremia and then, with seroconversion, some down modulation of the viral load.

One question with hepatitis C, as I alluded to earlier, is the frequency of antibody-negative chronic carriers. And I think we'll see some data from Alpha later and we'll also hear data from the Whole Blood sector that supports that these immuno silent prolonged seroconverters are not very common. Most of the pickups that have been observed, where follow up of the viremic seronegative donors has been pursued, have demonstrated that the cases that are found as RNA-positive are seroconverting. So for example, in the early alpha NGI study, they identified 20 RNA-only plasma donors and when they followed these donors, 16 of them seroconverted, really in a time course exactly as predicted from the earlier modeling work in the range of

50 or 60 days, on average. There were only four people who were not documented to seroconvert and none of those were followed beyond one month. So really, these are probably just failure to adequately enroll and follow these cases. So really, no evidence from this study of a prolonged immunosilent carrier. There have been case reports. The French, for example, documented a case and reported at the recent ABB meeting of a donor that they detected who was viremic and failed to seroconvert for several years and in fact, had transmitted HCV for years prior to that. But I think these cases are very rare.

Nonetheless, there are still reports coming out that are raising concerns over delayed seroconversions for HCV. Most recently, there was a paper in Blood just three or four months looking at an injection drug user cohort from Amsterdam. And in this cohort they were following a cohort of 358 injection drug users and this is a very high prevalence population. It was actually an HIV cohort that they further studied for hepatitis C. And 88 percent of these individuals were HCV positive at enrollment, so very high prevalence and of the seronegatives 19 seroconverted on follow up. And what the study then did was to go back and test the samples in the freezer from

the prior three years from these 19 seroconverters and they detected RNA in 12 of the 19 cases prior to seroconversion. In seven of the cases it was the kind of typical viremia that we've been talking about prior to seroconversion, but they actually had five cases, all of which were HIV-negative in whom they did detect intermittent, low-level viremia for a year to up to three to four years prior to seroconversion.

One of the concerns about this study is that it was the intermittent nature of the detection of the RNA. There were often skips between detecting and then not detecting. And sometimes the subtypes would even shift so at one time they detect a 1B and then the next sample from that donor, that drug user a year later would be a type 3. So it's something here that sort of raises eyebrows about the accuracy of the results, but nonetheless, it's additional data to support earlier concerns about the possibility that there may be in some populations low-titer immunosilent carriers.

We've initiated a study in collaboration with Bruce Phelps at Chiron looking at populations who are at high risk for evidence of immunosilent infections and in some recent work we've tested over 1,000 samples from various populations from San Francisco: a VA

clinic population that has an 18 percent HCV prevalent, young injection drug users, people who are just beginning to use drugs in San Francisco, 45 percent prevalence; and then an older injection drug user cohort that over time has a 90 to 95 percent seroprevalence.

What we've done is to take the seronegative samples from these populations and test them using a minipool screening strategy similar to what we're doing in the blood supply. We're actually screening pools of ten using the Gen-Probe assay to see how frequently do we detect viremic seronegatives. And we did detect eight cases. All of these except for one have very high viral load and are probably seroconverters. And they're typically found in the populations that we know have very high incidents.

So this needs further study, but when we have tested, for example, in this cohort here, where we detected four out of 72 seronegative samples with the minipool strategy, we further tested the samples that were negative, so the 68 samples that were negative on pools of 10, we tested them with individual donation testing and did not identify any additional infections. So we're not finding evidence of low-titer viremia in high risk populations that would not be detectable by

the current minipool testing.

Just a brief comment on hepatitis B. I think as most of you are aware, with hepatitis B, the surface antigen tests have improved so much that the sensitivity of the antigen tests are down in the range of 2,000, in some of the new chemiluminescent assays, 1,000 per mL. And because the antigen tests are so sensitive, they've narrowed the window fairly dramatically in the viral load during the pre-antigen phase is very low.

From some work from, again from some of the early cohort studies, the time from exposure for hepatitis B to antigen is around 56 days and some limited work on pre-seroconversion RNA or DNA work we can narrow that window potentially by about 20 days. But the problem in terms of minipool testing is illustrated by the next slide, again, from Sue Stramer and Andy Conrad at NGI looking at HBV seroconverting panels. What one finds is that the viral load during the pre-antigen phase is extremely low, averaging only 400 or 500 copies and consistently less than 5,000 or so copies and that's because the antigen test detects any samples that have greater than 3,000 or 4,000 copies. So this is a problem for minipool testing because the

dilution inherent in doing pool testing to a great extent dilutes you out of the ability to detect the viremic pre-antigen phase.

So in summary, for the three major markers that we've been worried about and predominantly focused on, we understand the time from infection to seroconversion. For HIV, it's probably in the range of 16 days to antigen and 22 days to antibody. For HCV, about 70 days, for HBV about 50 days. The nucleic acid tests applied particularly to mini-pool can reduce these windows by in the range of five days prior to antigen for HIV, probably in the range of 50 to 60 days for HCV and perhaps by a couple of weeks for HBV. The doubling time is an important parameter as we model back and project yield. For HIV, the doubling time as indicated is probably around 30 hours. For HCV, it's probably in the range of two to three hours. For HBV, it's relatively slower at about four days. The viral load for HIV, it's a very rapid dynamic ramp-up, so from the point of being able to detect it to peak antigen we go from 100 copies or less to in the range of ten million. For HCV, this prolonged high titer viremic phase averages 100,000 to ten million copies. For HBV, the

viral load during the pre-antigen phase is very low, only 10^2 to 10^4 copies. And for this reason, as we talk about usefulness of window period detection of minipool screening, for HCV, it's perfect because it's a very high-titer, prolonged viremia, so mini-pool testing is really the answer for HCV. For HIV, it's only marginally effective; it will only further reduce the window by about half because there is this relatively slower ramp up, lower level viremia that minipool testing is not detecting. And for HBV, it's essentially unsuitable because pool sizes in the range of even as few as 20 samples dilutes you out below the ability to detect the pre-antigemic phase.

Now as we try to estimate the yield, the incremental yield of these assays, we then have to bring in the sensitivity of the test and I think we'll see a lot of this. These tests, as indicated, are extraordinarily sensitive. Fifty percent detection limits of probably 10 to 20 copies, 95 percent detection limits for each virus of within the range of 50 copies.

So as we project the yield which Ed is indicating we have to close here, we have to incorporate the data on the window period closure, the sensitivity of the assays and the doubling time parameter to project

the yield both of minipool and single donation testing. And just to briefly summarize, for HIV, if we could add minipool testing with sensitivities in the range of about 500 to 1,000 copies, we would project that we'll pick up about six HIV infections per year. If we shifted to single donation testing, we would pick up around nine, so an increment of an additional to 3 to 3.5 infections. And for HCV -- let's skip this, I'm sorry.

For HCV, we project that we would pick up around 54 donations by going -- I'm sorry, we'd pick up around 53 donations per year in the U.S. whole blood sector by introducing minipool testing and single donation testing would only detect an additional one or two infections, or 54.

And just a final slide, I recently supplied these numbers to Jim AuBuchon who has done a cost effectiveness analysis on the minipool testing versus single donation testing and it's kind of interesting that if we benchmark relative to p24 antigen where we estimate, Jim has estimated about $1.8 million per quality life year extended, minipool nucleic acid testing is actually more cost effective than antigen testing. If we assume about $8 per test to detect both

viruses in the context of approximately 20 member pools, we estimate about $1.2 million per quality life year. Transitioning to single donation testing is still -- increases the cost per quality to about $1.8 million, but it's actually still more cost effective than p24 antigen testing. So although in some original analyses one would have predicted, and I did, that this would have been a worse endeavor, given the relatively low yield. In fact, in minipool and single donation nucleic acid screening actually stacks up pretty comparable to p24 antigen testing, and in fact, better than some of the other recent issues such as solvent detergent plasma.

I'll stop there. Thank you.

(Applause.)

DR. TABOR: Thank you very much, Mike, for that every interesting talk. The next talk will be given by Dr. Paul Mied in place of Dr. Hewlett. He'll be speaking about NAT implementation and regulation in the United States. Dr. Mied is the Deputy Director of the Division of Emerging and Transfusion Transmitted Diseases.

DR. MIED: Thank you, Ed. May I have the first slide, please? I'm going to summarize for you,

briefly, FDA's role in the regulation of nucleic acid of NAT for pool plasma and for individual units under IND in the United States.

As we've already heard, it's well recognized that NAT is currently the most sensitive method for virus detection in the antibody-negative window period and that implementation of NAT could further reduce the window period for HCV, HIV and HBV resulting in enhanced viral safety of blood and blood products and also an additional public health benefit in providing early diagnosis and referral of donors for medical treatment.

Now this slide says everything takes longer than you expect, even when you expect it to take longer than you expect. Now, you know, this is true for just about everything today, except for NAT, it seems. It was, as we heard this morning earlier, from Dr. Tabor, it was in September of 1994 that FDA held a workshop on the feasibility of gene-based testing to close the window for HIV and at that time due to technical and throughput complexities, NAT in any form seemed a long way off. However, a little over three years later, screening of source plasma pools for HCV and HIV-1 was initiated under IND and now barely five years later we

can say that the licensure of the first NAT method may not be very far off after all.

For NAT, the whole process is backwards. Normally, clinical trials are done under IND at a limited number of sites. Then the test is licensed. Then FDA recommends it for widespread use and the new test is then implemented, but even now as we've heard, NAT as an investigational test is being used prior to licensure, to screen nearly all blood and plasma in the United States. This isn't the normal course of implementation of a new test.

So how has all of this happened? Well, due to the complex and customarily labor-intensive nature of NAT, the approach of screening minipools or small pools of plasma, rather than single donations was initially considered to be the more practical and feasible format due to the technical state of development of NAT and the concept of pool testing during the fractionation process of plasma for further manufacture.

By 1997, some countries in Europe had initiated voluntary screening of pooled donations of plasma using the nucleic acid-based test method and also a directive was issued by the European Union that HCV RNA testing would be required in Europe for all plasma

for fractionation by July 1, 1999. And that HIV-1 testing of such plasma would be required at some unspecified later date.

This move created an impetus in the U.S. to implement such testing for blood and plasma, and this was made feasible in part by support from the NHLBI through contracts for developing such tests here in the U.S. Screening of source plasma pools for HCV and HIV-1 RNA under IND was initiated in early 1998. Pool sizes currently range from 96 to 1200 donations and at this time virtually all source plasma in the U.S. is being screened by pool testing for HCV and greater than 80 percent is being tested for HIV-1 RNA by a nucleic acid-based test.

A significant portion of this testing is preformed by a central testing laboratory or a testing service. Some manufacturers are also testing for HBV, as we've heard, although this is much more limited than HCV or HIV. A number of measures were taken by FDA to implement nucleic acid testing in the U.S. FDA views minipool testing as a form of donor screening and this position was endorsed by the Blood Products Advisory Committee at the March 1997 meeting. FDA developed guidance outlining regulatory approaches for

implementing pool testing and discussed them briefly at the September 1997 BPAC meeting. These regulatory approaches address the use of NAT as a commercially available test, as a testing service and as an in-house test.

This guidance document, "Application of Current Statutory Authority to Nucleic Acid Testing of Pooled Plasma", was issued on November 26, 1999. FDA also developed in July of 1998 and published a technical draft guidance for industry on validation of nucleic acid tests, "Guidance for Industry in the Manufacture and Clinical Evaluation of In Vitro Tests to Detect Nucleic Acid Sequences of HIV-1 and 2." There was a comment period and, following the incorporation of comments, the revised guidance either has been or is just about to be posted on the CBER website and published in the Federal Register.

In September of 1998, we held a workshop to discuss NAT for HCV and other viruses and at a BPAC meeting in March of this year, we discussed the issue of NAT implementation for whole blood and transfusible components under the IND mechanism. At the September BPAC, we discussed regulation of NAT testing for Parvovirus B-19.

Nationwide screening of whole blood donations was initiated under IND in early 1999. Pool sizes ranged from 16 to 128 units and you'll notice these are smaller pools than for source plasma. At this time, over 95 percent of whole blood donations are being tested for HCV and over 65 percent for HIV. I'll let some of the other presenters give you their up to the minute numbers for positives found and total numbers screened later this morning, but it's interesting that the false positive rate has been found to be similar to serologic tests.

Now this sign here says look out ahead and this sign says look out. So look out. NAT, not only for source plasma, but also for whole blood is here now.

Now these next two slides list the licensure requirements for test validation from that technical guidance document that I talked about to give you a sense for the type of studies that are ongoing under IND. The tests should be demonstrated to be manufactured consistently under GMP with appropriate quality assurance for the kit and kit components. The purity, identity and functional activity of primers, probes and other components should be determined and

specifications should be established. Conditions of specimen stability should be established. Methods for collection of specimen, transport, testing, et cetera should be validated. And there should be in place a validated mechanism for identification and retrieval of positive specimens in a pool as well as for the identification of the implicated donor.

Instruments used in generating pools to perform the tests and software used to calculate results should be appropriately validated. Analytical specificity will be demonstrated in studies of potential interfering substances in the assay. The tests should meet the analytic sensitivity requirement of 100 copies per mL for the pool and 5,000 copies per mL for the original donation.

Analytical sensitivity will also be demonstrated by testing of commercially available seroconversion panels and low-titer panels developed by FDA. The clinical sensitivity, specificity and reproducibility of the assays should be established through clinical and laboratory studies. Finally, the tests would be subject to lot release requirements for licensing.

Now FDA has developed panels for HCV RNA

and HIV-1 RNA. At the present time, a WHO standard for HCV RNA is available. It's a lyophilized antibody-positive specimen from a single donor. The CBER panel is an antibody-negative specimen, genotype 1B. One of the panel members has been calibrated against the WHO standard so that one international unit equals four genome equivalents per mL. The CBER reference panel covers the range of 5 to 100,000 copies per mL. The WHO standard for HIV-1 RNA subtype B has been established and has been defined as 100,000 international units per mL. There are two CBER panels that are available. One is antibody-negative plasma specimen and the second is a cultured virus specimen spiked into HIV-negative human plasma, covering a range of 10 to 250,000 copies per mL.

The general study design for NAT validation involves screening of somewhere in the order of 300,000 to in some cases more than one million donations in at least 10,000 pools. Blood and plasma centers need IRB approval for NAT screening of donors. Informed consent is obtained from donors and they are recruited into follow-up studies to confirm their test results and to resolve their infection status.

Donors are being followed up for six months for HIV-1 and HBV and for 12 months for HCV. A

validated supplemental NAT that is of the same or can be another technology is being used to confirm results and look-back is being carried out for NAT-positive only results.

A number of other issues have been identified regarding implementation of NAT under IND. For example, NAT requires several days more than conventional antibody tests due to logistics, that is, testing by centralized laboratories and other technology limitations. Consequently, certain blood products, for example, platelets and some red cells, are expected to be released on the basis of serology during the initial phase of the studies under IND, that is, Phase 1. This is necessary to prevent shortages of those critically needed blood products. This phase, however, which is expected to be of short duration, will be followed by a phase where all components are released on the basis of both NAT and serology and this would be in Phase 2. This should be occurring very soon and we'll be hearing more about this later this morning.

Recipient concerns are being managed by local IRBs of hospitals and transfusion centers. Cost recovery has been permitted by the FDA under the IND due to the high cost of NAT testing and the national scope

of the studies. Product labeling will be permitted. FDA recently sent a letter to at least one of the blood organizations and some blood banks who inquired, saying that the units can be labeled as -- and something like "screened by an investigational NAT test for HCV whose performance has not yet been established," or something to that effect.

However, no enhanced safety claims are permitted during the study phase. This is going to allow a distinction between NAT screened and unscreened units which will coexist during Phase 1. Other issues are that donors are counseled on the basis of confirmed investigational test results and deferred until their clinical status is resolved.

I already mentioned look-back. This would be consistent with existing guidance and would be for a period of three months for NAT-only positives. Other issues that are anticipated in the future are NAT for other viruses, for example HBV as we've heard and Parvo B-19 and the increasing push towards single unit testing by NAT. Also, we've had a dialogue with various sponsors regarding potential replacement of existing tests such as the HIV-1 p24 antigen assay. This last issue was actually discussed at the Blood Products

Advisory Committee meeting in March of this year.

So in summary, NAT for HCV, HIV and HBV has been implemented under IND for both whole blood and source plasma. More than 90 percent of whole blood and nearly all source plasma are being screened by a NAT method for HCV and HIV.

There are several implementation issues relating to the donor, the recipient and product management and the move toward universal release of products for transfusion based on NAT. These issues need to be addressed in the clinical trials that are done and hopefully they'll be discussed at length during the workshop today.

So I think I'll stop there and thank you very much.

(Applause.)

DR. TABOR: Thank you, Paul, for those excellent remarks. The next speaker is Dr. Michael Chudy. Dr. Chudy will be speaking on NAT implementation, regulation in Europe. Dr. Chudy is from the Department of Virology, Paul-Ehrlich Institut in Langen, Germany.

DR. CHUDY: Mr. Chairman, ladies and gentlemen, first, thank you for this invitation to this

meeting. May I have the first -- yes.

However, even in the period after introduction of virus inactivation steps into the manufacturing process, some episodes of virus transmission have been occurred, so several ongoing efforts to improve the safety of plasma products. But in my view, not only plasma derivatives, also all sero-components because until now no virus inactivation steps are implemented.

There's a development of sensitive methods for the detection of viral genomes, the question arose as to the application of NAT could contribute to a further increase in the virus safety.

Now the events in Germany, you see there's a German blood bank in Hagen, one of the greatest blood banks in Europe with over one million donations per year. They have implemented pilot studies, in general, in 1996, screening and anti-screening of all blood donations for HCV, HBV and HIV. In consequence, the Paul-Ehrlich Institut initiated a phased plant procedure. This is the first step of exchange of information. And you see the next step was proposals with written comments by the manufacturers and then in September a hearing in Paris and later comes the

conclusion of HCV NAT implementation for the label concentrates, erythrocytes and platelets and later now for the quarantined plasma.

What was the reason of this decision? I can do it very quickly because we have also heard in the first talk today, we have performed in our Institut studies with seroconversion panels and that is my present review of the definition of seroconversion panels. Regarding to HBV, you can see that not all available panels in my view are relevant to this investigation. I think from nearly 40 panels, only 14 were relevant.

And you can see and Michael Busch also mentioned this in these figures that NAT can reduce the window period is not clear over three weeks and you find in this period very low titers of survivals. The other situation is for HCV, summarizing for several panels and you see the reduced window periods of over 50 days and a second thing is that we have found very high titer in this period, so when we define the detection limits of the tests regarding single donation of 5,000 units per mL, you can see that all donations, positive donations can be detected.

The next slide summarizes -- you'll see

that also the detection limit for the tests, now is a commercial test. You know that Amplicor is a detection and made the final copies. The incidents that are also pilots that reach from over three million donations in Germany.

Now the events in Europe. First, in 1994 the implementation for HCV NAT for plasma pools. Later, it comes for some other immunoglobulins. Such immunoglobulins would not have in their manufacturing process an activation step for viruses and now the regulation for all plasma pools since July this year.

You see that this paper is an addendum to the regulation of medicinal products derived from human plasma. I will go in detail to this regulation. You see the time scale and you can see that from the beginning, from the discussion in the biotech working party until the date for coming into operation that took nearly two years.

What are the contents? The first part are introduction of comments so it's clear the impact of the NAT for virus safety and there is a potential to reduce the virus load and now I think the NAT has reached a stage that it allows to go and test for plasma pools.

The first recommendation was at the time

that from July 1st of this year batch release only platelets derived from plasma pools tested and found nonreactive for HCV by NAT and there's also a regulation for plasma- derived -- plasma products used as excipients that means an example albumin as a product from the fractionation of plasma and then albumin is an additive to a recombinant Factor VIII and then the recombinant Factor VIII for us also, under this regulation and so the next recommendation was pretesting of minipools.

The next three points, regulation for such batches of products released before July and the next point was evaluation and approval of the test methodology and validation data by the competent authorities and the definition of the level of the NAT, at least 100 units per mL for the production pool.

I just show this to direct your attention to three points regarding blood donation center regulation and regulation for plasma derivatives. You can see we have no virus inactivation step for the level components, but we have an inactivation step per regulation for these products. A very important question is the time available for the tests. You know the platelets have a very short shelf life and so it's

very limited to perform the NAT and there are no time-limited principles to test plasma pools for production of plasma derivatives.

You see also the regulation for blood donation center. They're referred to single donations allow look-back evaluation. We have regulated only the level of production pools, but if pretesting of minipools is recommended, also look-back is possible.

So to summarize, now available, reference preparation of HCV calibrated in units, I think John Saldanha will go in detail later, you see the gold standard and some other national standards and also a standard from the EDQM, this is the last one, prepared and provided for the European manufacturer. The Paul- Erhlich Institut has also performed an all-national standard. In my view, it was a commitment for the blood donation center for -- prerequisite for the implementation of the NAT test.

Okay, until now there are no specific NAT guidelines available. The background was -- or the two guidelines for analytical procedures and we see that HIV NAT is a limit test and for validation these features are recovered, specifically sensitivity, robustness and also quality assurance. I will not go in detail, but I

will show you now the recommendations, our own recommendations for blood donation center for the validation and later the European OMCL network has also prepared a paper, "Guidelines for Validation of NAT Positive Detection of HCV RNA Plasma Pools" and I have listed here the European Pharmacopoeia monograph. There is written a special point to recommendation of implementation of an internal control for HCV entity. That is a very important thing in my view.

Now, very briefly, some results of the implementation in Germany, that was a frame, you know that, and you see here that all the documents have the prove by our institute, that was a lot of work, but was the situation? Nearly a hundred blood donation centers for each validates to commence to our institutes and you see that some blood donation centers have performed more than one procedure.

Seventy-one centers perform the NAT on site and 25 have external test laboratories. These test laboratories can be divided in external diagnostic labs, seven, and in six blood donation centers with an additional testing capacity for other samples, and there's a great know-how in this field.

Some points to the use methods, you see

most of them, 66, use the commercial test, the COBAS Amplicor. Eight use the modified COBAS Amplicor. That means that they replace the extraction procedure of the kit by another and only ten perform in-house methods like Taq Man, Light Cycler and others.

You can see the link between methods and pool size. We have Amplicor and modified Amplicor, Tax Man, and Light Cycler, and other, and in this we have the pool size and the red is the minimum pool size, from single donation testing up to ten pools, and then 20 in blue and 30, 40 is the yellow; up to 48. And the green one is the maximum possible pool size of 96 and you can see that with Amplicor most of them perform single unit testing or pool maximum of ten donations. And this modified Amplicor, it is possible to increase the pool size from 48 to 96. Only one uses the Light Cycler, it's an important surprise for me and some also tested with Tax Man; it spans all the pool sizes.

Okay, that is my last slide. That's now the situation for the regulation in Europe. HCV mandatory is clear is the regulation for the plasma products. We have in Germany the regulation also from April of this year in Switzerland and Austria and in the near future in the Netherlands. There is an HBV and an

HIV testing as a self-commitment procedure and the most manufacturers of plasma derivatives and also in our great blood banks in Germany. For HIV, there is a special situation, I think. It's more interesting for manufacturers as plasma derivatives. There's an activation step of soil and detergent and now comes into debate Parvo B19 testing. It's under investigation. In the beginning of October this year, the first hearing in a biotech working party where all manufacturers give their future ideas in this field. And routinely all great manufacturers implemented B19 testing for high-titer donations. The other viruses are not so interesting, I think, because there is a question if they're transfusion prevalent viruses.

Thank you for your attention.

(Applause.)

DR. TABOR: Thank you very much, Dr. Chudy, a very nice talk. The next speaker is Dr. Masaharu Nakajima. Dr. Nakajima is Director of the Pharmaceutical and Medical Safety Bureau in the Ministry of Health in Tokyo. Dr. Nakajima will be speaking on NAT implementation and its regulation in Japan.

Dr. Nakajima.

DR. NAKAJIMA: Thank you, Chairperson,

ladies and gentlemen. My name is Masaharu Nakajima from Japanese Ministry of Health and Welfare. I am very honored to be invited here to have the opportunity to speak about our Japanese experience on NAT implementation for blood products.

First, I would like to introduce the outline of our blood program. Japan has a population of 125 million and blood donation was done 6.1 million times last year. The donations were totally non-remunerated and the blood collection was done by the Japan Red Cross, JRC alone. Donations consisted of about 25 percent of pheresis, 44 percent, 400 milliliter of whole blood and 31 percent 200 milliliter whole blood. Plasma products,

self-sufficiency rates are 26 percent for albumin and 60 percent for globulin. Most of import is from the United States, I think contributing somewhat to U.S.-Japan trade balance.

(Laughter.)

As in other countries, our blood program is very unique, difficult and sensitive, including moral issues in Japan and we are discussing a new framework of blood program to include self-sufficiency and safety.

Next. The Japanese regulatory scheme on

blood products is mainly composed of the Blood Law and Pharmaceutical Affairs Law. The Blood Law regulates blood donation conditions such as donor health and facility requirements and give license to blood collecting organizations. The safety issues are regulated basically by the Pharmaceutical Affairs Law. The main body to discuss the issue is Center of Pharmaceutical Affairs Council and minimum requirements of biological and blood products dictates necessary tests for manufacturing procedures.

A good manufacturing practice, GMP, is required as usual and a virus validation guideline is made to apply to evaluate virus inactivation and the removal procedures.

Minimum requirement of biological and blood products and viral validation guidelines are applied to products delivered in Japan regardless of its original country. Therefore, products not satisfying the requirements cannot be imported nor used in Japan.

Other requirements may arise according to the situation as administrative guidance which is not legally compulsive, but the strength of requirement depends on the conditions of the cases. A rapid development of technology and the diversity of its

influence requires these kinds of measures.

The next shows the history of introduction of screening tests in Japan. After the introduction of these screening tests, post-transfusion infection decreased step by step, especially after the introduction of HBC antibody and HBS antibody tests in 1989. Fulminant hepatitis caused by HBV-positive transfusion has not been reported. HCV antibody tests decreased post-transfusion hepatitis very much.

Also, although HIV-2 antibody tests were introduced in 1994, there has been no case of HIV-2 reported in Japan and this year, the NAT for three viruses were introduced and window periods shortened substantially. But it was very sad that very recently, just before the implementation of NAT, two cases of HIV infection through transfusion occurred which was our window period donation.

Next. Recent history surrounding NAT begins donation sample storage since September 1996 which enabled confirmation at look back in our huge deep freezers built in northern part of Japan to keep frozen samples for ten years. JRC began plasma pool testing of source plasma for fractionation in November 1997 for three viruses, namely HIV, HBV and HCV. And techniques

in the system were developed, improved and stabilized.

Our source plasma in plasma for transfusion are collected from the volunteer donors in the same blood centers and some for transfusion are used for source plasma later. Central Pharmaceutical Affairs Council decided the policy to implement three NATs for blood products including transfusion products in October 1998.

Then finally, three NATs were implemented to transfusion products except platelets component because of the short shelf life of 72 hours in Japan. As a trial in July 1999 and all donations had begun to be screened in October 1999.

Next. There are two NAT centers of JRC. One is in Tokyo and the other is in Hokkaido which is a northern island of Japan. The samples are gathered and transported to those NAT centers according to the area of donated centers, blood centers. One more center is being built in the near future. The NAT system extracts and detects three viruses including both HBV DNA and HCV and HIV RNA at the same time using a so-called multiplex reagent system at the present situation.

Five hundred serologically screened samples are pulled and tested. If it turns out to be positive,

which means that one or some of the three viruses detected, then related samples are stopped delivering immediately and the virus and the original sample is pursued.

Serologically screened samples are used to avoid cross contamination by high titer samples. The pool size will be reduced in the near future. The NAT centers can test to 20,000 to 30,000 samples per day. The sensitivity of the test is less than 100 copies or international units per mL and 50,000 per mL at 500 pool. Most of the genotypes listed here are covered by this system.

Next. Japan is composed of four main islands and many small islands including Okinawa. Samples are transported twice a day from blood centers to two NAT centers by trucks on the ground and aircraft. The third NAT center will be located in the western part of Japan.

Next. Until now, serological window cases are detected by the NAT. Among the source plasma samples of more than five million most frequent was the HBV, 1 in 72,000. The dominant genotype was C, 78 percent; and B, 12 percent in contrast to Type A and D dominant in western countries; and five mutations were

found. The close relationship between pre-cor mutation to stop E antigen production and found hepatitis was observed in Japan.

The frequency of HCV was 1 in 535,000. The dominant genotype was 1B, 70 percent; and Type 1A, which is common in western countries, was very rare. The frequency of HIV was 1 in 2,631,000. Whole donation screening was done on 1.2 million samples up to now and HBV, 8; HCV, 3; and HIV, none were detected. It is said that as high as seven percent of the population are HBV carriers and half of 300 million world-wide HBV carriers are in Asia. In Japan also, it is estimated that 1.4 million are HBV carriers and 17 percent of deaths by hepatoma is from hepatitis B. Therefore, we consider it is necessary to include HBV in Japanese NAT system.

Next. As a conclusion, the next step of NAT we consider would be, first, quality assurance and quality improvement; and second, reduce the time necessary for testing including logistics; and third, although accurate estimation of cost is not calculated yet, to make the test more cost effective; and fourth, a single donation testing and automated system; and fifth, to cope with emerging pathogens and how far and to what extent.

The evaluation of NAT still needs careful consideration, such as the relationship between NAT data and infectivity, but the results obtained so far and theoretically NAT is very promising to play a role as a fundamental test of pheresis in an attempt toward the goal of totally safe blood products. Thank you for listening.

(Applause.)

DR. TABOR: Thank you very much, Dr. Nakajima, for a very clear and interesting talk.

I'd now like to introduce Dr. John Saldanha who is well known to all of you because of his leadership in developing standards for RNA detection for these viruses. Dr. Saldanha is from the Division of Virology at the NIBSC in Potters Bar, just outside London.

DR. SALDANHA: Thank you, Mr. Chairman. I'd like to thank the organizers for inviting me to speak today, and I hope in my talk I'm going to give you a brief introduction to standardization of NAT assays and the quality assurance of these assays. Now we've heard from previous speakers about the implementation of NAT, and what I'm going to show in this talk is that this has only been possible because of the establishment

of working reagents and international standards.

May I have the next one, please. Why do we need to standardize these assays? I think you're all familiar with the fact that NAT assays are very sensitive, and the sensitivity can vary from laboratory to laboratory. We were also concerned as a control laboratory that there would be discrepant results between manufacturers of blood products and the control authorities which could lead to problems with the release of the final products. So these really were the early discussions we had.

And before, as you realized, before you can routinely introduce NAT, you need to have some sort of standardization of these assays.

Next one, please. And how do we go about standardizing assays? I think the first thing to do is to use the calibrated reference reagent which is also known as the run control to insure reproducibility from run to run and to establish an internationally accepted standard, and these are normally WHO international standards.

Now at NIBSC, the way we develop working reagents is to first of all start a collaborative study which involves manufacturers control authorities'

independent labs; and based on the results of these studies, we prepare a working reagent and the working reagent is at a dilution that can be easily picked up by the majority of labs. We then evaluate the reagent by sending it out to laboratories and getting results back on a monthly basis, and then finally move to the stage of developing an international standard.

Next one. Here I've listed some of the collaborative studies we've done at NIBSC, starting in 1990 with a study done by Janet Butman to look at the variation and sensitivity of assays to detect HIV DNA, ending with the bottom which are the three studies to establish the international standards for HCV, HBV and HIV.

Next one. On this slide I list some of the reagents that are available. We have working reagents for most of the commonly looked at viruses which is C, HIV B19 and we also have a multiplex reagent which has four of the viruses. In fact, we now have an agent which has hepatitis B in it as well. And there are two working reagents for HIV RNA and a working reagent for HBV, and I'd like to point out that there is a leaflet outside by the registration desk which lists these reagents and for those of you who are interested in

information on ordering them.

May I have the next one? And I think as a previous speaker mentioned, we now have WHO international standards. The reasons for establishing international standards, I think are quite clear. At the moment we have several working reagents which are either calibrated in genome equivalents, and in the U.S.A. I think you're very fond of copies per mL, and some people talk about PCL detectable units per mil. And it's very difficult if you try and compare results from different laboratories to find some sort of common ground. What the WHO does is to establish an international standard which is calibrated in a commonly accepted unit, and this is a very arbitrary unit called the international unit, and then the next step is to calibrate all the existing working reagents against the international standard.

Next one. At present we have three international standards that have been established. The first standard was the hepatitis C virus international standard which as was mentioned previously is a lyophilized preparation off of the genotype 1A isolate and each vial contains 50,000 international units. This is defined. This standard was established in 1997. In

October this year there were two further standards established: the hepatitis B international standard which is a genotype A, subtype adw and this standard contains one million international units per vial. And finally, the HIV-1 international standard which is based on a CBER material which is a positive donation diluted in defibrinated plasma and lyophilized. In this standard, each vial contains 100,000 international units. So at present we have three international standards that have been established and we're planning to establish the standard for Parvovirus B19 in the near future.

Next one. Okay, and I think Michael Chudy showed this. Having established the standard for hepatitis C virus RNA, we then calibrated some of the commonly available working reagents against the standard and this table shows the calibration and international units of the standard such as the

Paul-Ehrlich, the NIBSC standard, the CLB Pelispy, the standard from the Institute Superior di Sanita in Rome and the CBER Panel No. 1 which is part of the panel that May Nguyen has established. And on the right hand column you can see the calibration of international units. So it's now possible if a laboratory uses any of

these working reagents to directly compare the results with a laboratory using one of the other reagents. So I think you can see the value of calibrating everything in a common unit.

One of the things that is confusing is the correlation between the international unit and genome equivalence. And the international standard has been assigned a titer to 10^5 international units per mL and this is not dependent on the sensitivity of the assay. It's defined as having some international units.

In contrast, when laboratories quantitate samples in genome equivalence or copies per mL, the figure they end up with is very dependent on the sensitivity of the assay. So what this means is that the ratio of genome equivalence per mL to international units will vary depending on the sensitivity of the assay. And I haven't got the slide here, but I have data which shows that it can vary anything from one international unit being equivalent to three genome equivalence right up to eight genome equivalence. And I think the only accurate way of trying to decide this is to run a sample in parallel with the international standard and get a direct correlation in IU.

May I have the next one, please? Okay,

I'll switch slightly and talk about the quality control of NAT assays. Michael Chudy talked about the validation of NAT assays, and I won't go into this, and as Michael sort of said, there are guidelines from the European Pharmacopoeia that pertain to the validation of NAT assays. Another way to insure the quality of the NAT assay is to use a calibrated working reagent or a run control in every assay run. And finally, participation in proficiency studies is to be recommended.

Next one. One of the things we do -- we established a working reagent for hepatitis C virus RNA back in 1994, and this sample was sent out to different labs, and we recommended testing the sample neat, 1 in 10, and 1 in 100 dilution. The sample contains roughly 4,000 genome equivalence per mL which by calibration against the international standard is just 700 international units per mL. And laboratories return results to NIBSC every month. We analyze the results, and send out a report on a yearly basis.

And I think in the next slide I'll show you some results which give you an idea of what happens. Now these bubble plots represent the number of positive assays over a time period, and you can see that

laboratories can either pick all of them up as in A or fail to pick any of the samples up as in D. I think this illustrates the use of using a working reagent. In fact, in laboratory B the assay improved over time by changing the extraction method.

Next one. Finally, this is the result of a recent proficiency study that we run in the U.K. with five laboratories, and we had a dilution panel of genotype 1 and a genotype 3 sample, and you can see that most laboratories were able to pick up between 20 to 50 international units per mL in this proficiency panel. This was a blind panel that was sent five labs. And we're hoping to send out similar panels twice a year to labs for the proficiency.

Next slide. This slide briefly shows the distribution of the working reagents. We started off with HCV and we reached a peak of the distribution in 1998, and that's dropped a bit this year because people have calibrated their own in-house working reagents against the international standards, so they don't need to use a commercial reagent. We have reagents to B19 and the use of that is increasing which I think shows the interest in this virus. HAV is pretty slow at the moment and we have just started sending out samples for

HBV.

Next one. And on this slide it shows distribution of hepatitis C working international standard and the B. And you can see that the use of the international standard has increased as people calibrate the assays in international units.

And finally, I'll talk a little bit about the introduction of NAT testing and some of this has been covered by Michael Chudy. One of the original reports in 1994 you're all familiar with was on the transmission of hepatitis C by intravenous immunoglobulins and the introduction of testing steps, and following this CPMP urged marketing authorization holders to develop and validate similar assays. May I have the next one?

And again, there were recommendations on intramuscular immunoglobulin testing in 1995 and recommendations by the CPMP but no final date set.

May I have the next one? And this is a slide that Michael showed and this is really the discussion in the biotechnology working party on the introduction of nucleic acid testing. And I wanted to show you this to try and illustrate how the biotech working party arrived at the figure of 100 international

units per mL for the tests.

I think we might skip. So again, some of the things that were discussed in the biotechnology working party back in May 1997 were the problems with using assays of different sensitivities which I've talked about, the level of control that should be used, and the EAPPI and EPFA proposed a detection limit of 1,000 genome equivalence per mL, while the control authorities wanted to have more like 400 genome equivalents per mL which was equivalent to 1 in 10 dilution of the working reagent.

And this data was based on some of the data we got back from laboratories who could pick up 400 genome equivalence in all assays, and these were several manufacturers and some control authorities. So the reason for setting the limit at 400 was based on hard data that we had collected over several years.

Next one. And on the 20th of February 1998 with the establishment of the international standard which was established in October 1997, we then said that the level could be set at 100 international units per mL which was equivalent to 400 genome equivalents or a 1 in 10 dilution of the NIBSC standard.

Next one. And what was decided was that

the run control should be positive in every assay run, and the level at which it was set was critical. If the level was too high, then laboratories with less sensitive assays could pass batches of blood products which would fail in other laboratories and this was a concern. And conversely if the level was set too low, this would result in the majority of assays failing and having to be repeated. So it was quite critical at which level the standard was set.

Next one. And to show that it was -- that 100 international units per mL was achievable, we shared some data that was collected during the collaborative study to establish the international standard, and you can see that most of the laboratories could pick up less than 100 international units per mL in 95 percent of the assays. That was 95 percent detection limit.

Next one. And so based on all this data and after all this discussion, the CPMP said that from the first of July batches would have to be tested for HCV, and the limit would be 100 international units per mL so this limit was based on hard data rather than plucked out of the air.

Could I go to the last one, please? So I think what I want to conclude with is that it was very

important to set up international standards and working reagents which were calibrated before we could think about introducing routine testing of NAT. And I'll finish up by doing some advertisements, I think. The next NIBSC/EPFA workshop which is going to be on May 10th and 11th next year is going to be held in Madrid, in Spain, and it's sponsored by the Spanish Medicines Agency and that would be followed on the 12th by the SoGAT workshop which is a WHO workshop on the 12th of May.

Thank you very much.

(Applause.)

DR. TABOR: Thank you very much, Dr. Saldanha. I enjoyed your talk.

We will now take a break which I think is listed on the agenda as too short. I think we ought to have at least 20 minutes because of the possibility of lines where you get coffee. You can get coffee in any of three places. There's a cafeteria on the floor below this which you reach by going left after you go out the doors and down a stairwell. There is another cafeteria that you reach by going straight back after you leave these doors, as far as you can go and then ask directions. It's on the second floor. And there's also

a Starbucks Coffee stand that you would pass on your way going straight back from these doors. So we'll start again in 20 minutes. Thank you.

(Whereupon, the meeting went off the record at 9:53 a.m. and went back on the record at 10:17 a.m.)

DR. TABOR: For anyone who is just outside the doors, could you pass the word that we're about to begin?

We're beginning approximately or nearly 20 minutes after the scheduled time to begin. And because of that the second session of the morning will run 20 minutes later than scheduled.

The next session is about the industry's experience with NAT screening of donors to date, and it's divided into two parts. The first is primarily related to screening source plasma, plasma for further manufacturing derivatives, and the second part dealing with blood bank screening which, of course, involves both whole blood and its components and the resultant recovered plasma.

The moderators of these two sessions are Dr. Andrew Dayton who is in the Laboratory of Molecular Virology at CBER and Dr. Robin Biswas who is in the

Division of Emerging and Transfusion Transmitted Diseases in CBER. The moderators of this session will introduce the speakers and because of the time limitations, I want to remind the speakers to try to limit their talks to eight or nine minutes, if at all possible. The two portions of this session will be followed by a short discussion period, and I realize that it's a short discussion period, so that if there's interest in further discussion, we can continue the discussion after lunch.

I'll turn over the microphone to Drs. Biswas and Dayton.

DR. DAYTON: Okay. Well I think everybody seems to have largely returned to their seats. Why don't we just get started. Let me remind you again that we have about ten minutes per speaker so if each speaker would aim for about eight or nine minutes, we could stay on schedule.

In the first section on Plasma for Further Manufacturing, the first speaker is Andrew Conrad from NGI.

DR. CONRAD: All right. I'm going to sort of set the table for my colleagues from Alpha and Baxter, and we're going to describe some of the

collaborative studies that we've performed. We actually began doing testing under IND in June of 1997. I just wanted to get that out.

What we're going to do is I'm going to explain the methodology and a little bit of data and let my colleagues describe the findings of these large clinical trials that we conducted under IND.

Basically, our concept was that source plasma donations are currently tested, were currently tested for a variety of serologic and antigen tests. The antigen and antibody testing methodologies failed to detect what we called the window period donors. So because the window period donations were not always removed, and have the potential to derive, I mean, to transmit HBV, HCV and HIV that we could find, implementing NAT testing would bring an additional level of safety.

So what we wanted to do was demonstrate the effectiveness of using the source plasma screening with NAT testing in a pooled format. The pool, we feel, is an important advance in that it makes logistics much more simple and I think as you'll see later that we cannot take pooled testing to new levels in the future.

Basically, this is the system that we use

at National Genetics in collaboration again with now Alpha, Baxter, Bayer and others. We use a system, a robotic pooling device called the TECAN and we make these sort of complex pools. What we do is we lay 512, up to 512, samples on the deck of the TECAN. Now it's important to note that 512 is just 8 by 8 by 8, but you can do any symmetrical number. You can do 7 by 7 by 7, 6 by 6 by 6, and use this algorithm to make smaller pools. It's important to know that because if you want to dial in better sensitivity, you can use smaller pools. But later, you'll see I'm going to advocate another way to get better sensitivity. So what basically happens is the samples -- my laser pointer sort of got a weird point on it -- the samples are placed on the deck in eight groups of 64. Each of these groups are then placed into what we call rows, columns and layers. These are primary pools. The rows, column and layers are then taken and placed into a master pool. If the master pool is found to be negative, then you can assume that all its members are negative, well, below cutoff. If it's positive, by testing the row, layer, and column, you can arrive at the single positive donation. In this case, you can see that Z3, Y3 and X1 was positive and that results in an intersection here

and a single positive donation identified.

Somebody is chain sawing something.

(Laughter.)

So basically that's the algorithm that we've employed for these clinical trials. What's important is the bigger the pool, the higher the sensitivity of the assay has to be so what we have -- the sensitivity of the assay in our FDA submissions for HIV was a mean detection of 1.4 copy per mL and a 95 percent detection of five copies per mL and this is using the UltraQual 2000 which is based on a 2 mL for reaction assay.

For hepatitis C, here are the numbers. We've got 1 international unit; six international units and this is based on a 1 mL assay. Why I keep telling you how many mLs we prep from the master pool is going to be important, and it's going to become very important in a minute, and you'll see why. For HBV which is now under IND, we're testing under IND, it's about five copies with a mean detection. The 95 percent at the time I made the slide wasn't done, but now I checked all the forms and it's around ten copies for 95 percent detection.

What's important about this is earlier I

said that as you tool down -- this is an 8 by 8 by 8. This is a 7 by 7 by 7, 6 by 6 by 6 and so on pool -- you can see that the sensitivity on the per reaction basis drops mathematically. This is a person with HCV, and their viremia is so high with HCV it doesn't really matter. But what I came to the revelation about recently was that instead of making the pool smaller, what I should do is test more of the pool because if I tested 10 mLs or 5 mLs I could get all of the efficiency of pooling and still have to do one test. So really what we're going to move forward in the future is in order to increase the sensitivity, instead of making the pool smaller which makes the costs higher and makes the efficiency less, but what we should do is test the whole pool. So our focus is going to shift from pool sizes to the amount of pool we prep, and I think that's going to be the fundamental change that we perform in the next few months as we add new and better technologies on. If we can prep more, we can make pooling more and more sensitive and eventually you'll have numbers of 0.1 virion per mL because you're testing 10 mLs or more. I think that's going to be an important fundamental change.

Like I said, there's economic reasons and

there's practical reasons before this because the cost of pooling at 512 with a frequency of, say, 1 in 100,000 is about 41 or 47 cents per donation, so economically, this can make tremendous amounts of sense and if we can get the pool sensitivity down by testing more of it, it becomes a safe and economic mechanism for intervening.

Basically, our methodology, just a brief summary of how it works, is we do everything in duplicate because we think that redundancy is the best form of quality control. So for every virus we do two separate primer sets. We use John Saldanha's multiplex control as well as the other standards. All the samples are first checked for virus and then they're checked for the presence of an internal control. This is just an example. Here's a positive sample on both, a negative sample and what's important about a negative sample is that the internal control has to show. So this is all done on an automated Southern Blotting machine with a computer scan and that's basically what the data looks like. Here are the positive controls and they're done in quadruplicate. One is a WHO standard diluted. One is our own working control.

And then just a brief discussion setting the table again for my associates. In the INDs that

we've performed, we've found only four positive donors out of about 100,000 donors for HIV. We found 32 positive donors out of 100,000 donors for HCV. And we found 11 positive donors out of 43,000 for HBV. And I wanted to mention something about HBV because we won't talk about that as much in the future. Of the HBV people we found, now it's up to 17, 14 of them were S antigen negative but cor antibody positive. That's because the plasma industry doesn't test for cor antibody. They test for S antigen. Of the three other people that we found that were cor antibody positive and viremic, but not S antigen positive, the highest level of viremia was 43,000 and I know Mike Busch quoted my own slide with Sue, but now we think that we have found one guy who is a little bit higher, up to 43,000 with no S antigen. The lowest person had 500 copies.

So these are the prevalence rates that we so far detected under these three INDs. For the specifics on these data I will now turn it over to Don Baker and Charles Heldebrant.

DR. DAYTON: So the next speaker is Don Baker from Baxter.

DR. BAKER: Well, the wonders of modern technology. If I could have my first slide, please?

Fantastic.

Dr. Mied indicated that the general experience is that everything takes longer and costs more, I think I would certainly say that for PCR testing, contrary to that indication, our experience has been that while it may have taken longer, it actually probably costs less than we initially anticipated to introduce this technology.

The experience I'm going to talk about today covers approximately 22 months of testing. In that period we took, tested approximately 2.6 million donations. Now this is not an unscreened population, obviously. These are individuals who have passed our normal donor questionnaires, have been excluded for all the risk factors that are examined. They are also seronegative, so all these individuals have been tested for HIV, HCV, HBV. From that, we constructed a little over 5,000 master pools, and those of you who can do the math quickly, 5,000 times 512 comes out to approximately 2.5 million and change.

Of these, the donation disposition, in other words, the time from the donor appearing at our collection center to the disposition of the plasma unit is shown in this slide. In 1998 that was averaging

approximately 50 days. Now as you know, the source plasma industry uses a 60-day inventory hold, so we were getting the results back on average within that 60-day period and in fact, we were not releasing any donations until we had our PCR tests.

In 1999, that average had dropped to, year to date, is 36 days and we're projecting that in 2000, the average will be 25 days. Now clearly if you're a blood banker those figures don't look particularly good; however, for those of us in the source plasma industry this is fine because it is well within our hold period.

For HCV, of those 5,000 odd pools, we had 330 pools which initially tested positive. From those in retest, when we broke them down as Andy indicated, we only found four in which there was a false positive which is quite an acceptable rate of false positivity.

This resulted in us being able to exclude 126 individuals who would have otherwise contributed to the pool. So from the perspective of a manufacturer, what this means is roughly five individuals a month, five to six individuals a month, we're excluding who would have otherwise contributed donations to our manufacturing pools. So this certainly a worthwhile test.

In addition, to the extent that HCV is a potential surrogate marker for other blood-borne pathogens or for risk behaviors that one wouldn't want to see in a donor population, certainly the impact of this test is magnified and we're quite happy with the HCV testing.

HIV, on the other hand, is less dramatic in terms of its impact. Again from the 5,000 odd pools, we found 21 that were initially positive. The reason, obviously, for the much fewer number is, of course, that p24 testing is used and the shorter window period for HIV to seroconversion. Of those, only 17 were confirmed positive. On a percentage basis, it looks like we have a higher false positivity rate with HIV, but I think this is more probably just the statistics in small numbers. And this allowed us to exclude nine individuals who would have otherwise contributed over that period.

So in summary, the system works. It is robust. We certainly haven't seen a problem with either the data transfer or resolving these in the time lines we'd set for ourselves, and the false positive rates are very low for these kinds of tests, reproducible and timely. Our expanding test menu. We have initiated our

HBV testing. We anticipate on initiating Parvovirus testing next month and the jury is still a little bit out on the timing of HAV. We anticipate that this test will offer much less value to the manufacturers, but it is still on our menu.

Thank you.

(Applause.)

DR. DAYTON: Thank you very much. Our next speaker is Charles Heldebrant from Alpha.

DR. HELDEBRANT: Thank you, Mr. Chairman. I'll go through the first two fairly quickly and we'll get right on to it.

I'm going to talk about a summary of our clinical trials of HIV and HCV screening of source plasma for further manufacture. And that's just me. Go on to the next one.

Alpha Therapeutic and National Genetics conducted prospective clinical trials to determine the safety and effectiveness of source plasma screened, of pool samples with the NGI, UltraQual, reverse transcriptase, HIV and HCV, PCR assays. As Andy indicated, our samples were pooled into master pools of not more than 512 samples and tested with the NGI assay.

Individual samples of each source plasma

donation were tested with our current FDA approved serology screening. All of our new donors were screened by serology first. Serology positive donations were excluded and were not PCR tested.

In our program, a new donor that has passed two donor screenings by serology and PCR is classified as a qualified donor. Qualified donors were concurrently screened by PCR and by serology.

All of the donors detected by PCR during the prospective screening were offered enrollment into a follow-up study to confirm infection. All PCR or serology reactive samples were disqualified from manufacturing use.

To talk about assay sensitivity, when we carried out the trial for HCV we used a 1 mL sample size with four reactions per assay. The analytical sensitivity in genome copies per mL is 20. That translates to approximately five to six international units. The number of virus copies per individual donation required for 95 percent detection when diluted 512 fold in a master pool is approximately 10,000, or about 2500 international units.

For HIV, we use a 2 ml sample size for reactions. The analytical sensitivity is five copies

per mL or approximately 2500 virus copies per individual donation for 95 percent detection.

In this particular study, just give it a twist there, if you would, just give it a 90 degree twist. There we go. This shows you four of the donors who we identified in the prospective HIV screening. At Day Zero what you find -- this is the day when they first became PCR positive. The black bars here represent all prior and subsequent samples available for each of these individuals.

You can see that prior to the first PCR detection for these four individuals, all of them were negative when tested with PCR. They were PCR positive, then became p24 positive and in the case of the two individuals who enrolled in the study, they were followed until they became antibody positive, confirming infection.

We have a similar slide for the HCV seroconversion. This shows 22 subjects who were identified. Again, individuals were PCR positive at Day Zero. Prior donations are listed here. Some of them have extensive prior donation histories. As Dr. Busch and many of you have seen, there are extensive window periods. The red bar here representing the time when

the individual became HCV antibody positive. You can see that these individuals can donate a significant number of highly viremic units into the pool. Of particular note is Donor No. 5 who never became antibody positive. He was antibody negative at this point and became PCR negative at this point.

Next slide. In terms of our clinical trial results for HCV and HIV, 342,000 donations. We removed 85 HCV positive donations and six HIVs. For those of you who like to count viruses that are removed from the pools, it's almost 10^{12} copies of HCV and almost a billion copies of HIV.

The clinical specificity as designed into the protocols was 100 percent. The clinical sensitivity of PCR for HIV is 100 percent. The clinical sensitivity of antigen and antibody was 27.8 percent.

The clinical sensitivity of PCR for HCV in our protocol was 70 percent. However, we assume that all HCV positive donations were active infection, which is not the case.

The clinical sensitivity of antigen or antibody for HCV was 42 percent. The mean reduction of window period for HCV, 57 days; and for HIV, greater than or equal to four days.

In terms of genotypes detected in our study, 77 percent were Type 1; 18 percent, Type 2; and five percent, Type 3.

In terms of HIV screening methods, one of the objectives of our trial was to provide data that p24 antigen testing can be replaced by PCR testing. To this end we looked at 288 HIV-1 infected samples, either from seroconversion panels, look backs, low titered samples. In this particular group we had 288 samples. When we look at these by pool PCR, HIV-1,2 antibody using look back, inventory hold and the qualified donor standard which is available to manufacturers of source plasma for further manufacture, we got them all.

We got the same result with individual PCR and antibody. Individual PCR did not detect three antibody positive samples in this cohort. Pooled PCR and HIV-1,2 antibody detected 87 percent. The addition of p24 antigen to pooled PCR added nothing and no sample in any of our studies has been p24 antigen positive and PCR negative. With p24 antigen alone and HIV antibody, we detected 200 of the 288 or approximately 70 percent.

One fairly interesting advantage of this study, when we were analyzing some of these, we actually came upon four donors that we would classify as eclipse

donors for HIV-1. These donors were identified with a very weak PCR positive result which is repeatable on these four donations. They're followed by anywhere between three and five PCR negative, p24 negative, antibody negative donations in what we would surmise is the eclipse period. These donors then were followed by a classical window period seroconversion where they are p24 positive only, followed by p24 antigen and then antibody seroconversion so that one of the benefits from this is we believe that we've actually identified what we believe to be the eclipse period here for four naturally detected donors.

To conclude, source plasma donor screening for HCV and HIV by PCR testing of pooled samples and appropriate serology of individual samples is safe and effective. It results in the detection of virtually all window period donations. It increases screening specificity, it increases screening clinical sensitivity, and for HIV-1 is superior to p24 antigen testing.

The addition of PCR testing of pooled samples to source plasma donor screening, coupled with inventory hold, lookback -- those are components of the quality plasma program -- virtually eliminates HCV and

HIV donations for manufacturing pools.

The results of our trials show that the screening of source plasma for HCV and HIV by PCR testing of pools of up to 512 samples and serology of individual samples is safe and effective.

Thank you.

(Applause.)

DR. DAYTON: Thank you very much for that excellent presentation. The next speaker is Alan Liss from Centeon.

DR. LISS: Thank you very much. And now for something completely different. We do not use NGI for our PCR testing.

(Laughter.)

Instead -- next slide, please -- as you may have heard a number of times, we have an in-house developed test that originally was developed in our laboratory in Germany, and you'll be hearing from Dr. Thomas Weimer this afternoon about some further developments. We have two laboratories, one in Knoxville, Tennessee, where we do all of our U.S. testing for plasma and another one in Marburg, Germany. In Germany, we started our PCR testing in July 1997; in the U.S., in April 1998 under an IND and to this date

we've around five million collections in our pool protocol.

Slide, please. Overall, our PCR objectives are the following three. Screen all PCR, screen with PCR, all serology-negative samples; interdict the PCR-reactive units prior to their pooling and use; and then, for our donors, to notify them and counsel them with this PCR information.

I'd like to present to you some of our on-going raw data. We do test, as noted, with HBV, HCV and HIV. We've been able to identify, up until this date of around November, 56 positive units which we traced back to 29 donors, interdicting these number of units; 517 HCV from 144 positive donors; and five positive HIV from four positive donors. So the bottom line being an interdiction removal of 1,139 units that probably would not have been caught by serology.

Again, this is also going on in Europe and just to give you an idea of some of the numbers there. They have 21, 140 and 0 for their positive units, interdicting another 161 units from what they test in Germany.

Since we are currently in the IND, and it has a clinical end to it, this is what we have to do for

what we call our clinical substudy follow up. We recruit or try to recruit, at least, our PCR reactive donors into the study, follow them monthly, and sample for PCR and serology until they seroconvert. I'd like to show you some data from our substudy. Currently, we're running about -- under a 30 percent enrollment rate. We try to get everyone back. They don't, but our numbers are building. And again, this is raw data and we're in the middle of the study, but we're seeing, in general, a majority of our reactive, PCR reactive donors to be seroconverting. There are some studies that we're looking at now where there seems like an anomalous number of HCVs who have completed the study but not seroconverted. We're looking into the details for that, and we hope to be able to identify exactly what's going on for these cases.

HBV. Again, we have one person who's completed but not converted, and we're looking into the reason for that as well.

Speaking of HBV, since we're one of the few companies to be doing this and have some numbers, we see some importance in trying to follow up and look at the rationale behind doing HBV and is it saving us any window period of time. This is simply reflecting some

panels. And this is the number of days between our first PCR reactive HVSAG nonreactive sample and our first PCR reactive HVSAG reactive sample, again, in panels, so not every panel is represented by the same time lags in between. If you look at the numbers here and draw an average, it's pretty close to the data that was presented earlier today of a potential window savings of in the high teens. And when we have our real data with our real donors, what preliminarily we found is it's in the same ballpark.

Next slide. So in brief summary, we feel that we're in the middle of a very strong and positive PCR IND. We are effectively screening 100 percent of our plasma. We are interdicting plasma from our manufacturing pools that otherwise would have gotten through if we had just used standard serology. And we think and we intend to use this to further help our donors, as well, in helping identify their diseased state earlier than with standard serology.

Thank you.

(Applause.)

DR. DAYTON: Thank you very much. Our next speaker is Brian Renoe from Bayer/Roche.

DR. RENOE: As Alan said, we're not testing

all of our samples with NGI as well. We're using Roche for ours. Let's go to the first slide.

The testing I'm going to talk to you about is happening at the Raleigh Test Laboratory in Raleigh, North Carolina. We got started on this in HCV in September of 1997. I'd like to just kind of give you an overview of what's happening. We're getting samples from 120 plasma centers. If you could just show the next slide, please.

The samples come to us every morning. We get about 10,000 samples by Federal Express. We go through this flow diagram, and it's not to really abuse you with a flow diagram, but I just wanted to point out that we tried to keep it as much in parallel as possible. The extraction of the nucleic acids for both the HIV and HCV is happening virtually simultaneously, and then we go through a parallel amplification detection process.

The entirety of what you see here for HCV and HIV is a turnaround time of about four and a half days so far, so if we get a sample from the plasma center, the results are back in their hands in hard copy form in about four and a half days.

Next slide, please. First, let me tell you

a little about HIV. HIV, we only got started on this year. We elected to use an established reagent manufacturer for the kits so we're using the Roche HIV-1 Ampliscreen, Version 1.5. This kit is sharing the Multiprep specimen extraction or up to the extraction before amplification with HCV. We're using 96 sample minipools. So far in the last three months or so we've done about 390,000 donations, 60,000 donors from 120 plasma centers.

Next slide, please. I don't have a lot of data then to show you, but we have been able to identify eight positive donors in that period of time. And most of them were in the first couple of weeks. Of those, we've got three that are HIV-1 NAT-positive, p24 negative HIV-1,2 antibody negative and then you can see the rest. I won't read through it. We've been able to enroll two of those first three donors and one in the category of NAT positive, p24 negative antibody positive at this point.

Next slide. This is just to show you, we saw most of them very much up front. I think this is probably the experience of most people is when we got started we kind of cleared them out of the pool of donors that were out there. So virtually all of those

were within the first few weeks of HIV testing.

Next slide. What we've tried to do is just a quick quantitation on some of those, and we were able to go back and catch about five samples here, six samples, and you can see that it runs a titer in terms of copies per mL everywhere from 60 to about 600,000 copies for mL. So it's certainly a range of effective -- I guess I don't want to say infection, that's what I'm hesitating on -- but effective titers.

Next slide, please. Okay, we go to HCV. We've got a lot more experience there. We got started in September 1997, and this is dated through November of this year. Currently, we're using -- we got the Roche Ampliscreen kit, Version 2.2. Same sort of data, 120 plasma centers. We looked at 3.6 million donations so far which represents well over 200,000 donors.

Next slide. Here we've got a lot more people that we've been able to -- we found as NAT positive, 208 NAT positive antibody negative donors so far. Ninety-six of those have been able to enroll in follow up studies. Six of them are pending enrollments. Last time I talked with the FDA, they were concerned with the number that we weren't able to locate, and so we went back and actually tried to relocate a number of

these donors, and actually 90 of that 208 just won't respond to letters or telephone calls, so we're considering them virtually lost to follow-up at this point. Five of them declined. Five agreed to participate but didn't show up, and then you can see the last six have other reasons for not being there.

(Laughter.)

Next slide, please. What it amounts to for HCV, on a monthly basis, we're looking at something like 11 or 12 donations, I'm sorry, donors per month that we're identifying as NAT positive. We're doing the same sort of thing. These people are supposed to be serology negative before we see the samples, so these samples would not have been normally caught, unless we were doing NAT screening.

Next slide. Of those that we've been able to follow up, 62 of them have gone to seroconversion. Again, the same sort of data. We're seeing 57 days as the average window period. One donor, interestingly enough, that we identified fairly early on has still not seroconverted. At least the last time we saw a sample was May of 1999 of this year and we still are seeing NAT-positive, but antibody negative on that particular donor. So, next slide.

This is just to give you a histogram of the seroconversion time, if you will. Sometimes when you put up a number, everybody thinks it's happening at a very nice normal distribution. Obviously, it's, there's a lot of spread here and this is pretty much an echo of what you've seen from the other studies.

Next slide. We also wanted to go back and just see what kind of range of titers on those donors that are coming up as NAT-positive we are actually finding or measuring. So we picked a number of donors here that during the 30 days prior to the NAT positive result had a negative result. And you can see that those cover a fairly broad spectrum of concentrations when we do -- when we concentrated them here.

So it's a little bit of a surprise to us because we didn't expect to see quite as many highs, but we were pleasantly surprised by the number of lows since they're virtually below our 95 percent detection limit.

Next slide, please. Okay, so a conclusion. Basically, the NAT -- using NAT to identify suspect infectious plasma donations certainly seems to be working for us as a part of the source plasma industry. When you couple that -- again, I'm seeing the same things in combination with the Amplicon donor programs

that are in place, inventory hold, QPP, viral inactivation procedures and viral remover processes -- we think this is a very effective tool for removing these NAT positive and suspect infectious donations from the plasma that goes into our products.

Thank you.

(Applause.)

DR. BISWAS: Okay, we will now go over to the session on blood components for transfusion and our first speaker is Christina Giachetti, Senior Program Manager of Gen-Probe in San Diego, California.

DR. GIACHETTI: If I can have the first slide, please? Okay, my presentation is on the TMA, HIV, HCV assays and the assays for HIV and HCV that earn a detection.

Our assay objectives are to have a sensitivity of at least 100 copies per mL for each target, detection of HCV and HIV subtypes and closure of the seroconversion window, specificity of higher than 99.5 percent, incorporation of an internal control for monitoring the assay in each sample, discriminatory assays for resolution of multiplex assay reactives, and we're going to use the same assay formulations for our semi-automatic system or the enhanced semi-automatic

system, as well as for our future fully automated TIGRIS instrument.

Our assay protocol contains the following steps and here are pictures of the instrumentation involved. First step is pipetting of the samples with a TECAN, auto pipetting the TECAN pipette calibrators, the specimens as well as the target capture reaction. Next step is sample processing, of course, viral lysis and RNA capture with the help of the target capture system. Next is amplification with the transcription-mediated amplification, detection with the hybridization protection assay and finally reading the results using a luminometer.

Our technology is all hybridization based. The first step is target capture where the target capture reaction is added to the specimen. This would produce lysis of the virus and then the presence of capture oligomers or capture of our viral RNA to a capture oligomer which also will attach via a poly DA and poly DT test to magnetic particles. And then we use magnetic separations to separate our captured RNA from the rest of the specimen.

Amplification used in transcription-mediated amplification which utilizes two enzymes,

reverse transcriptase and T7 polymerase, amplifies RNA or DNA targets, produces RNA Amplicon, is beneficial, very efficient and isothermal.

Hybridizations were used, hybridization protection assay that use acridinium-labeled probes to be able to hybridize with our Amplicon. After hybridization, there is a selection state where the label in the unprotected, unhybridized probe gets destroyed while the label in the probe that is hybridized to the target is protected and followed by detection with chemiluminescence.

We use the dual kinetic analysis to be able to differentiate internal control signals versus targets seen not in this sample. We do this by the use of two different probes with different kinetics of light-off. We label our internal control with a flasher probe with very fast kinetics of light-off and we label our probe, probes to the target, with a glower probe with slow kinetics of light-off. We use exponential tight feed algorithms to be able to calculate the signals from the target versus internal control.

This contains an internal control in the samples of assay calibrators, three negative calibrators, three HIV positive calibrators and three

HCV positive calibrators. Calibrators are run at the beginning of each run.

The internal control is an RNA transcript which is another in nature specimen with a target capture reagent; is detected with our internal control pressure probe; controls for HIV and ACV RNA capture amplification, and detection; and monitors performance of reagents, operator and instrumentation. Our initial internal control failure rate in our hands is 0.5 percent and a repetitive internal control failure rate, indicative of inhibition, is extremely rare.

Our assay validity has two criteria: sample validity as well as run validity. In sample validity, the internal control signal must be higher than the cut-off in nonreactive samples, but the internal control signal is not used in reactive samples.

In run validity, seven out of nine calibrators, (2 out of three of each kind) have to be valid and also have a criteria center of less than ten percent of invalid random results in the complete run. Calibrators are used to calculate the floating cutoff. Receiver Operator Curve analysis of the specificity and sensitivity data indicates the for cut-off to obtain higher than 99.5 percent on specificity and sensitivity.

Our calculations indicate that cutoff between 45,000 and 72,000 for the multiplex assay will give these bodies of sensitivity and specificity. Results for the discriminatory assay show 39, between 39,000 and 255,000 and for the assay discriminatory, it's between 44,000 and 526,000. This indicates of the assay where variations of the cutoff will not be by modifying our specificity and sensitivity.

I will switch now and talk a little bit about assay performance. I will show data from an analytical sensitivity and specificity and later on in the afternoon session Sheryl McDonough will give some results regarding clinical sensitivity and reproducibility.

We evaluated an analytical sensitivity of five different lots of reagents using in-house analytical sensitivity panels for HIV as well as for HCV. Here, we see in all the cases with five lots of reagents, we have 100 percent detection of the 100 copies per mL level and of the 30 copies per mL levels, we are higher than 90 percent.

Results for HVC are similar. Again, we have 100 percent detection at 100 genomic equivalents

per mL and we have higher than 90 percent of the 30 genomic equivalents per mL and results are very similar for the different lots of reagents showing reproducibility.

To compute the sensitivities, we have tested several nucleic acid standards in panels, like in this case, I show results from the CBER panel for HIV where we are able to detect the 100 copies per sensitivity panel members as well as the ten copy and the 50 copy.

For HCV, we have tested also CBER panel as well as dilutions of the WHO standard. Again, we show that we can detect a positivity to the ten copies per mL panel level for the CBER panel and were able to detect down to three international units per mL from the dilution of the WHO panel. This confirms our analytical sensitivity.

We did a lot of hybridization for subtype detection. Here, is a summary slide where we show about 200 different viral isolates and 200 different specimens infected with different HIV subtypes, which were tested and the assay was positive in all of them. As an example, I can show results from the subtype CBER panel where we have made serial dilutions of the panels and

test which is our limit of detections. In all the cases, our limit of detection was always lower than 100 copies per mL.

We also looked at sensitivities of those who have prepared separate transcripts and here we show 100 percent positivity rate for transcript for subtypes G, A, H, as well as O.

HCV subtypes also were evaluated with clean colored specimens as shown in this slide, as well as with RNA transcript and here we show again 100 percent detection for HCV1a at 100 genomic equivalents per mL as well as for HCV2b.

Specificity was tested in normal plasmas as well as in samples. We put in interference substances. The summary slide for specificity in normal plasma as we tried a lot of variation shows initial reactive rates for the multiplex assay is 0.79 percent. HIV discriminatory at 0.23 and HCV discriminatory 0.15. In all the cases, the repetitive reactive rate is zero percent for 100 percent specificity. In each, internal control failure rates also are shown here: 0.31 percent for Multiplex, 0.65 and 0.70 percent for the discriminatories again with zero percent of repetitive initial control failure rate.

Conclusions from our assays are we have 95 percent detection of 100 copies per mL level for both targets. Fifty percent detection as a copy for HIV and five genomic equivalents for HCV. Detection before seroconversion was 16.3 days before antibody for HIV and 7.5 before antigen. For HCV, an average of 32.8 days.

Subtype detection was demonstrated. Also for a specificity, we have 100 percent specificity with zero percent repetitive reactive rate in normal blood donor populations. No interfering substances were found so far for the zero percent repetitive internal control failure rate and no adverse reactivity without infectious agents or autoimmune conditions.

And our program has been partnered in part with a grant of the National Heart Lung and Blood Institute.

Thank you.

(Applause.)

DR. BISWAS: Thank you very much. We'll now hear from Susan Stramer, Director of Labs at American Red Cross, Gaithersburg, Maryland.

DR. STRAMER: Thank you. Before going through the American Red Cross experience with NAT, what the major users -- that includes myself, Sally Caglioti

of BSI and Mike Strong of Puget Sound, we got together and decided we needed standardization. So the three of us developed what I call a first generation list of definitions, so that in going through the blood banking experience with NAT, we report data uniformly and I would like to go through those definitions and Sally and myself pretty much adhere to them. Mike will take another generation to meet the requirements of the definitions.

Okay, firstly, we'd like to define a yield sample which is a seronegative donation that is confirmed positive. Let me just say before I go further, I do have handouts of the definitions that I will make available at the back of the room. Anyway, so moving from a yield sample which is really the purpose of our study to identify seronegative donations, we move into what is a confirmed positive result and those are termed confirmed positives by three different definitions. One, positivity by a supplemental NAT assay. In the case of the Red Cross, we're using PCR. Two, positivity on an alternate sample that is plasma and the positivity can be the primary NAT, an alternate NAT and serology or positivity on a follow-up sample.

Next. We define an initially reactive

result as a result that doesn't repeat. For example, a pool that is reactive that cannot be resolved to individual donations. We define a repeatedly reactive result as a pool that is reactive that does resolve into one single donation or a pool that does retest is reactive.

Next. We define a repeatedly reactive unresolved pool of result as a pool that is initially reactive. The pool then resolves, but we cannot identify a single reactive donation.

Getting more complicated, we have a repeatedly reactive nondiscriminated donation and that is a repeatedly reactive result that is one that resolves to a single donation and this applies to the Gen-Probe users with the use of the discriminatory reagents. We cannot identify that multiplex reactivity as either HIV or HCV.

And lastly, we have a donation that was reactive in a pool, reactive as a single specimen and did discriminate by the discriminatory Gen-Probe reagents. However, we cannot -- we do not have any corroborating positivity that is by the alternate NAT assay, an alternate sample or follow-up. Now you will see how all of these fit into a presentation of

specificity where specificity for a pool is defined as a negative pool result or all the negative pool tests divided by the negative pools tests plus the false positives. You can apply the same principle to individual donation specificity. And I will show you results for both.

We also will report on test failures. A test failure as Christina Giachetti described for the Gen-Probe assay can be an internal control failure. It can be assay calibrator failures or it can be failure of the user to follow the manufacturer's product insert.

We also run external run controls so we also can have an external run control failure; that is, where the external run control does not meet expected specifications. However, the results from the calibrator standpoint or the assay itself are valid.

Now to run through this with the Red Cross NAT program, we are using the Gen-Probe Multiplex assay plus discriminatory reagents. We implemented on March 3rd of 1999 in phases which I will go through. The entire Red Cross system began testing then by June 7th. Each of our assays, assay batches, contains the kit calibrators as defined and four external controls: an HIV, HCV negative and one used to demonstrate that p24

antigen testing may not be necessary.

Phase 1 program and Phase 2 programs for this presentation, what's important is pool size and the difference in seroreactive testing. For our Phase 1 program, we tested pool sizes of 128. For our pre-Phase 2 program, let me call it that to avoid confusion, pre-Phase 2, we test pools of 16. The other difference that's important for this talk is seroreactives were not included in the 128 program, but seroreactives are included for the pools of 16.

Next. Okay, the pooling algorithm for Phase 1 stretched to a pool size of 128. Resolution, again, following the definitions, we repeat. Instead of retesting pool, we test the component primary pools. If a pool is reactive, we test the individual donations.

The results of our Phase 1 program included about 2.4 million donations tested. We identified by definition ten confirmed positive donations. However, three of these must be excluded for our yield definition because when we went back and tested the alternate sample, we found them antibody positive. What does this mean? This means that we detected a test error in our primary antibody screening as Dr. Busch referred to earlier.

So our adjusted yield for our Phase 1 program of 2.4 million donations was 1 in 334,043 for HCV and we detected zero for HIV. Our internal control failures were actually a log lower than Christina Giachetti reported. We had 3.4 percent invalid runs. An initial reactive rate of .14 percent. That is reactive pools that did not resolve.

Next. Going to our pre-Phase 2 program, we're creating pools of 16 resolution. You take the reactive pool and just dissect out the donations. To go through the data, we've tested 1.6 million plus donations in over 100,000 pools. We've had 231 reactive pools that divide into 172 results that were not reproducible, that is, did not resolve to single donation and were termed initially reactive. That's a rate of .17 percent. The product resulting from an initial reactive pool is released. We had 59 repeat reactive individual donation samples, those that resolved from reactive pools.

Next please. Of those 59 repeat reactive seronegative donations and it's important to say in these algorithms I've excluded the seropositives. Those I will include in another summary slide. This is only dealing with yield samples. So of the 59 repeat

reactive single donations -- seronegative donations, they divided into 30 that were HCV discriminated using discriminatory reagent, 2 HIVs based on discriminatory testing, six in which discriminatory testing could not be completed because the sample volume was depleted and 21 results that were -- even though we had a single donation identified by the Multiplex test, both discriminatory tests were nonreactive. Of these two categories here, what's important is these 30 resolved into five HCV yield samples and these two resolved into 1 HCV yield sample.

Next. The 59 on the HCV arm, just to look at these specifically, of the 30 that discriminated by the discriminatory reagents, 14 were confirmed by supplemental NAT. But I just want to say supplemental NAT may not always be the gold standard because of potential source two contamination. For example, in nine of these they actually had to be reinterpreted as negative because plasma was negative by NAT and alternate NAT and follow up could not corroborate the results. But in five cases, we did have very high titer viremia by the supplemental NAT test. Plasma was reactive and we had follow up. So this translates into our yield of 1 in 321,912 which is very comparable to

the Phase 1 study.

Next. For HIV, we had two HIV reactive donations, interestingly enough, both confirmed by our supplemental NAT test. However, one was a source 2 contamination, that is, the plasma was negative and follow up was negative at seven and 12 days. However, we did have one that was -- will be probably be a high titer viremia sample although I don't have the quantitative PCR results back yet, but at follow-up Day 7, the NAT result reproduced. So for our Phase 2 study, we have a yield of 1 in 1.6 million donations.

Next. Interestingly enough, discriminatory QNS and nondiscriminated tests, all of the ones that we have supplemental information for did not show any evidence of being NAT positive, so the NAT result could not be reproduced.

Next. So to summarize all the data I've shown you for our two programs looking at seronegatives, the first program, screened 2.4 million donations, the second one, 1.6 million donations. Numbers of pools, you can see quite a few. A higher number of pools than our pre-Phase 2 study since we're testing a smaller pool size. Initial reactive rates were comparable, .14, .17. Our yield for HCV, 1 in 331,000 for Phase 1. 1 in

321,000 for Phase 2. Our HIV yield, we only had one in the Phase 2 for 1 in 1.6 million donations or if you look at the entire program, that's one in about four million donations for HIV.

Next. If you look at the sum of all the false positive categories that I went through, actually, our Phase 1 study was perfect in terms of false positives. We had zero that resolved to single donations that weren't confirmed, but we had 53 in our Phase 2 or a relatively low repeat reactive rate of 1 in 30,372. That's lower than any serological test that we do actually. However, going from Phase 1 to our pre-Phase 2 study, our positive predictive value dropped from 100 percent to 8.5 percent. Specificity has been good, but decreased a little for pre-Phase 2 and our single donation specificity actually for Phase 1 was 100 percent and just under 100 percent considering these 53 that resolved to single donation.

Next. Looking at test failures, number of invalid assay runs, 3.4 percent as I said for Phase 1; six percent for pre-Phase 2. This includes our external run control failures which have been pretty consistent at about 1.3 percent. Internal control failures, again, for both phases of the study very low, although a little

bit lower in our pre-Phase 2.

Next. Looking at the ten positives that we got out of the Phase 1 study, including the three that were antibody positive, just to show you the total data, all ten were confirmed by alternate NAT. We have follow-up on 70 percent of the samples. Consistency among the way the TMA assay runs, whether it's in a different pool size or it's the individual donation, always runs about 9. Discriminatory reagents run a little bit higher. Our viral load range from 180,000 to 59 million. Actually now, I think we have one at 72 million copies per mL and an index donation and we've seen all genotypes.

Next. Just looking at the S to COs of false positive, they cover the entire gamut of the dynamic range of the assay, but most of them do cluster at relatively low S to COs.

Next, please. Now, if we look at all the seroreactive results we've had to date, excuse me, not to date, in the first month of our phase, pre-Phase 2 study, we looked at about a thousand samples. Interestingly enough, NAT really compares well with the supplemental tests that we do. If we look at HIV against NAT positivity, we see about six percent

positive. That's about the same rate we see to confirm by Western blot, so NAT and Western blot perform very comparably. If we look at NAT relatively to HCV antibody, we see really the same expectation. If we look at RIBA positives for HCV, we see about 60 to 65 percent. If you back out those, it should represent resolved infection or be negative for RNA. You would expect about a 45 percent repeat reactives that would be NAT positive. And that's exactly what we see. If you look at the unexpected findings, those I've put in gold. Here we've had some NAT negative repeat reactive Western blot positive individuals for HIV, but both of these fall into the traditional category of patterns that don't represent true infection, that is, a pattern of GP-41, GP-120/160 and both of those have that pattern, so we believe that these are probably false positive results.

Conversely, we've had some NAT false positive results in antibody repeat reactives and those are represented here. Similarly, for HCV, we've had some NAT false positives, but certainly, in this category, we've had both NAT -- excuse me, antibody false positives and this category again represents resolved infection. Those are RIBA positives.

Next, please. And again, this just shows you the S to CO distributions of seroreactive, either on NAT negatives which are obviously less than one on an S to CO with a NAT test. These are the S to CO values again clustered around nine for HCV and for HIV, we have some very high values. These values down here represent what we believe are false positives on the test.

Thank you.

(Applause.)

DR. BISWAS: Thank you very much, Susan. Next speaker is Susan Caglioti, speaking for -- I'm sorry, Sally Caglioti for America's Blood Centers from BSI, Scottsdale, Arizona.

DR. CAGLIOTI: Thank you. I will be reporting today on the experience that we've had using the higher arm Chiron Gen-Probe, a TMI assay that's been described in the America's Blood Center Study.

First slide, please. There are three investigators in our program: our laboratory at Blood Systems, the Blood Center of Southeastern Wisconsin, Florida Blood Services. We have a total of 56 sub-investigators that send samples to us for testing. Through the end of November, we've tested about 1.4 million donations.

Next slide. This is just a summary of our test algorithm which shows -- most of the samples that I'll be discussing have been tested in parallel with serology, i.e., serologic results are not known at the time of the sample testing. Test in 24 member pools. Any reactives are resolved to individual samples with a repeat of the pool times two. Any individual reactives are tested by both the discriminatory assays described by Dr. Giachetti and then any discriminatory positives are tested with the original in it and an alternate sample, if available, by supplemental NAT and by follow up.

Next slide. This just shows our yield for the first several, first nine months of the study. We've tested, as I said, about 1.4 donations. We've seen four confirmed HCV positives for a rate of about 1 in 340,000 and two cases of HIV for a rate of about 101,000 and 678,000.

This is a summary of the HCV positive cases which we've had four. They're all young Caucasian females. Three of the four have been repeat donors. Risk factors, you can see here are one had recently had a body piercing, as well as a dialysis tech. This one has a sexual partner, IV drug user. This denies any

risk factor at all and this person has a seropositive. You can see the viral modes are pretty consistent with the exception of this one. And time to seroconversion has been in the range of 32 to 48 days.

These two cases are HIV NAT confirmed positive, seronegative cases. Both are 22 year old black males. One was a repeat donor and previous donation was in 1993, a directed donor only. All risk factors denied here. Lower viral load. This person claimed to have a homosexual experience two weeks prior to the donation. Time to follow up for p24 in this case was six days. To antibody positivity was 16 days. This donor would return for follow up in ten days, had both p24 and antibody reactivity.

Next slide. The next couple of slides show comparison of serology with NAT data. This one is Western blot. As you can see, we had in this time frame 303 HIV EIA repeat reactive donations. The data in the next several slides are just from our organization and blood systems donations and all of the centers for whom we test. We had 16 positive Western blots, 15 of which were NAT positive. I'll mention that this one donor that was negative in the pool was positive in a single donor setting. The indeterminants here, much like Sue's

were all cases where they were not normal patterns, all nonviral bands only on the Western blot.

Next slide will show the RIBA results that you can see. We had 463 samples from a little over a 1,000 HCV repeat reactive donors in this time frame. Eighty-two percent of the total positive RIBAs were positive by NAT. One hundred five negative by NAT. Of these 105, we looked at 60 and tested them by -- in a single donation setting. These were all pools. And about 25 percent of those were positive by single donation testing. These indeterminants of which there were eight RIBA positive, we have indeterminant NAT positive. Two of them, the only reason that they were considered indeterminant is because they had very strongly positive SOD bands which by package insert, even though the four HCV bands were there, would be considered indeterminant. Three of them were four plus C22; two were C33, and one was an NS5.

Next slide. Now I'll go through the algorithms that Sue went through or similar algorithms and show you what our specificity looks like. We've tested about 30,000 pools of which 607 were reactive for a reactive rate of about two percent. Repeat reactive, remember that we do repeat test our pools, we have 594.

If you take these pools and break them down into the individual donations, of these 594, 531 of these pools had an individual that was positive by both multiplex and discriminatory or 89 percent. Four had no single reactive donors and 59 had donors who were reactive by the multiplex assay, but not by the discriminatory assay. So as far as looking at false positives, this group, this group and this group would all be considered false positives.

Next slide. Looking at those tested as single donations because in our setting if we don't have enough samples to test a 24 member pool at the end of the day, we test individuals samples. We tested about a little over 7500 donations. We found 114 positive, about 1.5 percent. Of these, 92 or 80 percent were positive by the discriminatory assay, here representing the false positive group would be these 22 which did not discriminate to a positive.

Next slide. Of the 594 reactive pools, we broke those down into the individual samples, a little over 14,000 samples, 828 of which were multiplex reactive. Of those 635 discriminated by either HCV or HIV. One hundred fifty-nine were nonreactive, 34 QNS. Again, these 159 would be considered false positives.

Next slide, please. Looking at those samples that were discriminatory nonreactive but multiplex reactive, 22 came from those tested as singles. One hundred fifty-nine came from those tested as pools. So there were a total of 181 that were discriminatory nonreactive, but multiplex reactive. Of these, none were seroreactive and of these -- now this number should be changed. We have, of the 181, we have follow up samples on 95 of these individuals, and all of them are negative.

Next slide. Looking at those that are discriminatory reactive, both multiplex and discriminatory reactive, 92 came from those tested as singles. Six hundred thirty-five came from those tested as pools, a total of 727 discriminatory reactives. Of these, obviously serology positives, most of which are HCV, 15 of which are HIV. This is the group that we're most interested in. These are the 44 serology nonreactive, 44 of which were HCV. This data comes before the HIV case that we talked about earlier. So this is the group that obviously could be considered yield samples.

Next slide. In looking at these, we were unfortunately not able to do both follow up and

supplemental testing on all of them, but of the 44, three of them confirmed positive for HCV. The rest of them, I think we can consider as false positives or those for which we have no corroborating evidence. We do have two samples that were positive on a supplemental assay which is the Roche Ampliscreen and the Roche Monitor assays. Both of these samples that you see here, one was negative by follow up, one was not tested by follow up, were positive on the Ampliscreen test, but negative on the Monitor. We think these are sample contaminations.

This column shows seven samples on which we had an alternate sample. So we think again, these are -- these false positives are really derived from sample contaminations because an alternate tube from the same in-depth sample was negative.

And then we have a number of them that were negative by either supplemental or follow up and six here that you see that were either not tested by either follow up supplemental. These we had some that were invalid runs, repeated as nonreactive; two that were reactive on an invalid run, Q&S for repeat and one donor who did not come back for follow up.

Next slide. Looking at invalid runs and

for a total of a little over 1100 runs, we're seeing a total invalid run rate or failed run rate of over 14 percent. Now I will tell you that if you look at this by month you will see a marked decrease and our data for the last couple of months looks very much like what Sue reported in our total invalid or failed run rate is about six percent. You can see the reasons here for the failures. Kit calibrators, kit controls, instrument failures, about 30 percent due to technician error, external control failures and this is the five percent rule which has now become the ten percent rule of those internal control failures within one run. So this number really has come down to about six percent.

Next slide. In looking at and in keeping in tune with Sue's definitions, this is just our data in terms of the definitions that we're trying to standardize. So we're looking at an initial reactive rate of 1.9 percent, generated from both pools, those samples tested as pools and singles. So this is numbers of tests with 1.9 percent. Repeatedly reactive pools, since we don't repeat the single test is about 1.9 percent. The repeat reactive unresult pools which Sue described the definition for, those are pools which have no individuals that are confirmed. 1.01

percent. Repeat reactive nondiscriminated donations. We have some from each one of these two types of sample tests. We had a total of 181 for .02 percent. And then the false positive, this really should be repeatedly reactive, or those that we consider false positives. We had 41 total or .005 percent.

Next slide. In summary then, our specificity, we're looking at both pool specificity and I think Sue showed the formula for this. We're looking at a pool specificity of 99.94 percent and then of an individual specificity of 98.93 percent.

Thank you.

(Applause.)

DR. BISWAS: Thank you very much. We're going to switch speakers. Michael Strong will speak first and then Richard Gammon.

Dr. Strong comes from -- will be speaking for Roche American Blood Centers Group. He's from the Puget Sound Blood Center in Seattle, Washington.

DR. STRONG: Being from the older generation, I won't have nearly as much data to present as the previous two speakers.

Slide. The Roche Group is comprised of 13 test centers throughout the U.S. with an annual

collection volume of 4.5 million from 150 test sites and you can see the distribution here across the country.

Next. This is our total yield at this state. We're in the process of breaking out the data to conform to the new definitions. We have 2.1 million units that have been tested with a yield now of ten HCV positives, four of which are confirmed by the previous seroconversion definition and an additional six that are in the category of positive on the basis of a separate sample, for example, the plasma unit being positive. Not all of our donors will have follow up donations to allow us to put them into the seroconversion positive category.

Next. Here's a breakout of the donors that have been picked up through HCV screening. First donor back in June, we have started the testing as of April, so we're still only a little more than six months into the testing scheme. These initial donors have all seroconverted. We don't have RIBA data on every one, but you can see here on a couple of these they are indeterminant reaction rates for RIBA with C33 positivity. And here a donor that has elected not to participate in the follow up and of course, like the other tests that we perform, we do run into donors who

not only don't want to participate in the study, but also get quite angry when you call them and tell them about their test result.

Next. Here's the last five donors that have been picked up, a couple again, who have yet to have a second sample drawn, but were positive on the plasma unit and again another donor that has elected not to participate in the study. This yield basically fits the new definition.

Next. In terms of test performance, again, we haven't broken out according to the new definitions into the different categories. Our turnaround times are about the same as they've been in the last few months with the test turnaround time ranging from 10 to 14 hours, a turnaround time for final resolution because we go through a separate set of tests to get to resolution of up to 72 hours. Again, this pretty much depends on the distance that these samples have to travel to the test laboratories. And then finally, our estimated false positive rate, based on total donations, again, and not yet complying with our new breakout of definitions is less than .01 percent and our run failure rates range between less than one percent up to about five percent and as previous speakers mentioned, that

part of it is due to learning curve. We certainly see a decrease in run failures as the laboratories get up and running and get more accustomed to the test themselves.

Next. So that's a very quick update of the Roche data and the next time we get a chance to meet, which seems to be quite frequently these days, we'll hopefully have a breakout with the new definitions.

Thanks.

(Applause.)

DR. BISWAS: Our next speaker is Richard Gammon for the Association of Independent Blood Centers from West Palm Beach, Florida.

DR. GAMMON: He's getting the laptop set up right now. While we're waiting, I just want to take the chance to invite anyone to come down and visit. I grew up in the Northeast part of the country and when I left the airport yesterday from Florida it was 80 degrees and sunny.

(Laughter.)

DR. BISWAS: While we're waiting I want to congratulate all the speakers on the sort of catching up a bit on time. But sometimes advance of technology does hold one up.

DR. TABOR: This is the modern equivalent of when someone turns the slide carousel upside down and the cap isn't on it. I've seen that happen and someone suggested that people get up and tell jokes until the slides are put back in order, but I think in this case, Dr. Biswas and Dr. Dayton, perhaps you could entertain some discussion while we're waiting.

DR. DAYTON: Yes, actually, I do have a question that I'd like to address to Dr. Heldebrant. In fact, you may even want to come to a microphone. Where are you? Why don't you come up to a microphone. I was very interested in the data that you provided on the eclipse periods where in HIV, where you had an initial weak PCR reactive. I think you had four of them and then you had it disappear for several donations and then reappear?

DR. HELDEBRANT: Yes.

DR. DAYTON: And that has tremendous implications for mechanisms of infection depending upon how the people were infected. So how were those individuals infected? Were they transfusions or IVDUs or sexual activity?

DR. HELDEBRANT: As far as we know, they denied transfusion. They denied IDU. We would assume

that they're environmentally or behaviorally acquired, but beyond that we don't have any specific data on any of the four to determine what their --

DR. DAYTON: If it's some kind of parenteral introduction, it's not terribly surprising to see a little peak and then a clearance, so that's interesting. For sexual transmission, that's very interesting, if it's sexually transmitted because we all think of sexual transmission as involving the transmission of a very small number of viruses. So the implications are if it's sexual transmission, the implications are either they had an open wound and there was a large amount of the initial inoculum which seems hard to believe, or that there's an initial burst and then a nonspecific clearance, possibly, then the eclipse period and then the acute phase, even before you get antibody. Maybe we can talk in private about that afterwards. I'd certainly be interested in pursuing that.

DR. HELDEBRANT: Yes, those are remarkably interesting samples. In a way, we kind of wish we had a lot more data on the individuals who donated them, but we have what we have.

DR. DAYTON: So are they now lost to follow

up or --

DR. HELDEBRANT: I have a feeling we could identify them if we had to. Currently, they're lost to follow up since they have seroconverted to antibody.

DR. DAYTON: Uh-huh, but they are --

DR. HELDEBRANT: But they're permanently deferred as donors so they're lost to our system.

DR. DAYTON: But they are contactable?

DR. HELDEBRANT: I would assume so, yes.

DR. DAYTON: Okay, the carousel is still upside down, apparently.

DR. BISWAS: Is Alan Liss, could Alan Liss, I have a question for Alan.

DR. TABOR: Come up to a microphone.

DR. BISWAS: It's a bit unfair, we're hogging the questions.

DR. TABOR: Chairman's prerogative.

DR. BISWAS: The question I have is of the HBV DNA positives that you have, have you done any antibody testing like anticor or anti-HBS and will you be doing it?

DR. LISS: We are doing anti-HBS Ag antigen.

DR. BISWAS: Anti-HBS Ag.

DR. LISS: SAG antigen, but no antibody test.

DR. BISWAS: No antibody tests?

DR. LISS: Right. We were compensating the core and so forth.

DR. BISWAS: Okay.

DR. LISS: Nice suggestion.

DR. BISWAS: Yes, I think so. I think it would be --

DR. LISS: It's been suggested before. It's a matter of putting it into the scheme.

DR. BISWAS: Okay. Dr. Gammon?

DR. GAMMON: We're all set to go here. Could I have the next slide, please? The Association of Independent Blood Centers or AIBC, testing began on all donations collected on April 1, 1999. As of November 30th, we have tested about 235,849 donations from 23 collection sites. We have tested about 9800 master pools for a 3.30 percent reactivity rate in those pools. Now out of the 324 pools that were positive, 13 of them or approximately four percent of the reactive pools were nonreactive upon individual multiplex testing, giving the specificity of the assay at our center of 99.9 percent.

Next slide, please. Average turnaround time for specimen receiving to result reporting is about nine hours, with a range of 6 to 12. Again we tested 324 pools. The number of reactions of units tested by discriminatory assays was 332.

Next slide, please. Now for the data presentation. Okay, in our experience, all 20 donors who were EIA positive for HIV as well as NAT positive for HIV were also Western blot positive. All 118 donors here were EIA positive for HCV and NAT positive for HCV were also RIBA positive.

Next slide, please. This slide just shows these were donors in which the quantity we tested was insufficient. It speaks to the limitation of specimen when drawing one PPT tube and the difficulty of having donors return for collection of additional specimen on a voluntary basis.

Next slide, please. This slide shows donors who were EIA serology positive, but NAT negative. I just want to make a couple of comments. We had 97 donors, 97 donors who were EIA positive for HIV, but not confirmed by Western blot. We had seven donors who were p24 positive for HIV, but not confirmed by neutralization. We have one donor who was EIA positive

for HIV and p24 positive, however, neither of the confirmatory tests panned out.

We also had 17 donors who were Western blot indeterminant and we had 118 donors who were EIA positive for HCV, however, they were RIBA negative. The above cases may speak to the problem of serological false positives which defer donors at blood centers every day.

There's also 31 donors here who are EIA positive for HCV and RIBA positive and this may potentially speak to situations seen with a donor who has cleared the virus so it's not detected by NAT or a donor who has a low level viremia below the level of detection or problems inherent with pool testing such as an inhibitor versus dilutional effect.

Next slide, please. These are our sub-study donors. We've had nine of the 19 donors in the sub-study return.

Next slide, please. A little bit about the specifics of our sub-study donors. Twelve of the donors positive for HCV by NAT; eight on follow-up were negative for HCV by NAT and EIA negative. We do have one donor that was just in October was positive for HCV by NAT and on follow-up bleed was also positive for HCV

by NAT. I'm going to talk about that a little further. Three of the donors under this category did not return. They're either first or second donors. We have a donor who is positive for HIV by NAT. This was a first time donor that did not return.

Next slide, please. We have six donors that were positive for both HIV and HCV in the study. Three were positive. Three were EIA positive as well as Western blot positive for HIV. Two were negative for Western blot but positive for p24 neutride and one is EIA reactive/RIBA positive. These donors have not returned either and were either first or second time donors.

All donors who do not return for additional testing were either first or second donors in our study. Five bleeds after the initial donation is the maximum number of returns that we have had so far.

Next slide. This is a slide showing the signal to cutoff ratio of the individual Multiplex testing of our sub-study donors on the initial donation. And I want to point out this is the last donor here that initial donation was in October that was NAT positive for HCV on the initial donation as well as the follow-up bleed in November. And you can clearly see

that the signal to cutoff ratio at 8.3 is higher than any of the previous signal to cutoff ratios.

Next slide, please. This is that same individual donor, again, a donor from Tennessee who the signal to cutoff ratio of HCV was 17.3 on the October 18th donation and on November 18th was even higher at 19.08. And we're working closely with Gen-Probe and I'm working with the Medical Director at the blood center from Tennessee to get this donor back in for additional testing.

Next slide, please. This slide shows five cases out of the 236,000 donations so far in which the pool, NAT pool was negative and the EIA result, initial EIA test was positive and so the NAT laboratory did individual testing and the individual testing was positive by discriminatory testing. And of particular note are two, in this case here in which the RIBA was positive for HCV. This case here in which, from Florida, in which the Western blot was positive for HIV and I just got a call this morning, actually during the conference that the Western blot here for this donor here is positive now from this donor here also from Florida. Again, these are specimens in which the pool was originally negative, but the individual was positive

as well with discriminatory testing.

Next slide, please. In summary, we've tested about 236,000 specimens and out of those we have one donation so far that may have detected HCV and increased their conversion window period and follow up testing is in progress.

Next slide, please. Thank you. I don't know how well this turned out. This is a gentleman practicing the ancient art of uroscopy, the study of the urine, and I hope we progressed a little further since those days. Thank you very much.

(Applause.)

DR. DAYTON: Well, let's open up this entire session to questions. I guess if you're going to field a question, please go to the nearest microphone because no one can hear you otherwise.

We have a question back here.

AUDIENCE PARTICIPANT: Yes. Focusing on the how shall I say -- not to be anti-semantic about it, but there's a variety discussion about what is a true positive, what is a true negative. And one of the questions I would have is given the phase one nature of much of the samples that were done, were any of these units transfused and what happened to the patients as

far as their seroreactivity NAT reactivity as far as resolving some of these issues and what will be actually defined as a truly infectious donor?

DR. DAYTON: Do we have anyone who has data on that or would otherwise like to respond?

Susan?

DR. STRAMER: I walked all this way to say that I'm going to defer that until this afternoon. So Larry, you're going to have to eat lunch and come back.

(Laughter.)

DR. DAYTON: Okay, so that's to be continued. Do we have any other questions?

AUDIENCE PARTICIPANT: I'd like to follow up. Dr. Biswas asked the question in terms of the HIV test at that time you have initial positive and identical nucleic stage, don't see anything. And then you see about four days ahead of the p24 assay. I wonder if that could be the sensitivity and the virus goes to the target cell, was maybe a test, like a PBMC type of thing to indicate that you may be can detect HIV.

DR. DAYTON: I'm not sure I understood the question. What exactly are you asking?

AUDIENCE PARTICIPANT: I was asking about

if you detect in the whole blood such as like PBMC maybe you can detect HIV RNA in the target cell.

DR. DAYTON: You're asking in that eclipse period, can you detect it in the target cell?

AUDIENCE PARTICIPANT: Right.

DR. DAYTON: Well, Dr. Heldebrant?

DR. HELDEBRANT: We don't have any samples of the PBMCs or the whole blood from these individuals. These are plasma pheresis donors, so all we can say is that in the plasma that we would screen, we can't find it and we've looked for it repeatedly with very sensitive individual testing and haven't found it. It may be in the target cells. I don't know. We just don't have any data on that.

DR. DAYTON: Thank you. Any other questions or comments or -- well, I guess then that means we've answered all possible questions and we all need to --

DR. BISWAS: I've got one question. Mike Busch, Mike, my question is I guess I should have been able to extrapolate it from your very, very excellent presentation, but what is the longest window period for NAT testing for the three viruses? Now, I understand for HCV there may be intermittent and there may be some

people who never seroconvert, but what is the longest period from exposure to NAT positivity that you have seen for HCV, HIV, and HBV?

DR. BUSCH: That's a tough question, because there's not too many studies where you have serial samples from exposure through to NAT detection. Most of these studies have screened by NAT and are following people out and finding the duration of viremia prior to antibody. In terms of people who have a discrete exposure and then how long does it take for them to become detectably viremic, there's not much data. In HIV, I would suspect that's no more than two or three weeks. With HCV from the transfusion cases it seems like it's consistently detected within, again, about two weeks of exposure, you detect RNA.

DR. BISWAS: I thought that from one of the slides that if one sort of did a little arithmetic, that the longest, zeroing in on the longest, window period time to NAT positivity was about four weeks, 28 days or something like that?

DR. BUSCH: I don't recall that case.

DR. BISWAS: Okay.

DR. BUSCH: It's a tough question.

DR. BISWAS: I have to go back and look at

that slide.

DR. TABOR: I'd like to underline Dr. Biswas' question because I think we tend to talk, it's not just your data is fantastic and we're obviously all indebted to this kind of study, but we tend to talk in averages and means when we talk about window periods and I think the actual extremes of the window period, both the beginning extreme as you were answering and the end extreme that Dr. Biswas was asking are very much of interest to people trying to work out a regulatory position. So maybe in the future you could include that in one of your talks.

DR. BUSCH: Yes, I actually had some slides at the end, it was giving an overview of a study that we have developed and looks like it's going to fund and go forward that's actually, involves a variety of elements, but probably the most relevant to your question is we've now with the help, particularly of alpha, compiled a much larger number of seroconversion panels that are just phenomenal, literally well over 100 HCV and about 50 HIV new panels that are being extensively characterized and then they will be studied both by the single donation and different minipool size NAT assays to understand how far back prior to antibody from a much

larger mix of panels can we detect any evidence of viremia and then in collaboration with Harvey Alter we're going to be doing infectivity studies similar to the study that was done, but with again a larger number of closely spaced plasma samples to ask the question of is there any infectivity prior to the ability to detect the virus by even single donation NAT.

DR. TABOR: The answer could be even simpler, I mean even using the panels that we have available now, it will be interesting to know, get the answer to that question, I think it would be very useful.

It's terrific that everybody has got us back on schedule. It's really rare for that to happen, and it's time to break for lunch. I need to tell you that it has been said that the cafeteria -- I think it's correct -- the cafeteria downstairs is not serving lunch, even though it's open for coffee, because of asbestos removal in the kitchen area.

(Laughter.)

So you'll have to find your way to second floor cafeteria, and again, if you go straight back past the Starbucks stand until you can go no further and then turn left, and go until you find some escalators to take

you up one flight in the cafeteria right up those steps. We'll try to reconvene around one o'clock. Thank you.

(Whereupon, at 11:58 a.m., the workshop was recessed, to reconvene at 1:00 p.m., Tuesday, December 14, 1999.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

(1:04 p.m.)

DR. TABOR: This afternoon we'll begin with the session on implementation issues as seen by trade organizations. The moderators for this session will be Dr. Mary Beth Jacobs who is the Acting Director of the Division of Emerging Transfusion Transmitted Diseases and Dr. Paul Mied who is the Deputy Director of the same division.

We will have five or possibly six speakers in this session, followed by a panel discussion. I'd like to ask the speakers to try to keep their remarks to eight minutes or less so that we can keep it in the time frame.

I'll turn the microphone over to Dr. Jacobs and Dr. Mied.

DR. JACOBS: Good afternoon. We have two additional speakers, Dr. Celso Bianco, who is the President of America's Blood Centers, and also a representative of IPPIA. We're changing the order a little bit. We're hoping not to have to reboot. So our first speaker will be Dr. Richard Gammon. I'd like to ask each of the speakers to join us at the table after their remarks, and for the panel discussion, we're going

to be showing an overhead which everyone will see, giving some implementation questions which have come to our attention and will ask the panel to discuss those if they haven't come up in their individual remarks.

So first of all, Dr. Gammon.

DR. GAMMON: Thank you. I'm going to talk this afternoon about the industry perspective from AIBC's point of view. Our NAT laboratory is located at Citrus Regional Blood Center in Lakeland, Florida. The location was chosen as one to be least likely affected by inclement weather such as hurricanes or snowstorms and has access to multiple major airports including Orlando and Tampa.

Next slide, please. Beginning on donations collected on April 1, 1999, K_2EDTA plasma pools of 24 donations were tested. There was strict adherence to the Gen-Probe's transcription mediated amplification assay procedure and everything has been tested for HCV and HIV.

Next slide, please. This slide just emphasizes the uni-directional work flow in our laboratory from specimen accessioning to amplification to the chemiluminescence detection of a viral marker, if it's present.

Next slide, please. This is AIBC's pooling algorithm. The pooling TECAN first creates three in process pools of eight. It then aspirates from each of the three in process pools to create master pool of 24 donations. Each pool master in- process is weighed and the results are transferred directly into the NAT laboratory's information system. If a pool falls outside the weight range, the pool is voided.

Now I'm not going to go into detail about the rest of the testing, the remainder of the procedure. It was covered earlier today. Just to mention that again amplification detection of the RNA and HIV and HCV occurs.

Next slide, please. This is how specimens, the flow of specimen testing occurs in our laboratory. Specimens come in between 3 and 5 a.m. from the blood collection centers in our accession. A pool is created and tested and serological testing occurs concurrently. NAT results are available between 12 and 1 p.m. on negative pools and results are faxed or e-mailed to the various blood collection centers. The individual breakout testing then occurs, results are available by 5 to 7 p.m. that day and discriminatory testing occurs once a week and these results are used for donor

counseling purposes.

Next slide, please. All units since initiation of testing on April 1st have been released in Phase II. Phase II means that there is completed NAP and completed serological testing on all blood components including platelets upon release.

Next slide, please. As far as what AIBC does for look back. The collection centers conform to current look back policies and placed for serological testing of HIV and HCV for NAT reactive donors. Specimens NAT reactive, regardless of the serostatus, look back must be initiated.

Next slide. Consignees need to be notified of receipt of units from an NAT reactive donor and consignees are required to follow FDA guidelines and their standard operating procedures for notification of recipients.

Next slide, please. I was asked to talk briefly about a few automation issues. I just want to mention three of them briefly that were brought up by our laboratory. Apparently, we have had some problems with bent TECAN pipette tips that have resulted in pipetting errors. Resolution was that the deck was realigned. We've had some ilid block failures that have

resulted in pipetting errors. Apparently with the ilid block failures the pipetter ejects its tips and thinks it's picked up another tip when it has not. You end up with a tip eject error. What we've been doing is cleaning, repairing and replacing the ilid block and apparently it becomes a point that you may need to recreate the master and the in-process pools.

Next slide, please. We also have a piece of plastic that's called a frog leg and these have not been working as expected either and so you basically have replaced these. Apparently, in certain instances you can end up with a fatal error and then you need to recreate in-process in master pools and in all of these issues we've been working very closely with Gen-Probe on to get resolved.

Next slide, please. I want to talk briefly about software issues, especially those that are unique to AIBC. With the advent of NAT, AIBC felt the need exists for specialized computer programs to provide process control. We designed our own NAT computer software package with specialized programs to number one, identify pool samples which have not been individually accessioned; number two, validate the reading of the sample numbers in each pool; and number

three, to confirm the presence of 24 individuals samples in a positive pool for individual testing.

Now talking briefly about each of those programs, the pooled not accessioned program, this identifies any NAT sample which has been involved in the pooling step which has inadvertently not been accessioned. It's run after the pooling program and provides an opportunity to accession any sample missed. It validates the sample number based upon collection site, identifies date of receipt and allows for result reporting. It also is important in billing since billing is based upon the accessioning of samples. If the sample is not accessioned, we can't bill for it.

Next slide. Validate pool files. This evaluates the reading of the unique NAT identification number which identifies both the collection site and the individual donation on each sample. This program flags misreads allowing for the timely correction prior to updating the pooling files. A misread includes if you did not have the two digit alpha site code, each of our collection sites are assigned a two digit alpha code and if it does not have the seven digit donor number. This was a bigger problem especially early on in testing before collection centers were familiar with where to

put the labels.

Next slide, please. Master pool confirmation. This provides a mechanism to confirm that the correct individual samples have been selected when preparing to test the 24 individual samples from a reactive pool. After entering the master pool number for the reactive pool, the operator bar code scans each individual sample number from the sample selected. Should the sample be entered which is not a member of the reactive pool, the program notifies the operator and allows the opportunity to take corrective action.

Next slide, please. Results of the software, the pool not accessioned program, less than one percent of the 236,000 samples processed in 9800 pools bypassed initial accessioning. The validation pool files was most beneficial in cases of misplacement of the bar coded collection site-specific unit number and this was a bigger problem earlier on in testing. Less than one percent of the samples were flagged by this program.

Next slide, please. In the master pool confirmation, has insured correct placement for all members, 100 percent of the 324 positive master pools detected as of November 30, 1999. I refer you also to

these issues about the software, were presented at the ABB and are in the supplement for the meeting in an abstract form.

Next slide, please. Access to NAT testing. AIBC's IND allows for testing of approximately 500,000 units over a 12-month period.

Next slide, please. Our participants include AIBC members, non-AIBC members, free standing blood banks, and hospital-based blood banks. To my knowledge, most of the quals of blood collection centers looking for testing come to me. AIBC has never turned away anyone looking for NAT testing. We have collection centers going from 2,000 units up to 100,000 plus. So access is open to anyone that is interested in testing with AIBC.

Next slide, please. A little bit about the process of how access is gained toward testing through AIBC. Principal investigator, myself, was contacted. I sent out an investigator's handbook. The individual collection center needs to get IRB approval. They can usually use their own IRB if they have one or they can use AIBC's if they do not have one. Of course, there's a period of questions. They need to order supplies, get a donor number applicator, PPT tubes, develop their

standard operating procedures and we provide a model for that in the investigator's handbook. The donor number applicator needs to be validated and staff needs to be trained and documentation of that training needs to be in place in the NAT laboratory. And at that point in time they can go ahead and implement NAT testing on their donations.

Next slide, please. So how do we go about setting up an NAT laboratory? Well, last fall we had to determine what method we wanted to use. Did we want to use PCR? Did we want to use TMA? Those were the two methods available and are still available at this time. We also had to decide, we had to choose a site, build an NAT lab. AIBC has about 30 members and we had to pick where to put one. We decided to use an existing facility that had -- was fairly new, had an extra warehouse for storage space, so that helped cut down on cost and that's how we decided where to put our laboratory at Citrus Regional Blood Center. We had to obtain a clinical review officer and submit an IND. We also had to develop a client base. I mean building an NAT laboratory is expensive and the testing also generates a cost, so we have to have a client base to do the testing and the client base have to find a courier

system to get the specimens down to the laboratory in a timely manner. That's why the bulk of our specimens go through Orlando just because of the large number of direct flights. Once we got IND approval and IRB approval, we could go ahead and initiate testing. I put this little loop -- and that happened in April. I put this little loop of continuous because every time a new client wants to come on board, they need to get their IRB approval, go through what we talked about in the previous slide, then they can go ahead and initiate testing.

Next slide, please. In conclusion, our coordinator work flow has allowed rapid turnaround time and has allowed us to be maintained in Phase II since initiation of testing or units have been released in Phase II with completed NAT and completed serological testing. The result is minimal impact on product release. All platelets have been able to be
-- all cellular components, including platelets have been able to be released and fully tested and may potentially improve patient transfusion safety.

Thank you very much.

(Applause.)

DR. JACOBS: Thank you, Dr. Gammon. Our

next speaker is Kay Gregory who is Director of Regulatory Affairs for AIBB.

MS. GREGORY: Good afternoon. My statement is going to be very brief. We thought we'd like to hear more from the people who actually hold INDs and have some things to tell us than what you'd like to hear from us.

The AABB applauds the FDA's interest in maintaining the safety of the blood supply and its willingness to be flexible with regulating NAT. Historically as you've heard, FDA has required that it has to be licensed before it will introduce for blood bank screening on a nationwide scale. This is the first time FDA has permitted widespread use of a test under IND.

It has been a real challenge for everyone involved and FDA has been most supportive. There is, at least one lesson that the AABB believes should be learned from this approach to NAT. All of us, blood banks and the FDA, need to be more attuned to the concerns of the hospital transfusion services. Although there were attempts to keep the hospital transfusion services informed, the information really wasn't clear to them.

There was a great deal of concern about whether NAT testing of blood products constituted investigational use for patients, whether IRB approval was required and whether informed consent must be obtained for patients. While the FDA addressed these issues once they arose, it would have been preferable to have considered such issues in advance.

Finally, the issue of inventory control of a mixed inventory and the need to identify which units have been NAT tested and which have not is of great concern to transfusion services.

We appreciate FDA's recognition of this concern and willingness to address it by permitting a mechanism for determining which units have been tested and which units are still undergoing NAT testing for HCV. And we expect that the FDA will similarly address the issue of NAT testing for HIV as soon as that testing is widespread.

The use of nationwide INDs is another aspect that deserves careful consideration by the FDA in making future decisions. By definition an IND can be used during investigational research phases. In permitting testing, over 90 percent of the nation's blood supply is under IND, the outcome is perceived by

many as being presupposed and rapidly became viewed not as investigational, but as essential screening.

The AABB appreciates the opportunity to speak today and commends the FDA for holding this public workshop. We encourage FDA to be mindful of the potential impact on transfusion services to consider their needs and to avoid the use of nationwide INDs in any future implementation of new technology on a nationwide scale.

Thank you.

(Applause.)

DR. JACOBS: Thank you, Ms. Gregory. Our next speaker is Dr. Susan Stramer, Director of Laboratories for American Red Cross.

DR. STRAMER: Thank you. My presentation this afternoon will be briefer than the one this morning and I will speak more slowly.

(Laughter.)

I've listed the issues that we were asked to comment about from Dr. Biswas, so I will go through them and on many of them as asked by Dr. Sherman this morning will elaborate on more data than we have accumulated.

As we found through this morning, the

American Red Cross is testing for HIV and HCV in a multiplex test. Our product release criteria I will describe in greater detail, keeping with the definitions that a Phase I program is release based on serology and a Phase II program release of all components including cellular components based on nucleic acid testing.

I will discuss the laboratory turnaround time our product control and recipient follow-up for the index unit and let me just say I don't have a slide for look back or prior collections, but of the 12 index units that I identified this morning, six were from repeat donors. Of one of those repeat donors, we had a look back in which we were actually able to retrieve the plasma from the hospital. That plasma was tested by PCR at NGI and by TMA and was found negative and that look back occurred 60 days prior to the index donation.

Automation was nicely covered by the AIBC presentation and similarly at the Red Cross we have five different software systems that allow rapid and efficient communication from the point of sample entry into the laboratory through resolution of reactive pools and all five of those systems have been validated.

What is currently and desired for testing? Obviously, we encourage more automation because we are

currently doing a manual test and clearly the more automated we are, the better off we are and then I will end with the ease of requiring NAT testing as far as the Red Cross.

Next, please. I showed this slide earlier, but I'm going to show it again because it's relative now to how we define the phases of our program. Frozen product control, through all phases of the Red Cross program. Our Phase I program which went from March and ended in early September; our

pre-Phase II program which included the logistics of the product control program, but not yet included cellular product release, so that's where we are now and we will move after the first of the year into what we call the true Phase II program where we will control cellular products. But we are not there yet. We are testing pools of 16 as if we were in this program, but currently we are releasing cellular components based on serology. And we do test in one laboratory which represents a formidable challenge.

Next. This represents daily collections over a period of two months, September and October, although that's irrelevant because the cycle that you see, according to day of the week, represents the

reality of samples coming into the laboratory. And for those of you who can't see this, this line here is the 20,000 units per day. So the laboratory for each of these bars, over 20,000, including this one that's almost 30,000, this one laboratory is to break down boxes, releasing these 30,000 units at which they have to be accessioned, pooled, tested, resolved and results communicated back to the collection region. So this is quite a challenge.

Next. During our Phase I or pool size 128, this represents over the same period of time which was the end of our Phase I program, our median turnaround time from the point of entry into the laboratory to the point of release of results. So this is a 42-hour turnaround time and you can see how the peaks correlates to those peaks of the highest collection dates that I showed on the previous slide.

Next. When we went to pools of 16, it doesn't take very complicated mathematics to see that in this one laboratory our testing volume increased eight fold and it also included testing of seroreactives. So we also had more pools to resolve and when we could, based on an initial reactive results fed into us from our national testing laboratories, we pulled initially

reactive samples. So not only did we have the eight fold increase just based on the arithmetic calculation, but we had to deal with all the seroreactives and contaminants resulting therefrom. So this yellow line here represents the median turnaround time. The dash line here represents the median of Phase I at 42 hours. And in the beginning there needed to be a period of adjustment, but you can see now that we're trying to stay below the line and where this needs to be, maybe at 20 hours or less for us to control all products, that's what we need to determine and that's where we need to move.

Next. Because we are releasing cellular components based on serology, the point at which we get a NAT reactive pool at the 16 member stage is when we initiate product quarantine or we freeze products in-house or notify consignees that you may have a product that may test NAT reactive, but is pending further resolution. So what this slide shows us is based on red cells, the two different phases, the number of products and the percent at different locations. So you can see that greater than three quarters at high numbers now for our pre-Phase II are in-house about a quarter at the consignee for 99 percent of control of product. One

percent of red cells that were in NAT reactive pools were transfused, however, none of these confirms to a single NAT reactive donation.

Next. For platelets, giving you the same type of information, because platelets have a shorter shelf life and go out earlier. You can see the distribution is a little bit different for the two phases of study so that more products in NAT reactive pools were transfused. However, in the Phase II program, there were seven false, I should say, seven NAT reactive units that did resolve again to the unit level that were transfused. However, none of those were true positives. They were all false positives, based on the definitions I discussed earlier. There was one, however, in Phase I that was a true, confirmed yield HCV sample that was transfused to a recipient.

Next. If you look at the ten NAT reactives in our Phase I, just based on the products made, including this one that was transfused, and you total this all up, we did have product control based on single donation of 95.5 percent.

Next. In here I will show you the results of the recipient. So here was the donor who was negative by serology, had greater than 10^6 genotype 2b

HCV RNA. Pre-transfusion. The recipient was negative. Shortly after several hours after transfusion there was a two log reduction, but clearly you can see based on dilutional effects alone that this person received HCV from going to negative to going to the two log reduction of the same genotype 2b. Twenty two days later when the recipient was resampled, the concentration was 1.2 times 10^6, again genotype 2b. So clearly, this individual was infected. The donor who contributed this sample remains seronegative but NAT reactive on days 9, 39 and 81 days of follow-up.

Next. Lastly, just to show you how the Red Cross is structured, this is the lab that we run NAT testing. It's in San Diego, the National Genome Testing Laboratory. It's not in Alaska. It's actually in California, but all of these samples on a daily basis, 24 hours a day, seven days a week are shipped to this lab, which runs 24 hours, seven days a week and we do make NAT testing available to non-Red Cross facilities.

Thank you.

(Applause.)

DR. JACOBS: Thanks, Dr. Stramer. Our next speaker is Dr. Celso Bianco.

DR. BIANCO: We thank you very much for

this last minute addition to the agenda. I'm Celso Bianco and I'm the President of America's Blood Centers. We thank the Center for Biologics Evaluation and Research for the opportunity to make these public comments. ABC is a consortium of 72 not for profit community based blood centers that collect over half of the U.S. volunteer blood supply.

ABC members are very proud of their participation in the largest research protocol ever in the history of blood donor screening.

You can go to the next slide, Roger. And the next one. They are proud of their partners, the assay kit manufacturers, Chiron, Gen-Probe, and Roche and CBER.

Implementation of NAT IND continues to take a tremendous amount of energy and resources on the part of all participants. It could not have happened without an incredible commitment to common goals. It happened with very special people in many organizations. It could not have happened without the explosion of communication and transportation systems that took place in the U.S. in the last couple of years. And it could not have happened without the Internet and e-mail.

ABC members built 16 laboratories spread

throughout the country and are testing 6,000,792 samples a year for members and for hospitals that collect blood in their communities. According to a survey completed on December 1st, 100 percent of the 72 members were performing NAT for HCV; 44 of them were in Phase II, that is, all components were released on the basis of serology and NAT. For members in transition to Phase II, the vast majority of products other than platelets were released on the basis of NAT. These centers plan to be in Phase II early next year. Eighty percent of the 30 members using the Gen-Probe assay for HIV are in Phase II. Members using the Roche system are implementing the HIV assay at this time. And interestingly, based on the same survey, only seven of our members indicated in their packing slips that the units being shipped have been subjected to the research NAT.

We're excited about the preliminary results presented today because they suggest that those efforts were not in vain. NAT appears to detect infections that are not detected by the currently licensed tests. The preliminary results encourage us to continue to work diligently towards the ultimate goal of the IND research protocols, licensing of these assay systems by the FDA

and the application to the totality of the blood supply.

Unfortunately -- next slide, please -- some issues keep threatening to derail us from the steady path of licensure. ABC members would like to comment on these issues.

Next slide. The first is single donor testing. We agree with CBER's statement that considers NAT on the plasma pools as an interim step until individual tests are ready for licensure. The issue of single donor testing is not if, it is when. One manufacturer has indicated to us that they will file a PLA for individual testing using the current manual system, or as they call them, semi-automated system. Manual testing at the level required to screen 13 million donations of whole blood and many millions of donations of source plasma would increase the workload by 16 to 24 fold. Compliance with cGMP would become practically impossible. This is unacceptable.

It should be noted that these proposals are being made as Congress expresses great concerns about errors in transfusion medicine and the Institute of Medicine publishes its "to err is human" report on medical errors. The National Heart and Lung and Blood Institute has funded a major contract for the

development of automated instrumentation for individual blood donors screening by NAT. When these or any other instrument in the corresponding tests are ready and licensed and reimbursement covers the increased costs we will be ready. Any attempt to skip the orderly evolution of NAT screening that may place blood donors and recipients at increased risk because of potential human errors will be vigorously opposed by ABC members.

The next slide. The second issue that concerns us is pool size. One manufacturer has chosen a pool size of 16 plasma specimens while the other has chosen a pool size of 24. Considering the characteristics of NAT, these differences are not statistically significantly. However, there have been attempts to suggest that a slightly smaller pool size would provide a higher sensitivity. We believe that FDA has the authority to prevent such misleading claims from being propagated and respectfully request that it does so.

CBER could contribute to the avoidance of these type of issues by providing manufacturers with a specimen that constitutes the minimum sensitivity standards. Manufacturers would be required to adapt their systems to meet these standards regardless of pool

size.

Next slide. The third issue, ABC members are extremely concerned about conflicts between intellectual property and public health. These conflicts are illustrated by two recent events. In one, Chiron, the owner of the patent rights to the HCV sequence chose Gen-Probe for the manufacturer of ACV NAT and indicated that they will not license any other manufacturer. ABC members recognized that intellectual property rights and royalty reimbursement are motors of progress in our society. However, monopolistic control of public health is not an acceptable practice. We hope that Chiron and Roche will come to an agreement that serves other donors and patients. And actually, there's a beautiful statement that was made by Karen Shuse-Lipton, the Executive Director of the ABB in the most recent ABB news publication that refers exactly to this same issue.

In the other incident, the discoverers of the so-called SEN-V virus published their findings in the New York Times and in their Internet site. They have not yet published a single paper in the scientific literature or made a single presentation at a scientific meeting. They appear to care more about their stock

value than about the public health value of their discovery. I must emphasize that ABC members and I believe the entire blood banking community have as their goal the best interests of blood donors and recipients and will do everything in their power to remain true to this goal.

Fourth, and the next slide, we urge FDA to consider withdrawing the recommendation for use of HIV-1 p24 antigen assay when a facility performs NAT for HIV-1 under an FDA-approved IND protocol and releases products under Phase II. The contribution of HIV-1 p24 antigen and assay to the safety of the blood supply has been minuscule and NAT is theoretically superior and actually, in practice as we saw the data today for the detection of HIV-1 even when tests are performed in pools.

Removing HIV-1 p24 antigen for the least of required tests will allow testing facilities to focus on more important issues and will reduce some of the financial burdens.

Finally, we urge CBER to allow the use of NAT tests currently under IND as an additional, more specific test for confirmation of antibody screening for HCV and HIV-1. NAT results would not affect donor

deferral status and would greatly facilitate donor counseling.

The last slide. And finally, we want to congratulate everybody that contributed to the success of NAT implementation. Thank you very much for your attention.

(Applause.)

DR. MIED: Thank you, Celso. Our next speaker will be Sally Caglioti, also speaking on behalf of America's Blood Centers.

DR. CAGLIOTI: Thank you. I'm going to address the same list of issues that Sue described that we were asked to address today in the context of the America's Blood Centers who were performing the Gen-Probe Chiron and Gen-Probe TMA assay.

First slide, please. This shows a map of the centers that are involved and you can see here that we have three laboratories, in Milwaukee, one in Florida and one in Arizona. The blue dots represent all of the collection sites for whom we perform testing. As others have described the samples are flown in or driven into these three laboratory sites on a daily basis. The three sites work together to develop standard protocols for validation, for procedures, and for training. So

we're all using very common procedures.

Next slide, please. Going through the list of questions as far as routine product release and surveying our sites, of the 56 sites for whom we do testing, 55 claimed to routinely release products based on both serology and NAT results. However, 11 of the 43 sites that surveyed that responded to the question said they did have a policy for exception release based on serology. So I think the definition here is unclear as to how many sites are really releasing all the time based on NAT results and it occurs that it's less than the 55 of the 56.

We did have sites that did -- that are in Phase II that reported some significant product lost during implementation. Twelve of 43 sites that we surveyed reported some significant losses, primarily in platelet pheresis products during the first stages of implementation, when turnaround times were very long.

Next slide. Turnaround times vary. They obviously differ from one lab to another. We have a range of anywhere from 12 to 18 hours and these are means for test result. In other words, time into the lab and time out and then turnaround time from the time of collection until the time of release of the products

is anywhere -- ranges from 20 to 36 hours.

Recipient follow-up is in progress. I told you earlier that we do have six cases, six field cases. Of those, we have two cases where we haven't formed the recipients of the other donors. It is not appropriate to do so, so this is in progress. As far as donor follow-up goes, of the NAT reactives that we have for which we have not been able to confirm by discriminatory, we have about a little over half of the donors who have returned for follow-up.

Next slide. As far as just identifying issues that our hospitals have made us aware of, during the implementation, although we do feel like the implementation went very smoothly. The blood centers and hospitals for whom we provide laboratory services were extremely concerned about the delay and product release for a period of time. Product loss, the shortened expiration dates on platelets, as well as the mixed inventory that Kay mentioned, donor deferral and, obviously, the increase in cost.

Next slide. The laboratories as everyone mentioned, are extremely concerned about the lack of automation. The difficulty in adhering to current good manufacturing practices with the difficulties in process

control with these manual systems.

It was also very difficult in the learning curve for the new technology. We're not familiar with molecular technology in blood centers and this was something that we had to overcome. The logistics of centralized testing was something new. Many of the laboratories who are doing testing for others, this had been their first experience with doing so. And then the complexities of pool testing.

The next couple of slides I just want to elaborate a little bit on. The issue that I talked about this morning which is the issue of false positives which causes a concern for us because these now are people who are deferred, blood donors who are deferred and we have almost 300 of these in our system.

As you can see from this curve, this is a curve by month, the number of false positive incidents that we have had within our laboratory and you can see there's a spike and obviously this represents a learning curve of the test and we are coming down in the number of false positives that we're seeing. But obviously, we need to resolve this issue. It has donor impact. It has product impact. It has cost impact. And we are working very closely with Chiron and Gen-Probe and the

other laboratories to try to evaluate what's causing these.

Next slide. We found in looking at the relative position of these samples in the test system itself that 99 percent of these are caused by some positional effect within the test system, so in 47 of the cases, there was a seropositive right next door to that positive sample. In 15 cases, there was a seropositive sample either in front of or behind in the actual test system. Within the TTU, which is the testing unit, there were 33 and then there were 20 just randomly within that run. So I think that we know the reason for the false positives and we are working very carefully to try to reduce this number. And then lastly, I'll address the issue of availability. All of the Chiron Gen-Probe centers are accepting testing from both hospitals. Of the 56 sites that we test for, 12 of those sites are hospital transfusion services. So I'll close with that.

Thank you.

(Applause.)

DR. MIED: Thank you, Sally. We also have Michael Strong for America's Blood Centers.

DR. STRONG: I also will address the

questions that have been posed to us by the FDA. In terms of markers tested, the Roche sites are currently in transition to HIV. The majority of those sites will be up by the end of this month and we have the two final sites that will up by the middle of January.

In terms of Phase I to Phase II transition, our recent survey demonstrated that we have a majority in Phase II for HCV. We are just beginning the transition to HIV with two laboratories that are up. This is the time line for transition to Phase II by the laboratories that reported. There are still a significant number of labs that haven't determined when they will, in fact, be in Phase II.

Next. In terms of recipient follow-up, so far the ten confirmed donors that we have been all first time donors, so there have been no follow-ups to be done on those. And all of the products have been controls where there have been no release of positive products.

In terms of turnaround time, I mentioned this morning that we have a turnaround time of about 10 to 14 hours for the test. In terms of platelet release with a couple of exceptions, the majority of our laboratories are able to release product within 24 hours with the exception of those where we have a positive

pool and resolution testing has to take place. In that case, worse case seems to be about 72 hours.

Next. In terms of automation, our current system employs the Hamilton 1 AT Plus Pipetter. The COBAS sample core analyzer which has quite a lot of field experience now in Europe and elsewhere in the world, the new AmpliScreen data system which -- data management system which is currently being implemented in the majority of the Roche testing sites with a couple of exceptions is version 1 and the second version is to be implemented shortly.

In terms of desired automation, clearly for us automated sample preparation would be a huge improvement. Currently, it's all done manually on these pools and that's been a problem initially with failed internal controls. And of course, we also, we'd like to see software upgrades that would allow us to make this an integrated system from sample in to data out to connect with all of the different information systems that we currently employ in these different laboratories. There are a multiplicity of different software systems.

Next. In terms of test availability, once again I showed this slide this morning. We have the 13

sites distributed geographically throughout the country. That was one of the selection criteria by ABC for determining test sites. And you can see from the connections here with the colored dots where the collection centers are relative to a test setter. To pick our heartland representative here in Kansas City, you can see they have samples coming from Ohio in the central part of the U.S. and, of course, the further away, the more challenging in the turnaround time and platelet release.

Next. Now finally, I do want to give credit to all of the laboratories that are involved in this process. It's been a tremendous effort to coordinate all of these programs. The cooperation with Roche has been very good and it's been a real challenge to do that. But to give you some idea of where we stand relative to leadership and it's been about a year since that cold night when we first started, we also would like to just show you what the leadership has been like, and also to redefine window cases.

(Laughter.)

We have been all over the map.

(Laughter.)

Thank you.

(Applause.)

DR. MIED: Thank you, Michael, I think.

(Laughter.)

Next we have Chris Healey, speaking on behalf of ABRA.

MR. HEALEY: Thanks, Paul. As you may know, ABRA is the trade association and standard setting organization for the source plasma collection industry and just let me just echo what Celso said that we're proud to be part of the NAT success story. Although ABRA members and plasma collectors, in general, haven't been involved in the process of pursuing INDs for NAT, they nevertheless play an important role in fulfilling the IND requirements. First, collection centers are responsible for ensuring that adequate informed consent is obtained from each donor whose plasma will be tested under an IND. This is no small task when one considers that a single collection center may supply two or more fractionators with INDs that mandate the use of varying informed consent forms.

The same hypothetical center may also supply a non-U.S. fractionated for whom no informed consent is required because NAT may not be -- because NAT may not be considered an investigational test in

that country.

The second way in which plasma collection centers contribute to the success of NAT is by managing the look back process. Collection centers are charged with the responsibility of identifying prior donations from donors that have a positive test result by either serology or NAT. Because the time frames for obtaining test results vary by test, this process becomes more complex with the addition of each new test and NAT is no exception.

The complexity of the look back process is confounded even further by the growing list of events for which look backs are required. From the perspective of source plasma collectors, look backs and informed consent represent the greatest challenges in terms of NAT implementation. However, they also represent the greatest opportunities. ABRA believes that a substantial effort should be undertaken to standardize informed consent forms for NAT. This would simplify the process for collectors, fractionators and regulators alike without in any way impacting source plasma safety or compromising the donor's right to know.

Further, this would permit source plasma intermediates to move more freely between and among

fractionators and to meet market demands in a more timely basis.

Second, in light of the resulting reduction in window periods occasioned by NAT, FDA should begin reconsidering the look back, the current look back periods. Such periods currently range from six months and, in the words of Buzz Lightyear, to infinity and beyond. But with the advent of NAT and the industry 60-day voluntary hold, the question becomes what is an appropriate look back period?

A third implementation issue for the source plasma collectors is how NAT has impacted the administration of the ABRA viral marker rate standard. For those of you who don't know this past year ABRA implemented a revised viral marker rate standard that sets cut off limits and action levels based on the number of confirmed viral market positive donors at each center. Centers that exceed the standard for a single review period of six months must take corrective action and those that exceed it for two periods lose their QPP certification.

This standard was developed using industry-wide data that were collected just as NAT for HCV came into full implementation. In terms of implementing this

standard, NAT has added an unanticipated level of complexity. The NAT data must be aggregated by collection center and reconciled with each center's serology data. The fact that different laboratories perform serology and NAT makes this process even more arduous. Today, the viral marker rate standard has been fully implemented and the first formal six month review period will come to a close at the end of December, this month. ABRA is confident that the NAT results will be fully integrated into this process.

In closing, I'd like to reiterate that the source plasma industry is proud to be part of the NAT implementation process and we look forward to an on-going dialogue regarding our proposals for informed consent and reconsidering look backs. Thanks.

(Applause.)

DR. MIED: Thank you, Chris. And I believe as our final speaker for this session we have a representative of IPPIA.

MR. HEALEY: I think that the representative will be available to respond to any of these questions to be appropriated.

DR. MIED: Okay.

DR. JACOBS: Okay, we've got a period of

time to discuss some of the questions and Joe's going to show you on the overhead. I think as you look at the implementation questions, you'll see that many of these were covered by our speakers. We'd like to go through them briefly and see if we have additional comments on them. And we want to assure you that we've been listening carefully to the various specific comments that were directed to FDA about suggestions for how we are continuing to implement those and some specific ones were made by Dr. Bianco, by Kay Gregory and just now by Mr. Healey.

So looking at the first implementation question, it seems as those most people have talked about the timing from Phase I to Phase II. It seems though from the data from Dr. Strong that we're not sure about full implementation of HIV and I wonder if the different groups could comment specifically on what they think the time line will be for full implementation of HIV and at the same time, do they want to say anything else about the shelf life of platelets in Phase II.

We could start here. Sue or -- did you want to respond to that on full implementation of HIV in Phase II?

DR. STRONG: Okay, I'll start. Since I've

raised this ugly specter. I think the big problem for the Roche sites is software. The dreaded V word, as we say, validation.

Because we have a multiplicity of systems and people are trying to automate as best they can in order to eliminate errors from manual data transfer and to make the implementation easier, they have put off going to Phase II for HIV for the very reasons just mentioned. Platelets are a significant problem.

Also, to some extent, transportation and turnaround time for samples in and out for some centers has been a problem, so I think to be conservative, people have tried to do it in a fashion that doesn't overly extend the availability of blood components.

Obviously, some places have been able to do that more quickly than others and my guess would be that we will probably have everybody in Phase II within about six months. That's my best guess.

DR. CAGLIOTI: I'll just comment on the Chiron Gen-Probe. I think you've seen the data. There's a very high percentage of those that are within our IND that are in Phase II. However, many of them have these exception rules. I think to be 100 percent Phase II, I think it will mean that we'll have a license

test available from the FDA. So I believe that we won't be in 100 percent Phase II product release until we do have a license test of some sort because there are some organizations that just feel like they cannot get their platelets released in a timely manner due to all the complexities of the testing.

DR. GAMMON: I just want to make a comment that AIBC is currently 100 percent in Phase II. We felt that it was necessary to do and we were able to do that from the start. Thank you.

DR. STRAMER: Speaking for the Red Cross, a little bit larger volume in comparison to AIBC. We've been testing in one laboratory and as I've shown you from the number of samples arriving per day, the formidable challenge for us is having additional sites to perform testing. We are currently in process to bring up the second facility, very close to our first facility in order to split our testing into two and hopefully that will decrease our turnaround time, perhaps by half. What we need to do is clearly define what our turnaround time needs to be for labile components to make sure they're available for our consignees and then to go through the process of training and all of our procedures. So we hope that

will happen shortly after the beginning of the year, but I cannot tell you a specific date because we need to have a specific target and then we need our plans for implementation.

DR. JACOBS: Thank you.

DR. MIED: I'd like to go back just a moment to the question of platelet release and direct my question to Richard Gammon and perhaps also Sue Stramer.

Richard, you mentioned that automation problems with -- when you have those problems with the TECAN or the L.A. Block or you have invalid tests or errors which, taken all together, may not be that infrequent and you need to go back and recreate those in process and master pools.

First of all, a logistical question. Are the samples that went into that master pool somehow sequestered so that they can easily be brought back out again to create new in-process and master pool and secondly, if you do have an automation problem or an error or an invalid test, is that it for platelet release because now you're talking about getting into second day and maybe the third day. Under Phase II.

DR. GAMMON: Right. Well, a couple of things. We haven't, to my knowledge, we haven't

released anything. We have not released anything without being fully NAT tested. If there is a problem and we do do -- we have two runs a day, sometimes three runs, morning, afternoon and sometimes evening. So if there would be a problem or a loss of a run or a run would have to be redone it could be done later on that day or the first thing the next morning. So to my knowledge we have not released any components without NAT and serology testing.

DR. MIED: Now if you do have an automation problem or an invalid test or some type of error in performing the NAT test and you're releasing all products, including platelets, based on NAT in addition to serology, you do have to go back and reform those pools. Does that take release of platelets out of the question entirely?

DR. GAMMON: No, not entirely. We've been able to do it. If we can't do it that day, we'd have to do it the next day and you're right, it would cut a day off of the -- it would cut a day off of the release of the components, but the general agreement with the AABC members is to hold off in releasing those components, so the NAT tests are available. Now of course, all the members do have built into place an emergency release

form. If there would be an emergency that the hospital's physician could sign for an emergency release of components not fully tested.

DR. MIED: Sue, has that been a problem for the Red Cross?

DR. STRAMER: Again, we're releasing platelets based on serology, but to address the question of invalids and what happens. Firstly, as pools are created they're stored with their constituent donations. So if there was a reactive pool the individual donations are immediately accessible. If repooling needs to be done, let me say the only reason we would do repooling other than an inadvertent accident which hasn't happened, is because we do gravimetric checks in our pools and if they're not within the correct weight range, then the pool is considered invalid and then the samples will be repooled. But once the pooling is done and the pooled samples move to testing, if we have an invalid, those are added on immediately the next run. So they really shouldn't be a problem for platelets.

Let me say this also, and I don't know how logistically possible it is, we don't make platelets from each collection. So clearly there are other ways to overcome the issues of the small number of invalid

runs we've had, etcetera.

Dr. Tabor has a question.

DR. TABOR: Could we try to nail down the answer to that question a bit more concretely? Is AIBC already in Phase II for both HCV and HIV? Is the Red Cross hoping to have Phase II in place early next year for both HCV and HIV? And is ABC not going to have Phase II completely for both HVC and HIV until there's a licensed test?

DR. GAMMON: Okay, AIBC is in Phase II. We've been in Phase II since we began testing on April 1st and we test for HIV and HCV.

DR. STRAMER: The Red Cross tests all consenting donors for HCV and HIV. However, we're still in Phase I. A time line for Phase II has not yet been completely developed for implementation. We hope to do that, let me say, perhaps by first quarter.

DR. TABOR: You're referring to both tests?

DR. STRAMER: Yes. We do both tests because we run the Multiplex test, so we don't have a choice.

DR. TABOR: Okay.

DR. STRAMER: But we again, don't have a definite time line. It is our goal to be in Phase II,

obviously, as quickly as possible.

DR. TABOR: And ABC -- did I understand that correctly?

DR. BIANCO: You heard correctly two different answers and it's -- I think that what we have -- Mike Strong not too long ago said we are all in Phase II on a good day.

(Laughter.)

We have to recognize that those systems were put together as -- they were not final tests that a manufacturer had put together, had tested internally and all that. Those things have been evolving. And particularly, I think there are two major issues that we still have to resolve. One is logistics. If you saw the --

DR. TABOR: Celso, excuse me, I mean the reasons -- we've heard the list of reasons. I'm just trying to nail down the numbers.

DR. BIANCO: The numbers will come as we improve this software for the Roche systems and as the logistic issues are resolved for centers that have to travel a lot to get there. And so I believe that the prediction that Mike made of six months to get there is correct. I think that what Sally was trying to tell us

is that the inducement of a licensure would be a great motive for everybody to be in Phase II.

DR. TABOR: Obviously, if you were forced to do it, you have to do it. But that almost leads right into one of the other questions. We've now talked about 99 percent of the supply. Are the tests available to everyone and to what extent can someone who is not a part of your system obtain testing?

DR. BIANCO: To my knowledge, and I think I can respond the same for all the organizations that are sitting here, the tests have been available to everyone and at least, for instance, the example that I have in New York, hospitals that collect and have not used the system is because they did not finish going through their IRB or they didn't have enough encouragement to just move fast enough.

DR. TABOR: We still get letters or at least some letters at FDA from small outfits complaining that they are at a competitive disadvantage. Should we just send them the addresses of the large IND holders? That's good because that's what we've been doing.

DR. BIANCO: Definitely.

(Laughter.)

DR. JACOBS: We asked the question: has

contamination been an issue? I think we've had speakers address that, so I think now we'd like to ask the question what have you learned from looking at your data about contamination or other types of errors, and could you briefly comment on any changes in procedures, etcetera, and Sally, could you speak first?

DR. CAGLIOTI: I'm the ring leader of this. I think it's real clear, at least in the system that we use and I think the Red Cross has come to find since they've started to include seropositives in the pools and in the test system itself, but the contamination is coming at the individual test level within the test system within the run. We don't seem to get pools contaminating other pools and we don't seem to see a lot of pooling contamination of sample to sample within the pooling system. This all happens intra-run. So I think what we need to do is look at ways to separate the seropositive samples when we're doing the resolution testing, i.e., when we find a positive pool, perhaps look at the seroreactive samples and take those out of the run. So yes, we are looking for ways to try to reduce that. But it is an issue and it seems to only be occurring with the individual sample level, not within the pooling system itself which is I think what we were

concerned about before we started testing.

DR. JACOBS: Thanks. Sue?

DR. BIANCO: Actually, Mary Beth, just a comment. If we compare to the predictions that we had in the early days of NAT testing, we were very scared and actually I think that every one of these systems in the centers took a large number of precautions in terms of separating environments and engineering and all that, that have prevented those disasters from occurring.

DR. STRAMER: I'd like to reiterate what Celso said. As I presented this morning, our pool reactive rate or initially reactive rate for our pre-Phase II is only .17 percent. And that's comparable to the lowest test we do today for serology. So our initial rates are very low. Our repeat rates, that is, rates now to a single donation level, are only 1 in 30,000. That is clearly lower than any serological test we do and I'd like to think it is because as Celso also said, we've taken the precaution to really isolate and have a linear work flow. But since we -- the challenge did come when we included seroreactives. So until we're in a Phase II program, what we are doing now is obtaining the serological results from the NTLs and segregating, removing the seroreactives, testing them

singly, not even bothering to pool them and we've isolated those on isolated runs. So clearly, separation of seroreactives out of the pooling and during resolution also will help prevent contamination.

DR. JACOBS: Good, thanks very much. And I wonder if either Dr. Gammon or Dr. Strong would want to comment on that?

DR. STRONG: I'd be happy to. One of the things that we've learned is that you have to be careful when you walk across a room with a plateful of samples, that you don't trip and drop them and cross contaminate that you've got everything in your hands. I think a second thing is automation is clearly going to be a big improvement to this process. Any kind of manual steps, any kind of manual pipepetting will increase the chance of contamination. I think that a lot of these contamination aspects have been because of pipepetting problems. The texts have gotten very good, to be more careful, and that the learning curve has clearly been important in that. So there's still a lot to be learned, but the more we move toward automation, I think the better off we're going to be.

DR. GAMMON: And I just wanted to emphasize that I think minimizing contamination, a proper training

of staff. We have a unidirectional work flow and strictly adhere to that, when we have doors marked "do not enter", move in one direction and strict adherence to the manufacturer's insert all help to minimize contamination issues.

DR. MIED: Chris, you asked a question what is an appropriate look back period. I think we heard from Mike Busch this morning about his experience with look back. I'd like to direct this question, first of all, to you, Chris and then secondly, maybe to Celso and Sue.

Going back to prior collections, how far back are you going, how many cases have you done and what have you found?

MR. HEALEY: Paul, I don't have any direct data on the INDs and perhaps others in the audience can comment on that, but I think you heard from Chuck Heldebrant and Alpha this morning that 100 percent of all implicated units were retrieved under the industry 60-day hold and we've rolled that data out before, that the 60-day hold really affords an adequate opportunity to hold back all those implicated units with the NAT and the shortened window period makes that hold even more robust, so we're really able to get 100 percent of all

the implicated units. I hope that addresses what you're asking.

DR. MIED: Now there's prior collections. Some had not been, perhaps, previously NAT tested. Have you found NAT positives among those?

MR. HEALEY: I'm not sure I understand your question. The look back units, in other words, have they not been NAT tested? I can't answer that. I don't know what the individual IND holders have done with the units they have retrieved, whether they've gone back and eventually performed that on those, and what the results are.

DR. MIED: All right, Celso and Sue, how far back are you going, and how many cases do you have, and what have you found?

DR. STRAMER: Okay, I can go first. First, the IND, the simplest approach we took was to take what the FDA is already using, so for look back for HIV we used p24 antigen, since NAT is a marker of virus and so is p24 antigen. So we do look back for three months.

For HCV, we do look back for a year. That's the current guidance, correct me if I'm wrong, but that we are just applying what the FDA has already chosen as the most logical method.

From our 12 cases that I discussed, our index cases, our yield cases, six were from repeat donors. I don't have memorized all the collection dates, but interestingly enough, most of them did not fall into the look back period. Of the one that did, we were, knock on whatever, it was incredible luck that we could retrieve that plasma, that the plasma was sitting on the hospital shelf. We could call the hospital, get the plasma and have it tested. And that was 60 days prior to the index NAT reactive unit and that tested stone cold negative on every test we could throw at it, although there is a red cell recipient and that red cell recipient will be notified to be tested with encouragement with the consignee giving them the plasma results so that that recipient shouldn't be unduly alarmed. We had additional data that they're likely not infected.

DR. BIANCO: Yes, I don't know exactly all our numbers, but I believe whatever they are, they're very small. And I don't think that they have been published at the present time. We are using the same algorithm for HIV these three months, or eight weeks actually, use the p24. Algorithm for HCV, we are doing one year. I think there is a big difference between

what is done with source plasma versus our donors. Our donors come at bigger intervals, and so the look back has less meaning. Within the source plasma, the donor comes regularly, the plasma is there and there is -- they can collect the data and they can hold back those units more effectively. Now I don't think that we are ready in whole blood collection, at least I'm not ready to accept a certain look back period except for the numbers that Mike was showing us and that were, in essence, faulty.

DR. JACOBS: I think we could take just a few questions from the floor if there are any. While we're waiting to see if someone comes to the microphone, I'd like to thank all of our speakers. I think it's been a very interesting session and we appreciate your directly dealing with the questions that people are concerned about and I think we've gotten some very clear information. So if there's anyone who would like to raise an additional question, if you'd go to the microphone.

Well, I guess you've maybe anticipated -- is this someone coming? Well, he's going to a chair. Okay.

Please identify yourself when you ask the

question.

MR. SHERMAN: I'm Larry Sherman from the CAP. I would echo Kay's hospital perspective and from that standpoint ask if there have been any blood centers who have exchanged post-NAT implementation frozen product for pre-NAT product still out on shelves?

DR. CAGLIOTI: Larry, yes, Blood Systems did that.

MR. SHERMAN: Blood Systems has done that. Anybody else? The reason I ask is from a hospital perspective and patient perspective, it's intriguing. The hospital, let's say a patient is transfused right now, that wasn't done, that patient gets red cells and plasma will get tested -- red cells for which they pay, but the FFP from that donation for which they have paid for the testing probably is going to Europe for fractionation and the FFP that they've received was probably drawn by spring or winter and wasn't NAT tested.

DR. BIANCO: I'd like to make just one comment, Larry. We all did that with p24 antigen. This was a licensed test. We knew the outcome. We knew what was going to happen. In this case, we are in a IND mode and while the results here are very encouraging, I think

that we are all --

MR. SHERMAN: I agree fully and I agree NAT should be tested, but I'm simply saying that patient paid for the whole nine yards to be tested.

MR. TEGMEIER: Gary Tegmeier, Community Blood Center, Kansas City.

Sue, I have a question for you. You indicated that Red Cross is testing for HIV and for HCV for all consenting donors. I wondered what percentage of donor who are declining to be tested?

DR. STRAMER: From data we collected when we first implemented, it varied from one to two percent, but current estimates are probably one percent or less.

MR. TEGMEIER: So are you discarding the blood from donors who do not consent to be tested?

DR. STRAMER: Currently, we don't, but very shortly we will go to a policy of, if you consent to donate, you consent to the test and then everything we collect will be tested other than autologous.

MR. TEGMEIER: Thanks.

DR. JACOBS: Thank you again to our speakers.

(Applause.)

DR. TABOR: The next session will be on NAT

testing for Parvovirus B19. The moderators are Dr. Mei-Ying Yu from the Division of Hematology at CBER and Dr. Thomas Lynch, also from the Division of Hematology. The first speaker is Dr. Thomas Lynch.

DR. YU: I just want to introduce to you, Dr. -- probably everybody knows about him, but anyway, Dr. Thomas Lynch is our Deputy Director of Division of Hematology, CBER.

DR. LYNCH: Thanks, Mei-Ying. Good afternoon, everyone. This session actually raises an interesting contrast to some of the prior sessions in that we're now talking about a virus that is generally less clinically significant than viruses such as hepatitis C, but have a far greater prevalence in the general population and among donors.

As you may know -- the first slide, please -- as you may know, the Agency in considering NAT testing for B19, recently reached the conclusion that insofar as testing plasma for further manufacturing went, there would be no need to demonstrate the clinical effectiveness of such tests, i.e., no need for clinical trials under INDs as are being conducted for the more significant viruses.

We thought instead the testing could be

implemented as an in-process control and validated as an analytical method and that we could control the quality of such testing and the impact to the products through the licensing mechanism. Well, this left us with a need to establish some reasonable and objective standards for evaluating license applications covering nucleic acid testing for B19 and we've gotten together a discussion group among those who have been involved in the NAT testing for the other viruses, within the Agency, to come up with some guidelines in that respect.

Next slide. What I want to focus my remarks on today are aspects of the clinical, pre-clinical assay evaluation. There are other aspects to licensing a nucleic acid test such as facilities issues, control of your reagents, sample handling, shipping, storage and so forth. Those, I think, are all very much generic GNP issues that will apply across the board whether the nucleic acid test is being performed for hepatitis C or B19.

The clinical evaluation is out, as I mentioned, and this leaves us with the sequence of events that take place in order to evaluate the performance of the assay. Our internal conclusions are that analytical specificity, analytical sensitivity and

the precision of the assay to include reproducibility and proficiency of the testing lab should be performed as our current recommendations suggest.

However, the determination of what for a better term I've called preclinical specificity which in other types of tests involve the testing of random, healthy donors, and preclinical sensitivity which in current applications involve the testing of a certain number of known positives, need to be modified in the context of B19 screening.

Next slide. The reason for this is that NAT for B19 focuses not on the donor and the donor's clinical status, but on an effort to cap the contamination of manufacturing pools from which plasma derivatives are manufactured. And therefore, whatever objective standard is set for the performance of such a test should focus on the manufacturing pool. So therefore, as a first suggestion, we have proposed that whatever testing is done should be capable of capping the B19 DNA contamination of the manufacturing pools to less than 10^4 genome equivalence per mL and everything else will key off of this. So this is a central proposal.

Now we feel that that is a meaningfully low

number and there is some support from that, from the Vitex experience which I think we'll hear more about in a moment and the number of positives that will have to be rejected should be manageable. It should not paralyze the testing and release process for plasma.

With regard to testing for specificity, there's two components to this testing. One focuses on the familiar, random plasma donations and the point, of course, is to -- the expectation is that most of these will be negative, but when a positive is obtained that result should be confirmed with both repeat testing in duplicate and a confirmatory test, preferably one that involves the use of different primers targeted to a different region of the genome.

The second phase and finally, the confirmed positive should be titered in order to determine what the test actually picked up. The second phase would be testing of 100,000 plasma donations in the form of minipools. Now I'm making an assumption here, a reasonable assumption, that most testing initially will be done in minipools, much like it is for the other viruses. Any positives that are obtained should be confirmed by, again, repeat testing in duplicate and the use of an appropriate confirmatory test.

The sponsor should then trace the positive reaction back to the individual positive unit. Now this proposal is not being made for the implementation of an approved test, but identifying the positive unit was considered to be an essential part of validating the assay. So during the validation phase we think the individual unit should be identified and then the titer of the positive minipool and the individual donation should be determined.

Next slide. For sensitivity, there are basically three things: testing of 20 known positive donations. It's a small number, but we recognize that the availability of known positive units may differ from sponsor to sponsor and determining the titer of each that is tested.

The second phase overlaps perfectly with the proposal under specificity, testing of the 100,000 donations in minipools, confirming any positives, tracing the positive result back to an individual donation and determining the titers of the minipools and the donations. And finally, as the gold standard, all of the manufacturing pools that result from plasma that has been tested through the minipool process, should be tested and determined to be, in fact, less than 10^4

genome equivalence per mL.

We're bringing these thoughts and I want to emphasize that they've gone no further beyond that, these thoughts to you, in order to get feedback on the practicality of the proposals and the effectiveness of them as standards for judging license applications for B19 NAT testing.

I think I'll finish there. The first outside speaker is Aris Lazo, Dr. Aris Lazo from VI Technologies and this will start a series of talks that will describe actual real world experience with Parvovirus B19 and its testing.

Dr. Lazo?

DR. LAZO: Well, thank you very much and I'd like to thank the organizer for giving me the opportunity to be here and show our data from implementation of B19 testing in PLAS + SD.

Sorry, I went too far. I'm going to focus on the implementation of B19, testing for PLAS + SD by the polymerase chain reaction. However, I just want to mention very briefly that we have implemented hepatitis A testing for final product and we submitted supplement to our PLA to the FDA and was approved. On the label claim it states PLAS + SD tested by hepatitis A by PCR

contains no greater than 9.4 genomes equivalence with 95 percent confidence interval of 6.2 and 12.6. genomes equivalent per mL.

This testing has been done by NGI.

As you know, we have an on-going Phase 4 clinical trials and so far we have transfused 77 healthy volunteers with one unit each and there are 14 PLAS + SD lots that have been involved. We have no evidence of HAV transmission. We have 18 seroconversions for B19. All of them are associated with three lots of PLAS + SD that contains PCR titers greater than 10^7. Lots with B19 titers less than 10^4 have no volunteers transmitted with those lots has not seroconverted.

Therefore, Vitex decided to take the action to screen for B19. The available methods for testing antigen and antivirus are not appropriate, therefore, we decided to develop, implement and validate the CR testing for B19.

The testing is done at two levels. It's done at the minipool level and it's done also at the final product. Our manufacturing pool contains no greater than 2500 units of plasma. The plasma comes in cases containing 20 units each. Therefore, we formed the primary pools from each case containing the 20 units

from the pigtail from each of those units. And then from five cases containing 20 units each, we formed what we call the minipool which contains 100 units. Our first round of testing is testing the 100 units minipool. If that's negative, it goes into the manufacture. If it is positive, then we go and test the five primary pools. We discard the positive primary pool that contained the 20 units.

We have tested so far approximately 14,000 primary pools and we have found 342 of those 14,000 to be positive. If we assume one positive unit per primary pool, that will be the equivalent to 1 in 800 and this correlates with some of the previously published prevalence studies.

Once we have formed the minipool our PCR consists of two pair of primers. We use an internal control called PPV which is a porcine Parvovirus very similar to the B19. We spike the porcine parvo at the very beginning so it controls for extraction, amplification and detection, and the extraction is manual. We use a Kiagene kit and then we proceed to amplification and finally detection using gel electrophoresis.

I just want to make a point in here that

the number one lane is a negative sample. The number two lane is a negative sample containing the internal control and lane number three is a positive sample for B19 and contains also the internal control.

We also run blanks and we run a negative control every five samples in order to control for close contamination.

Once we develop the PCR we proceed to validation and I just want to validate the assay and I just want to mention we did this before the guidelines that we are discussing were available. What we did was for -- we tested -- we studied really about at least four parameters that included limited detection, interference, the specificity, and precision. The for the limited detection we used the National Institute Biological standards working reagent that was kindly provided by Dr. John Saldanha. The reason we used this reagent was because if an international standard is developed, then it will be very easy for us to calibrate this reagent against the international standard and assign units and then we know what the true sensitivity of this assay will be.

For that, we did four series of dilutions a half log apart and each series contained ten replicates.

Therefore, we tested 40 samples for all the -- for each dilution. And the window of the limited detection is the dilution in which 95 percent of the samples are positive. And in this case will be the working reagent diluted 10^{-1}.

Based on the same serial dilutions, we determined what is called the endpoint dilution or the ED50 and that what it tells you really is the dilution in which 50 percent of the samples are positive. In this case, using the same reagent will be the 10^{-2}. If we assume a Poisson distribution, then we will be able to -- we were able to calculate the genomes equivalence for mL in these samples and therefore our assay has a medium estimate of 81 copies of genomes equivalence sensitivity with an upper and lower 95 confidence of 49 and 135. I must say that these calculations were done based on a paper by Dr. Alan Heath which is the statistician from the National Institute of Biological Standards and Controls.

Then we proceed to determine the specificity of the assay and for that we spike our sample with pathogens that include viruses and bacteria. And then in one of the samples, in one of the replicates that we did, we spiked all the pathogens

except the B19 and then we amplified as normal. We didn't see any amplification. Therefore, those primers for B19 are very specific and they do not cross react with any of the other pathogens. Then we spike the pathogens plus B19 diluted 1 to 10. As you have seen before, 1 to 10 is very close to the limit of detection of the assay. And we amplify and we detected the five replicates 505. Therefore, these pathogens do not interfere with our PCR assay. Similar results would have been with the bacteria.

Interference was determined at the level of indigenous substances like hemoglobin, bilirubin, lipids, anticoagulants and nucleoside analogs. All the substances were spiked into the plasma and then we proceeded to do the amplification. As you can see, there is no interference.

Finally, we did assay precision at two levels, repeatability and intermediate precision. Again, we used the NIBSC reagent, diluted 1 to 10 which again is very close to the limit of detection. We got 100 percent positive results. Also, we studied the intermediate precision which include different operators, different days, different lots, Tax polymerase, extraction kits and again we didn't see any

effect.

In conclusion, I can say that we have developed and implemented PCR for hepatitis A and B19 that is cleaning of the plasma for HIV and B19 reduces or eliminates transmission of B19 and hepatitis A. And transmission of B19 by unscreened blood products are likely, of course, more frequently than we recognize. These are the people who have participated in this work and that's all. Thank you very much.

(Applause.)

DR. YU: Thank you, Dr. Lazo. Our next speaker is Dr. Thomas Weimer from Centeon Pharma.

DR. WEIMER: Thank you. I'm going to present pilot study results which were obtained in our Marburg location. Why is B19 an NAT target for plasma manufacture? It's a self-limiting infection with only a few clinical consequences. But there are certain risk groups like pregnant women and immuno-compromised patients who receive plasma derived products and there have been reported B19 transmissions for coagulation factors. In addition, this virus can reach tremendous virus concentrations in the acute viremic phase up to 14 logs per milliliter. And in addition, it's pretty resistant to physical-chemical methods for virus

inactivation and in addition, it's highly prevalent in plasma donations.

So in our first attempt to estimate what we would expect if we wanted to start screening for B19, we looked at about 53,000 donations with a very sensitive PCO, showing that 1 in 835 donations was PCR reactive. Most of those positives were at low or moderate B19 levels which is less than 10^7 genomes per mL. Those genomes refer to the NIBSC 97542 standard.

About 1 in 10,000 donations contain titers of greater than 10^7 genomes per mL, up to 10^{13}.

Due to the prevalence our B19 PCR screening targets the removal of those high titered donations and the detection and removal of such donations from further manufacturing will prohibit 9 logs or more from entering manufacturing pools.

What we do, we call high titer screening which is a PCR of defined limited sensitivity which was designed to detect and remove B19 positive donations, was greater than 10^7 genomes per mL. This screening is integrated into the current minipool testing procedures and the potential B19 peak load of fractionation pools will be reduced to less than 10^6 genomes per mL.

Next slide. We have done two pilot

studies, one in IND and one in the quality control department in Marburg which tested part of the plasma fractionated at that location. They came to pretty similar result of frequency of around 1 per 10,000 donations with a high titer donation.

Well, this results in fractionation pools and we compare 30 pools which were from B19 untested plasma with over 100 fractionation pools which were made from B19 PCR pre-screened plasma and if you look at your proposed limit, there are a couple of pools which would be at or slightly above your proposed limit. What we can see is that the peak virus loads, they disappear. The average goes down and there are more completely negative pools found after pre-screen. Next slide, please. We looked at the product and the product was antithrombin III due to the fact that it could be managed from production logistics and that the product is going to be used in a clinical trial which includes pregnant women. We compared 12 lots made from untested plasma with six lots of antithrombin III which were made exclusively from pretested plasma, from plasma pools you just saw. What we knew from validation studies was that the cumulative B19 removal factor in the manufacturing process for the ATIII is about ten

lots. Here are the results where eight out of those 12 lots made from untested plasma contained B19 viral DNA up to about 2.5 lots, I think, whereas all those six lots made from pretested plasma were PCR nonreactive. So this indicates that the virus reduction capacity of this manufacturing process in conjunction with the high titer screening for B19 results in an unreactive final product.

Now this is specific for ATIII. This may look like different for other products and we may only be able to look at those products once we have implemented our full B19 testing for all our plasma.

Final slide, please. So we feel that B19 high titer screening is a feasible approach to screen for B19 contaminated units. It will remove plasma units with high levels of B19 from manufacturing and it will significantly decrease peak virus loads of fractionation pools and the removal capacity is nine to 17 lots of virals. It compliments the current viral removal steps and it considerably reduces the virus burden in final products.

Thanks for your attention.

(Applause.)

DR. YU: Thank you, Mr. Weimer. Our next

speaker is Dr. Gerold Zerlauth from Baxter Hyland Immuno.

DR. ZERLAUTH: Ladies and gentlemen, if you have to commute to downtown Washington every day, you may not choose a Formula 1 racing car and if you have to screen plasma for the presence of Parvovirus, you may not think of PCR in the first moment because that assay has been developed to detect very few viruses in rare incidents.

Parvovirus, as we have seen, is present in high numbers and in many samples and you have to make provisions that you're not overwhelmed with work if you run a PCR on such samples. As we have seen, there are reasons why we should introduce screening, namely for the transmissibility that can be detected in plasma derivatives and mainly there is no reliable test and so we have to take what we have and that could be PCR or NAT.

We have seen that prevalence of very high titer samples is fairly high, 1 to 10,000. We know that individual donations can go up to 10^{13} per milliliter and overall -- one more, please -- and we know that pooled donations and Dr. Weimer has just show they can go up to the 10^{8} or 10^{9} equivalence per milliliter.

Next, please. So we decided to go with PCR for screening of Parvovirus and we wanted to detect and eliminate high viral load donations. We wanted to show that there is no impact on antibody distribution and we did not want to have any donor related follow up due to the short period of time of the viremic phase.

Next, please. In mid-1998, we developed a quantitative PCR assay system based on TaqMan and we validated that, of course, and started a limited study to evaluate a suitable cut off level to achieve this goal. I have shown these slides previously. We found this comprises about one million donations and we found that up, almost 60 percent on nonreactive in this test. This is a very sensitive test and has a cut off limit of 95 percent at about 10 to 15 genome equivalence per milliliter.

We found that another 35 percent are very low loaded with virus and only about four percent of the pools were higher than 10^6 per milliliter.

This is how the production pools look like in the same assay and you see that we do have up to the 10^9 of genome equivalence in production pools if they are made from unscreened material.

Next please. This is just a list of

results that we have got in tabular form. I'm sorry, thank you. This is from 1997 when we started looking into this business up to May 1999. We have tested 773 of our production pools in the scheme and as you can see 25 of them were nonreactive in our system and about 20 percent of the pools were higher than 10^6 per milliliter.

Go on, please. If you look at that 10^6 which is four percent, it's four percent, as I said, and that four percent causes 20 percent of our production pools to be higher than 10^6.

Just go ahead. One more. Now we had to design a parvo screening program which had the goal to limit the maximum parvo load of production pool at the level of the 10^4 genome equivalence per milliliter and the procedure that we designed was, as I said, to detect high load minipools in the first round. We needed a suitable cut off and that was 10^6, we decided, and we wanted to deconstruct the high load minipools to retrieve the positive donations in the second round. That spells that we needed a highest throughput system because four percent causes a lot of resolution of PCR, several thousand PCRs needed to resolve a single donation. And the program we designed was that we

intercept Parvovirus minipools at the level of 10^6 genome equivalent from January 1, 1999, deconstruction them with a newly design specifically for the purpose resolution PCR which is qualitative and has a limited sensitivity and we remove the implicated donations.

These are the combined results. Around the beginning of this year we started a program, intercepted the 10^6 genome equivalence per milliliter in minipools and removed individual donations. As you can see, there was all this plasma around that gave rise to spikes like this. But from May of this year, we now have a complete program to remove all donations that are contained in such pools and as you can see, we can now nicely reduce all of our production pools below the level of 10^4, actually 10^3 is the common level.

One more, please. We can achieve a six log reduction of our manufacturing pools versus the nontested material. We also asked the question whether the antibodies are influenced, the antibody level and this line should illustrate to you that the antiviral B19 antibody content is not affected by positivity regardless of level or negativity. So if we clean out the high load parvo donations, we do not influence the antibody level of our production pools.

So in conclusion, I think we can achieve a six log reduction of Parvovirus by using a PCR in an appropriate manner. We can now give the issue further to the people who develop virus inactivation removal steps because they have now a much better point to start with because we are only introducing up to 10^4 genome equivalence instead of 10^8 or 10^9. And finally, I think PCR shows to be one step to reduce parvo related risk factors.

Thank you for your attention.

(Applause.)

DR. LYNCH: Thank you, Gerald. The last speaker in this session is Dr. Andrew Conrad from NGI.

DR. CONRAD: Thanks. What I'm going to do is sort of change gears a little bit and do parvo a different way. Instead of looking at what happened in pools, we recently decided to go look what happens in donors because the question was how big a deal is a parvo positive donor? How long do they last and what do we do? So we began sort of a prospective study looking at following donors from a parvo positive.

If I can have the first slide? All right, so basically what we're going to do is use the same algorithms that you've seen described earlier to take

master pools, test them for parvo and then resolve them out to the individual donor. What we would do is go take that donor and go back and look into our archives because we see every sample that's ever been tested at NGI and we went back and looked backwards and forwards to see if we could look at the parvo history of a donor. What we wanted to do then is determine (1) parvo antibodies, how those antibodies affected viremia and (2) look at the DNA levels over time.

So prospectively so far we've been able to identify these in the last couple of days that we've been doing this, these seven donors. What we have is donor 1 just started, but donor 2, the blue indicates he was negative. The red indicates other time periods that he was PCR positive and this is over weeks. And so you can see that this guy is donating rather frequently and if he's one of those big time viremia guys, he's nuking an awful lot of pools. So these are the donation frequencies.

What we did was we took those original pools and by quantitating those pools that we could model what we suspected a parvo infection to look like and what we modeled was that there would be a rapid increase, a rapid decrease and then a slow and steady

decline. The curve of that decline we've postulated would take a long period of time. We originally thought three months, six months and now it's looking longer. Once we got these initial pieces of data we could actually go to the original donors and we quantitated that donor that you just saw. It was negative for two different donations. Within six days or seven days, actually, he jumped to a level above 50 million where our quantitative assay can't read. Four days later, he was back down to that 50 million level. Then we had a big lapse of a couple of weeks and he had dropped 10,000 where he has stayed now for months. So what we think is going to happen, is the interesting thing about the way is parvo is going to be treated in an individual donor. Now we've looked at these profiles, we just didn't have the original nonviremic one and most of the other donors that we encountered that were nonviremic already are really in this medium level and it's only probably 1 in 5 or 1 in 6 that you catch in the little peak.

So what we really do think is that the most effective method is to just clip the head off the beast because if you start trying to defer donors who have parvo infections that may not work because they're going to be long term positives. So you have to be careful in

trying to use them in resolution algorithms because if you get rid of everyone with low level parvo, you may exclude tremendous numbers of donors and you might keep them excluded. So it's not something that you can wait out the storm for a short period of time which we originally thought we could do, so that's it.

(Applause.)

DR. LYNCH: Thank you, Dr. Conrad. We have a discussion period now. We are running a little bit late, but I think we have about 15 minutes. If there are any issues from the floor, we could take those.

While we're waiting for questions, I'll -- John? Please identify yourself when you ask a question.

DR. SALDANHA: John Saldanha from NIBSC. I was very interested in the data that were presented because it seems like people were moving towards removing pools that have greater than 10^6 genome equivalence and all I'd like to say is we should try and harmonize what we're going to do because I think in Europe the Biotech Working Party in a couple of months is going to look at the whole issue of B19, the introduction. And I was interested, Dr. Lynch, in your presentation on your thoughts on the B19 and maybe we can try to get some harmonization going on the

introduction of testing.

DR. LYNCH: I think that's always a good idea and we'd be happy to get the thoughts of the CPMB on any of these issues.

DR. ALLAIN. J-P Allain, University of Cambridge, England. I have a question for Dr. Weimer. You showed that you had a sort of cut off at 10^7 geno per mL. I was wondering how you devised that particular cut off. Is it because you have any kind of data about the infectious dose of Parvovirus or is it because when you have a low virus load you count on the antibody present in the product to neutralize it or how did you come up with this particular figure?

DR. WEIMER: It was for very practical reason. This is the concentration at which gives us prevalence of 1 in 18,000 and that's the number that we can handle in our pooling system. If we go to lower figures, we will get too much positives and this needs too much work for resolution. And the higher the sensitivities, the more you get into cross contamination problems and I think for B19, the opposite is true then we heard before. The more you go into automation, the more you run into problems with cross contaminations due to the very high titers.

DR. ALLAIN: But I must say that is not quite satisfactory. You would like to know what's infectious and what's not to direct what kind of screening and how you can really set it up from a clinical standpoint.

DR. WEIMER: Our main focus was to reduce the virus loads into pools in the final product and this is the first step towards this goal.

MR. BABLAK: Jason Bablak with IPPIA. I have a question for Dr. Lynch. In your presentation to begin this session you showed a cut off level of 10^4 that the FDA was thinking about using and in some of the presentations by the companies they were somewhat higher and it showed that there was probably no Parvovirus in the resulting pools or resulting products. I was wondering if you could explain how the FDA came up with that number and if that might be a little conservative based on some of the manufacturing data we just saw.

DR. LYNCH: No, I think it's intentionally conservative. Let's be clear that we're talking about manufacturing pools when we're applying any sort of a numeric standard. You might achieve that by a higher level of acceptability in the minipools because presumably there's a Poisson distribution in the titers

of viremia in all of the minipools and you're diluting out to some extent the ones that may be higher than your manufacturing pools, so for instance, Dr. Weimer's data and also Dr. Zerlauth's data showed that testing with a relatively low sensitivity test, capping the minipools at a higher level actually resulted in manufacturing pools with a substantially lower titer than that.

Now with regard to the product, that of course is what everybody is interested in, but the plasma is only the starting point. As you know, the impact of this testing on any given product will depend on the process by which it's manufactured. I would imagine that for a product that's subjected to very limited purification, let's say the solvent detergent plasma, there's not going to be much removal of B19 from the starting pool. On the other hand, there's some very highly purified preparations out there where there may be a greater capacity for clearing residual virus in the plasma.

I don't have a firm proposal for that. One possibility would be to address any claims to final product safety on a case by case basis, but that's an issue that we need to visit in the future. The 10^4 number is partially from a desire to seek the lowest

reasonably achievable number. What we expected to see as a prevalence of minipools or manufacturing pools above that level and how much plasma would have to be rejected to achieve that level, and finally, sort of a Gestalt from the SD plasma experience that suggested that plasma at that level of B19 contamination may not be infectious, i.e., there must be low enough levels of virus to be neutralized by whatever antibodies exist in the plasma and maybe that will influence the segregation or partitioning of the virus during manufacturing. So that's basically the thinking that went into the number.

I'd actually like to ask either or both doctors Weimer or Zerlauth what a testing at 10^4 for the targeting 10^4 in the manufacturing pools implies for practical implementation of this test. It seems like in one context it was suggested that it was readily achievable and in the other that there might be problems in having to deconstruct too many pools.

DR. WEIMER: I think first of all we need to come to a common standard that we can talk in the same language of genome equivalence. I think this is still like in the early days of HCV testing and I don't know how all our numbers compare at the lower end. I think his 10^4 and 10^7 somehow complicated just reusing

the same standard. But I don't know how all other numbers to compare to them. So I would be a little bit cautious in that respect.

DR. ZERLAUTH: With respect to the numbers, we are using the same standard, the NIBSC standard, so I think they are quite comparative to these numbers.

With respect to feasibility, that certainly -- if you really introduced into the 10^4, you need to resolve high number of pools and that causes a lot of extra work. That eats up to ten percent of our current capacity, so that's not nothing. We have to develop a fully automated system. That's all done by robots. We don't touch those samples any more and therefore we can do it, but it's not an easy task and I think we have to go different ways as before, but it can be done and that system actually it's nothing special in it, it can be bought off the shelf and adding a few issues or items and then you can use it. So actually I think we will come to the same conclusion.

And once John Saldanha comes forward with his parvo WH approach, then we certainly can compare the numbers exactly. So overall, it seems to be feasible, but it is not an easy task. It's a lot of extra PCR to be done.

DR. CONRAD: I just want to say one thing. One of the tricks that I don't think people are fully using is that if you identify parvo positive donors, exclude them temporarily from the pools or shuffle them because the guy is high and then he comes low, so there may be ways to do with wisdom instead of just brute force of the PCRing the hell out of everything. I can't believe I'm saying that, but there may be ways with temporary donor exclusions that may allow us to lower those viral burdens in any manufacturing pool without having to test every sample.

DR. ZERLAUTH: But you don't have to exclude the donor. You just can't sort of intercept the donation. If you are fast enough, you can avoid the next donation.

DR. CONRAD: Well, the next three donations. When you learn the temporal distribution of the parvo, there may be ways at which to say okay, this guy is positive, let's wait whatever amount of time before you start reintroducing his plasma or you know, multiple units of his plasma and that way we can effectively without having to screen consistently and resolve the guys down at the 10^4, limit a few of the peaky guys and that will keep the viral titers below

10^4.

I think the people resolve that, the donors resolve that and there are long term cases I think around 10^4 copies at the individual level. So pools will automatically be diluted down more.

DR. ZERLAUTH: You're right, this is one aspect, but that's a bit tricky logistically to keep them intact. It's an obvious approach, but that's a little bit difficult to implement it. And one other aspect, I think, is very important, what we think is crucial. We have such a three dimensional pooling system. We are just getting, taking out all of the cross points, we just don't verify that. We just take them out in the routine screening and to achieve these numbers that we have shown you which includes about one million donations, we have to destroy 203 donations only, so that's very cost effective. We must not test all of them. We'll just take them out and destroy them. So that eases the workload. Otherwise, you're running into verification of loops forever.

DR. YU: I would like -- sorry, go ahead.

DR. LISS: Alan Liss, Cention. Just while we're looking at the strategy of developing a number, I think it's important from the plasma side to remember

what the target of that number is. If we're looking at a pool and we're throwing out, even if the number is one, if we're throwing out a pool, 5,000, 10,000, 200, versus a number where we throw out a donation, we have a strategically different way to look at it. We don't, I think, want to create a situation where we are -- while trying to pick a reasonable number at a pool level, we start putting us into a source limitation versus perhaps looking at a strategy where we identify a pool and a donation where we improve safety by throwing out the donation while still maintaining the proper supply.

DR. YU: Well, Dr. Zerlauth, could you just comment on how many samples, how many donations that you have to, you know, discard, based on this four log genome equivalent for a cut off.

DR. ZERLAUTH: After the full implementation of the three dimension pooling and its resolution, from May this year, up to now we had screened approximately one million donations and it took us 203 or so, something like this, donations that we had to destroy, not more than that.

DR. YU: So in terms of percentage?

DR. LYNCH: It's about 1 in 5,000.

DR. WEIMER: May I just comment on that? This is from a European perspective. If we look at the BPAC recommendation, we shouldn't identify donors, so we should stop at minipools of some science which may -- what is the smallest minipool? The smallest minipool? So you'll take the 64 times your 200 positive donors and this is the amount of plasma units you would have to discard under that BPAC recommendation.

DR. LYNCH: Yes, I think that's a point of clarification that needs to be made. The BPAC did agree with our suggestion that there was no need to notify the donor of a test result. There's no medical or public health reason to do so. However, that did not preclude one from tracing a positive result back to an individual donation. We are not requiring that or suggesting that it's necessary to identify single units in a fully licensed, implemented nucleic acid test for B19. If the manufacturer chooses to throw away more than one unit, resolve it to let's say a subpool of 10 or 20 or 50 or wherever the economies lie, they may do so. However, as a point of method validation, we think an important component would be to verify that a positive result is a true positive and that's best done by identifying an individual unit. Once the validation phase is done that

decision then reverts to the manufacturer.

Yes sir?

DR. CHIEN: David Chien from Chiron. Dr. Lynch has suggested a more conservative view, take a 10^4 genome equivalent test cut off, but the manufacturers right now suggest that 10^6 or 10^7 becomes from a practical point of view.

I wonder, you think that level, like the 10^6 or 10^7 may not have transmitted disease, it's an assumption that a recipient -- would be immuno competent a patient or immuno suppress the patient. So if it's going to the immuno suppressed patient, maybe we should be taking a more conservative view.

DR. LYNCH: Well, if we were talking about infusing the plasma, I would fully agree with that. I think we're several steps removed from what the patient sees in terms of the plasma derivatives, but your point is well taken.

DR. YU: Just one point, Dr. Chien from Chiron. I think you mentioned the cut off they mentioned from Dr. Thomas Weimer is 10^7. That's minipool level and then Dr. Zerlauth is 10^6 at the minipool level, but what we are talking about is the production pool with a cut off 10^4 genome equivalent per

mL, it's lower than that. So they are different.

DR. ZERLAUTH: And if I may add, this is just what we go into production, but then there are steps that might remove and inactivate the virus, also not very effective, but still there, so that's a different situation. Thomas Weimer has nicely shown their final product, at least for ATIII, is nonreactive for the virus and that's the actual target. And I don't have the data with me, but I can tell you that our anti D have been produced from such low level pools are also negative. So regardless of what starting level, the target final goal is to have negative or nonreactive products and I think Dr. Weimer with this limit and with our limit have shown that we can achieve that. That certainly is worth a try.

DR. LYNCH: I'm getting the hook from Dr. Tabor. I would just like to close the session by reminding you that the National Heart Lung and Blood Institute is sponsoring a workshop on Thursday the 16th on Parvovirus B19 transfusion medicine. I don't know that he's still here, but I saw George Nemo earlier, there he is, and who can provide anyone interested in further information.

Thank you very much.

DR. TABOR: We'll now have a 20 minute break and we have one more session.

(Off the record.)

DR. TABOR: We are about to begin the next session. The afternoon session consists of two parts, first a series of talks concerning the evolution of single unit testing, followed by a panel discussion.

Before we begin, there's an urgent phone call for Rick Chattelain.

The first speaker this afternoon will be Sheryl McDonough. Dr. Sheryl McDonough from Gen-Probe, speaking on the evolution of single unit testing.

DR. McDONOUGH: Good afternoon. I'd like to thank the organizers of the meeting for the opportunity to speak to you today. We can go on to the next slide.

I'd like to discuss some assay performance data that we've seen testing single samples, talk a little bit about the advantages of single donation testing, address some of the concerns that we're hearing about implementation of single donation testing and progress on implementation.

Next slide. I thought before I'd start that we would reiterate what the TMA, HIV, HCV,

Multiplex assay format looks like. As was mentioned in the morning session, we have fully automated sample pipetting and pipetting of the target capture reagent. Then we have sample processing steps which terminate in wash steps which are performed on a target capture system shown in the middle of the slide. We have reagent additions and incubations associated with amplification and detection steps and then the samples are put into the luminometer for a fully automated read and the report is generated.

Next slide. Regarding assay performance on single donations or undiluted samples, you heard the analytical sensitivity discussed this morning by Dr. Giachetti and here I'm just reiterating what she presented about the clinical sensitivity that we've observed in seroconversion panels, that is, testing undiluted samples in HIV-1 seroconversion panels. We see a reduction in the window period versus antibody by 16.3 days and this is out of a 22 day detection window and versus antigen by 7.3 days. With HCV, we see a reduction in the detection window by 32.8 days.

Next slide. In terms of specificity using individual normal donor specimens, we've tested over 4,000 samples using five different lots of reagents and

the initial reactive rate is 0.79 percent. As was mentioned, if you go back and retest those samples in duplicate, the repeat reactive rate is zero percent. The other thing that we can ask in these samples is to look at the internal control signal. In each tube, you'll see if the assay was performed correctly, and also if the sample can support amplification. We did see an initial internal control failure rate of 0.31 percent, but when these were tested in a single point we saw no repetitive internal control failures indicating that there's a low level of specimens which cannot support amplification.

Next slide. This is an example of a reproducibility study that we've done with the TMA assay. We've looked at in this case, three operators, three different sites, two reagent lots, two instruments and over a period of six days. When we look at lot to lot, variability, site to site, operator day to day, these are -- I should mention these are plotted as the percent CV of the signal to cut off at the sensitivity limit for the assay which is 100 copies per mL for HIV and 100 copies per mL for HCV.

If you look at the percent CVs, you see they're very low, lot to lot, site to site, operator to

operator, day to day, between run, percent CVs or between 11 and 16 percent and within run, about 12 percent for both analytes.

Next slide. So the assay performance that we have observed indicates that this test would be adequate to pursue in a single donation testing format. What are the advantages of going to single donation testing? I think we've already discussed the fact that there's an inherent improvement in sensitivity, basically because you're adding more of the specimen volume to the reaction. And the way that would be observed clinically would be an earlier detection of the virus.

Another advantage is that the turnaround time becomes similar to current tests. Samples can be brought in, tested and the negatives released immediately. It takes at least another shift to perform the pooling step prior to testing and the retest algorithm can become more straight forward when you're testing a single unit as opposed to deconvoluted pool. And this one is pretty obvious, you don't need to do the pooling, so you eliminate the need for that space and equipment and you eliminate some potential sources of error.

Next slide. Here's some data looking at sensitivity that we've seen in seroconversion panels testing samples, undiluted and diluted 1 to 16. So we have seven different HIV seroconversion panels in which if you look at the sample, the days detection, undiluted, versus a 1 to 16 dilution, you see anywhere from two to five days earlier detection and in one panel a 14 day earlier detection with undiluted samples.

We've also identified two HCV seroconversion panels in which undiluted specimens were detected six or three days prior to the test in the 1 to 16 format.

Next slide. This is just one way to look at Alpha Therapeutics' data. The HCV seroconversion panels that they tested and this is looking at days between the first positive PCR result and days -- and the day when the sample became -- had a copy level of greater than 1200 copies per mL. And you can see most of the specimens -- many of the specimens show a difference of five to ten days and some of them a little lower and even one showed a 30-day difference between the positivity at the first undiluted or the first sample versus 1200 copy per mL.

Next slide. This is an HCV seroconversion

event that we detected during our IND testing with the Multiplex assay. This individual, if you look at the top, was seronegative on Day 1 and did not seroconvert until Day 88, during follow up testing. The sample was tested at each follow up point, Day 9, 14, 21, 27 and 88 and in each case was positive when tested undiluted in the Multiplex assay, actually in the discriminatory assay.

When those samples were retested in a 1 to 16 dilution, the first two samples were positive, but the next sample is negative, the Day 14 was negative. Day 21 was positive again. Day 27 and Day 88 were negative at a 1 to 16 dilution, implying very low copy levels in some of these samples.

The samples were also analyzed by a quantitative PCR test and helps clarify what we were seeing in this particular conversion event. The yellow dots represent the copies per mL of HCV in these different follow up samples. First of all, the first sample we found was almost 10^6 copies per mL, so very strong positive. The next sample, about a week later, it had dropped in titer over a log. And by the 14th day was undetectable in the assay, implying very low copy level.

Seven days later the copies again were up around 10^5 copies per mL, dropping to undetectable again, just a few days later and were up around the 400 copy per mL range at Day 88. This type of fluctuation in HCV R and A titer was not known about a year ago and I think we're just starting to hear that this is not an uncommon event. We've certainly heard that this has happened in other cases, in other seroconversion panels.

The red shows the signal to cut off for the undiluted TMA samples.

Next slide. This is a very early study that we've done with just a small number of IVDUs. This population, 30 different samples were examined and we found that most of these samples were reactive with HIV or HCV or both. Seventy-three percent were anti-HCV positive. Only 20 percent of the samples were not reactive for anti-HIV or HCV.

Next slide. We looked at five of the samples that were anti-HCV negative and we found by TMA they were RNA positive. When these samples were retested in 1 to 16 dilution they were all negative. Likewise, we found one hemodialysis patient that was HCV negative and TMA RNA positive, but negative again at a 1 to 16 dilution. So these are very early results,

small sample size, but it does look like in this high risk population you can find individuals pre-seroconversion with very low copy levels of RNA.

Next slide. What are some of the concerns for implementing single donation testing? Of course, some of the concerns with the pool testing now are actually alleviated by going to single donation testing, so we don't need to talk about turnaround time, but what you really end up with is the need to perform a great and large number of tests at each site and if you're performing testing right now you have to ask what's the need for increased space, what's the need for increased people, how do I keep them all trained and what reagents and instruments will be available if I'm going to go from a pool of 16 to now testing individuals, is that a 16 fold increase in lab space and people, etcetera. And what we've done is taken this as an opportunity to look at optimizing the instrument usage and personnel work flow to improve the throughput.

Next slide. The way that the testing has been done through most of the IND for the TMA assay is that if individual operator, using one TMA instrument system will run a rack of 100 tubes and that takes about five and a half hours to complete. We've shown that an

operator can just in a half hour with extra time actually do testing on 200 tubes, so what we're calling format one is one operator, one TMA instrument system, 200 tubes in six hours. That's fine for some laboratories, but it's certainly not adequate for single donation testing at other sites. And so we're now looking at a second configuration in which we add a second instrument system to the laboratory. We add personnel that start in staggered shifts and for this particular format it would be three technicians with an 8-hour shift each and the result is a 1,000 tubes per 10.5 hours. So we've certainly shown an increase in through put without the equivalent increase in space and personnel.

By staggering the three shifts throughout the day, a laboratory can now get 3,000 tests per day with two instrument systems. And I need to point out that with the Multiplex test that's actually 6,000 results because you get both an HIV and an HCV result.

Next slide. This is a nonartist rendition of the throughput schedule and this is just hours throughout the day starting with Hour Zero. Each of the columns represents a set of 100 tubes and the different colors represent the different operators throughout the

shift. So in this schematic, we're showing the first operator comes in and does the TECAN set up and pipepetting steps and passes those first 200 tubes off to the second operator. And then they move on and do the TECAN steps for the second 200 tubes, pass them on to the fifth and sixth 100 tubes and they have time for lunch and they go out and finish until they've done ten racks of tubes. Likewise, the second operator will perform the different steps of the target capture, move on, have time for lunch and complete. So each operator works an 8-hour shift. You get 1,000 results in 10.5 hours.

Next slide. Another question that we've been asked is reagent availability for single donation testing. Gen-Probe has completed a new manufacturing CBER level manufacturing facility that's 93,000 square feet. It has the capability of at least 80 million tests a year and the manufacturing scale is appropriate for either single or pool testing. We do have a backup manufacturing facility. It's about 12 miles away with about 50 percent capacity and we do maintain safety stocks of critical, raw materials and intermediates and final product.

Next slide. So just to conclude, I hope

I've shown you that the assay performance is appropriate for single donation testing in terms of specificity and sensitivity.

Next slide. Some of our initial studies confirm that you see improved sensitivity with a single donation test compared to a diluted format and that's for both HIV-1 and HCV. Some of the issues associated with pool plasma testing actually go away by going to single donation testing, including the turnaround time, release of labile components.

Next slide. By adding equipment and personnel scheduling appropriately, we can get a throughput of 3,000 tests per day per laboratory and so our work to address implementation of single donation testing will continue in the future.

Thank you.

(Applause.)

DR. TABOR: Thank you. That was a good introduction to something very new. The next speaker would be James Gallarda, Dr. James Gallarda from Roche.

DR. GALLARDA: Okay, I started out the title of my talk without a question mark at the end, but one of my colleagues pointed out that there's a great deal of uncertainty about evolving to single unit

testing so I thought that would be appropriate.

Next slide. What I'd like to do is talk about our view of moving to single unit testing from an in-pool testing from two perspectives. One is the feedback that we're getting from our customers on the issues we're doing minipool testing as it stands right now with our semi-automated system. The second is really to look at what is Roche's role in this, where do we see ourselves in the future on this. So our view is that there are three prerequisites required to move toward single unit testing. The first is that there be a sufficient incremental gain in blood safety by going to single unit testing over minipool NAT testing.

Secondly, the manufacturer has to provide high throughput automation, that the system have operational robustness and that there be dependable manufacturing.

And then thirdly, the manufacturer must have sufficient business potential to warrant the development of these systems, so I'd like to go into each of these three separately.

Next slide. Well, with regards to the incremental gain in blood safety over pooled NAT, there are pros and cons. On the pro side, you have high

sensitivity with undiluted samples and that potentially could increase the safety by further closing the window. Furthermore, there may be -- this may be indicated for low titer viruses that are especially early in pre-seroconversion. So on the pro side I think there is evidence that if you go to single unit testing, there might be some incremental gain there.

On the con side, this is going to be pretty costly. It is expected that the yield would be extremely low and the cost will be extremely high to go to single unit testing. And from the presentations today for all three viruses, for HCV, for HIV and for HBV, minipool testing has been demonstrated to interdict infectious units that would have escaped by serological testing alone.

So the conclusion we can say right now, it's to be determined. The pool NAT is increasing the blood safety. We've interdicted infectious units and single unit NAT has yet to prove incremental gain in blood safety and I think over the next year or so there will be some studies that can more clearly identify the contribution that single unit testing can have.

Next slide. With regards to the manufacturers providing high throughput automation and

operational robustness, Roche agrees with the ABC's position on this and that is -- frankly, when we talk to our sites, they don't want to do a semi-automated slash manual system for single unit testing. It's been traumatic enough getting to where we are, so we think that high volume automation is required and we have been looking at high volume automation. In fact, at ABB we had one system that we placed into Japan and that has automated sample preparation instrumentation as well as a three virus Multiplex capability using homogeneous PCRs, the detection comes from.

I think on our side, our PCR products, we've been in the PCR business for a long time and so far we feel quite good about the performance of these products, the low fail run rates and the low false positive rates and in the minipool NAT clinical trials, I think I would agree, someone said that the real success here is that we went to minipool testing because there was a need and this need was met by minipool NAT testing.

Next slide. And from our perspective, you know, having this experience now behind us is that the system that we've developed has accommodated 13 sites in the U.S. and five sites in Canada and we've tested over

two million samples at these sites in both large and small blood banks and so the bottom line, the feedback that we get from these sites is that okay, we've got this working and I think the real heros in this are the technicians who are doing this because there was a significant learning curve to get to where we are today.

And the fact is from the data that was presented today is that we have successfully interdicted numerous window cases with this PCR product and therefore this is the reason we're doing this is to prevent transfusion associated with infection.

Next slide. With regards to the manufacturer providing dependable manufacturing, we've had over 2,000 of the COBAS Amplicor and analyzers now placed in the field and in our system I'd say that that is an instrument that illustrates the dependability of the instrument side of going forward with any kind of further automation.

And on the kit reagent side, we just kind of passed a milestone. We're now making over 800,000 kits, PCR kits per year. So the conclusion here that I want to leave you with in this section is that Roche has the experience to move forward to single unit testing.

Okay, next slide. But there's the dark

side to all of this and that is there has to be sufficient business potential to warrant the development. So the bullet points are, due to the uncertainty of the ABC Patent situation, Roche will not further invest in automation. Therefore, we have no plans to develop products for single unit testing.

Secondly, Chiron has elected not to license HCV NAT for blood screening to any company other than the Chiron Gen-Probe alliance.

Having said that I want to assure our current customers that we intend to go full steam ahead with our on-going clinical trials for the COBAS Amplicor system, as well as the Japanese Red Cross trial which has this Multiplex, more automated system than we have in trials right now. We will continue those trials and we will continue to submit for regulatory review.

Finally, we will have full intention to support our customers without any change.

Thank you.

(Applause.)

DR. TABOR: Before you leave the podium, Mike, I can't resist asking for some clarification. How are you going to support your customers by not investing and not initiating new research?

DR. GALLARDA: That's a good question. I would say that the answer is that a year and a half ago we were asked by the customers to get into this business and the understanding was at that time we were going forward with minipool testing. That was the paradigm and that's what we were going forward with. I think that has demonstrated an effectiveness that everyone -- I think all the blood centers should be proud of.

We expect that those products are going to be licensable. We're going to file for licensure and then there will be the need to support those products and so that's what I mean by continuing to support the customers with a minipool NAT system that we have.

DR. TABOR: Thank you. Those are some really difficult ancillary problems that maybe we can go over in the group discussion and they probably don't only apply to Roche.

The next speaker is Dr. Klaus-Heinrich Heerman from the University of Gottingen.

Dr. Heerman.

DR. HEERMAN: First, I want to thank the organizers for inviting me to this lecture. Ladies and gentlemen, you have heard today and the last time NAT is very sensitive, especially if performed in a single

test. Therefore, the handling of this technique and fundamental extraction steps are susceptible to cross contamination. The surface area, the surface tension of the liquid is the only cover against the template cross over. And therefore easy and safe extraction methods are required in the same way only less expensive methods are qualified for high throughput testing especially in blood bank facilities.

First slide, please. I've developed an easy way to extract template RNA. After sample distribution of the plasma, the template was extracted by protease and a further step, the protease was denatured by heat and in a dilution step we get some streptomycin coated particles to capture the first primer for hybridization which takes the template. After several washings in a special magnetic rack, the template can be amplified or put in to the reverse transcription method. Finally, there is an easy extracted, easy detection by fluocin dye.

Next slide, please. In cooperation with Tobias Lateler and Michel Kohler from the Department of Transfusion Medicine, the test was adapted to modify pipetting work station and starting with microplated tubes and using microplates in all subsequent steps,

each donation is linked to its individual HCV RNA result. Thus, a simple data file is transmitted to a sample blood bank software where all data of one donation are collected in addition to other several particular results. For this way we have to develop some additional features.

Next slide. We could use a simple magnetic rack.

Next slide. On the bottom of microplate rack was fixed some magnetic stripes in longitudinal direction and there are -- next slide.

There are further disposals, a special waste disposal were introduced to the robot and for removal of the used disposal tips they -- we want not throw away these tips through the air. We train the robot to put the disposals back down in the rack again which was third from bottom with liquid, for instance, the diluted hypochlorite.

Next slide, please. This system of primers was used. If there were a peer contamination problem of the amplicons we are able to start a placement PCR with a primer set for HCV 4. Because these primers do not detect the small template of everyday performance at 3.0.

Next slide, please. It's very difficult to characterize the primer qualities. The PCR should not be otherwise affected by point mutations of the template. Here, you will see the amount of an alignment of up to 400 NCR sequences and I show you the deviations, the point mutations in the definite positions. We have changed all primers.

Next slide, please. We use safety primers and these primers have fixed -- we always use three or four primers. The primers have fixed five flesh ends or the same five flesh ends and they vary at three flesh ends.

Next slide, please. Here, I show you the limiting dilution curves of different forms of HEAD.

Next slide, please. The HEAD or extraction amplification detection is very sensitive. In the middle, there is 160 international units per mL. We only use 50 microliters for tests with eight international units for a test and this 32 genome equivalence.

Next slide, please. And the blood banks don't get very much, only a few positive specimens and therefore we use 201 specimens of our diagnostic lab to test the system and of this 201 we detect 196. Only

five we don't detect and therefore we have -- it looked for the titer of the specimen and the only footnote which the very diluted specimen, of this specimen, one cannot be retested from the in-house positive and pharmapositive assay. Two further could not -- reached from the Roche system and one of these five samples were from pathologic labs.

Next slide, please. On seven different days, testing of six replicates of two dilution series with different lots of reagents were performed and here are the results. The middle and the extreme -- and in addition of several individuals or possible individuals of TB test this, too, and with only minor infections.

Next slide, please. These are the results of a long time study of up to 28,000 donations. In the window period we found two positive donations and five donations have already antibodies and the drawback we have are these false positive results left. This is 1 in 2,000 reactions and if we perform other tests in the unit it's increasing three to 1,000 tests and therefore -- next slide, please.

We identify the major problem of our liquid handling of the robot and the pipetting tips have very small outlets, therefore the speed of the liquid is very

high if you inject it.

Next slide, please. Therefore, the future invention is to use magnetic tip and this is the shape of these different devices. This is a hepa tip and this is magnetic tip and you take it into the surface of the liquid and on this side you don't inject liquids.

Next slide, please. With the same chemistry we could have found the extraction by covering the magnetic tip and collect the beads in the solution by putting down the stick into the liquid and then you can wash easy with some additional wires with the stick and you put the beads into the solution for RT or TCR.

Next slide, please. These are the advantages of this system. There are variable specimen volumes up to more than 10 milliliters. There is an easier reduction of the template volume. You can leave the beads in 25 microliters. There are easy washing steps and the exchange of buffer is easy too. There are no pipetting steps and this is really more laminar procedure and the handling is more slower. With this system, you perform additional pools, but in another way you can pool after in the middle of the denaturation and have it capture individual probes and this is a major point. You have less plastic waste and the cost of our

system is about 4.5 dollars and if we lose many tips for washing, we are at about 2.5 dollars.

Thank you very much.

(Applause.)

DR. TABOR: Thank you very much. Our final speaker is Dr. Jean-Pierre Allain from the University of Cambridge, England.

DR. ALLAIN: Thank you, good afternoon. I'm first of all thankful to the organizers inviting me to share with you the data on the single dilution testing using TNA for the detection of HVC and HIV RNA.

We are in the process of doing two studies, one of them is targeting first time donors in the Southeast of England which is essentially looking into HCV RNA detection and the second is done in collaboration with the blood center in Durban, South Africa because of -- and directed to what they call high risk blood donors, essentially directed to HIV. So I'm going to update you quickly on both of these studies.

Could I have the first one, please. This is the study design of the U.K. study where we start with whole blood donation and as you can see here it's done in parallel with the antibody EIA testing so these samples we are testing in the study is -- are not

prescreened for serology. The objective of the study is three things, first of all, to assess the visibility and performance of TMA in testing individual donation. The second is to look at the clinical efficacy of the test and the third to compare with the currently on-going pool testing. So as I mentioned we are targeting essentially first time donations, although the pool testing with 96 member pools is looking at the entire population of blood donors first time and with repeat donors.

So basically the TECAN is preparing the pool, but also taking one in an archive and then what is left in the tubes is sent to us for TMA testing and then we on the second day have an initial result and then confirmation on day 3. However, our results are not taken into consideration of the moment, but for clinical distribution of the products.

Next one, please. So first we looked at the performance of the assay in terms of sensitivity and I'm not going to dwell on it because this has been presented previously. The only thing I would mention is that the sensitivity is very high, in some ways too high because we have difficulties by using rTPCR too much sensitivity for both HCV and HIV.

Next one, please. Just another rendition of what Sheryl showed about the timberline of testing. We have actually two technicians working on the project and, basically, it's comfortable for each and you can see here the various steps of the technique to do two runs of 100 tubes. However, we have tried to do three and it's quite feasible within six hours except that there is no time for lunch, but it is quite feasible.

(Laughter.)

The next one, please. So basically, at the moment and this is as of last Friday, we have run 304 runs of 100 tubes. Two hundred seventy seven or 91 percent we've added drugs. We were a little bit concerned as has been presented previously by other speakers of the relatively high member of invalid runs and in particular out of these 27 or nine percent we had 22 cases of failure of the calibrators and this was a concern to us. However, we had in some cases, as you would expect, in the learning curve, identified human errors and also some technical problems.

Next one, please. This is a little bit complicated but it basically tells you all of our results. Here, you have the actual run, so each individual is 100 tube run, each of the dots here. You

have in red, the round dots, the invalids and the triangle of the initial reactor and then that's the open and the closed represents the repeat when needed. In addition, I put here the invalid runs shown here and this is the actual number of these various results. You can see at a glance that we had essentially two periods of problems. One here, essentially which was initial reactive, none of them repeating and another one here which was both the initial reactive and invalid and as you can see, this corresponded also to a bad period of invalid runs. We have identified the reason for these here which was, it was in the middle of the summer, believe it or not, it was too hot in England for the selection process and now with the air conditioning running we eliminated that problem. Here, it was more complicated than it was, in fact, a combination of problems with instrumentation and in particular the washing system, and at some point the luminometer, but also kind of not quite strict enough adherence to the manufacturer's protocol. So the false were shared so to speak.

But except for these two periods, if you look when everything works well, as you can see the number of invalid runs is infrequent. We have very few

invalids and very few initial reactors.

And this is translated here in numbers. Out of nearly 26,000 individual first time donations we have screened, we have 1.23 percent invalids, excluding the invalid runs, which all repeat, were obviously no problem. The initially reactive were 0.48 percent, but as you can see here most of them were located in these two critical and difficult periods, but you can see that on repeat we had only three so-called false positives. In other words, none confirmed samples that were repeatably reactive and of note is that these three were during the period of problems with all the invalid runs. So as you can see here, 17 of these 20 were confirmed and the details are shown in the next overhead.

And these are the 17. As you can see in black, most of them were HCV as you would expect; one HIV and all of them were also seropositive and all of them were confirmed either by our in-house HIV or HCV confirmation. And the overall prevalence for HCV was 1 in 1700 donations which is exactly what we predicted from the epidemiology.

Next one, please. If we look at the -- now we compared that to the overall data as far as antibody was concerned, confirmed positive NTHCD. There were 24

during that period, but because we were doing the screening only four days a week, we tested, in fact, 14 and 11 were TMA positive, three negative which is basically corresponding to the 85 percent chronic infection as we would expect.

Next one. Okay, just quickly -- so that's for the U.K. study in first time donors. Now the South African design is different for several reasons. One of them is that they are testing for NTP p24 antigen. The other is because of the prevalence of HIV and to some extent, HCV we did it post-serology except for p24. So basically what has been done is that the p24 positive antibody negative were included in the study to see whether we could replace eventually p24 by TMA and also the negative. So on one hand, those were tested individually. Also, on site in Durban, they were put in pools of 24 and we tested both pools and individual samples.

Next one. So this is just a quick summary of the results. We've tested 1800 samples. We have .03 percent invalid. Initially reactive, we have 2.23 percent, repeat reactive 141 and confirmed positive, 26. As you can see here most of the confirmed positive were HIV antibody positive which were deliberately put into

the system by our South African colleagues as positive control and four of them were positive, but also were positive by p24. We haven't found at this point what we're actually looking for which is anti-HIV negative, p24 negative TMA HIV RNA positive.

Last one, please. So the question has been raised already how the feasibility of single donation and I've done some calculation as far as England is concerned. Out of 2.2 million donations per year, 85 percent being repeat donation and 15 percent being first time. So if we do it by all individual testing for repeat donation, that would require either 31, if it was a 3 run technician or 46 individuals which in the current situation in England seem a terribly high number of people, but if it's done by a pool of 16, then it takes either seven or ten individuals to do the testing.

In first-time donations, if you do individual, two or three individual can test all the first time donations easily and if it's in pool. So one of the options at the moment would be at least before the complete automation is available, one of the possibilities would be to do individual testing for the higher risk first time donation and the pool of 16 for the repeat donors.

Thank you.

(Applause.)

DR. TABOR: Thank you very much. We now have a panel discussion. I'll try to end the session on time. If the members of the panel could come to the table, please. I think we'll begin our discussion with the subject of single donor testing, but I would like to allow it to be free ranging over any topic connected with implementation of NAT.

I'd like to begin with a question for the panel and I'd also like to invite the audience to respond, perhaps some of the manufacturers.

I've heard concerns expressed that the advent of single donor testing is going to possibly cause delay in the filing of PLAs for minipool testing. I would like to get some comments on that, if possible. Perhaps Dr. Gallarda from Roche could comment on that since it's sort of related in a kind of opposite way to what he was saying.

DR. GALLARDA: Well, I can just tell you that from Roche's perspective, we intend to go full steam ahead with filing for submission for the Ampliscreen HCV and HIV products and our estimated target date for this is the end of the first quarter of

next year.

We have a pretty active group doing a lot of PCR assay development and I think the flip flop here is that we don't expect to have a lot of impediment in filing those because we're working actively on single unit systems. So I think our strategy is to provide what, apparently, is going to be an effective means to further close the seroconversion window, interdict NAT only units and that's the minipool system that we've developed.

DR. TABOR: Dr. Busch.

DR. BUSCH: I think that we did have a resistance on the part of the other manufacturer, Gen-Probe, and now a partnership with Chiron to really fully support minipool testing. They run a bandwagon, in part, I think, with encouragement from FDA and NLBI to move forward toward single donation testing and they felt that there was potentially going to be an acceptance of minipool testing. What I think we've seen though is with a lot of effort on our part and cooperation, I think, from Chiron and now Gen-Probe, is a commitment from that company to support minipool testing and bring forward a licensed product. They are, in parallel, pursuing an IND claim for single donation

testing, but recognizing that the industry, at least for the next few years is really intent on doing minipool testing until, I think, the efficacy and cost and reimbursement issues around supporting single donation testing come forward. So I think there has been movement. I would say that there's been great value that there's been a competitor out there. I think if Roche had not been there, if you guys had not come to the table two or three years ago and entered this business that there may well have been a sort of inexorable move to single donation testing even though it may not have been in the interest of the industry.

DR. TABOR: A question from the microphone on the floor.

MR. HALVERSON: Craig Halverson from Gen-Probe. I just wanted to confirm that Gen-Probe is going forward with -- in parallel, with individual donation testing as well as minipool testing and we do not expect that individual donation testing will delay in any way approval of minipool testing.

DR. TABOR: Thank you. Does anyone on the panel have any comments or questions on the general issues related to NAT implementation?

DR. CHUDY: I think we should encourage

single unit testing because I think in the future when NAT will replace the serological test. It could only be in the stage of single unit testing and in the moment there are times to act and I think this is the new solution.

DR. HELDEBRANT: Chuck Heldebrant from Alpha. I think based on our experience with minipool testing of plasma pools, I think it's premature to look at single donation testing by NAT as a way to replace serology. They measure different things. They complement each other. We look in the future to have minipool testing of NAT to help us cover the window period and serology testing to help us cover the more developed periods of infection. The objective we have is we want to be able to detect any unit from any donor at any time during the disease state. That's what we want to be able to do and as good as it is NAT doesn't give us that capability.

DR. SALDANHA: I'm not sure where the serology comes in, but certainly when we discussed the introduction of NAT testing it was always felt it was an additional assay to ensure additional safety. It was never the idea to replace serology. And as far as I think we're concerned in Europe, serology assays are not

going to be replaced by NAT as an additional assay.

DR. STRAMER: We may not necessarily agree with that in the United States as you heard earlier. I mean there are specific assays that we can target elimination such as p24 antigen that measures basically the same thing as NAT, ALT, perhaps as we look at hepatitis B screening anti cor. So we are hopeful, even those are not in your menu, except for ALT that we can move to eliminating some of our current tasks with the replacement of some more sensitive tests.

DR. KLEINMAN: I wanted to agree with some of the comments that Celso made previously about the move toward single donation testing. I think it is a question of not if, but when and I think that since we have demonstrated efficacy for minipool testing at a lot of effort, I don't see the need to rush headlong in the same rapid way into individual donation testing. I think we need to accumulate the data and we need to get better at our laboratory turnaround time and we need to as well get the automation up to the standards of serology testing. I don't think we should be going into systems that are semi-manual for individual donation testing given the potential problems they can generate and given the fact that at least the models predict only

a small incremental benefit. But I think once we get to more automated systems, that incremental benefit will be worth it, then we need to find a way to be able to pay for it. But that shouldn't stop us from doing it. Those problems will need to be solved. So I basically think that Celso's rendition of the problem was a good one but urge that we go on that course.

DR. TABOR: Question from the floor.

DR. ALLAIN: It's not exactly a question, it's rather a comment. I mean I was just going to say the same thing as Steve just mentioned that on the basis of our experience, the semiautomated system we are using currently for single donation in molecular virology laboratory like ours is difficult. We have problems and we need to solve them on a daily basis and so I don't think as it is the technology would be suitable for general use in blood banks, particularly knowing that the level of molecular biology knowledge in the average blood bank is often appalling, if I may say so, at least it is the case in England and in some other countries, including the U.S., I believe.

So I agree with Steve that unless automation is there, I think that would be the absolute necessity to implement it on individual donations.

DR. TABOR: Dr. Bianco.

DR. BIANCO: Actually just following those statements, I think that the FDA has done that in many ways in terms of looking at entire systems, not just the chemistry of a test or the molecular biology of the test, but the entire system and how it's going to work in the field and I think that more and more with the complexity of what we do, I think that as we look at NAT testing and licensure, we should look at the entire system, so we make sure that the outcomes are the outcomes that we want. We just don't have a test that in the model somewhere in a corner works very well, but when we throw it to the world that may not perform as we need it to perform.

DR. TABOR: I'd like to ask a question about NAT testing for hepatitis B virus. We've got people from quite a number of countries here, Dr. Nakajima from Japan, we've got at least two people from Germany, Dr. Heerman and Dr. Chudy and can some of you bring us up to date as to what is going to happen with NAT testing for hepatitis B virus in your countries?

DR. NAKAJIMA: I'm sorry, I didn't quite understand what do you mean by "what's happening" --

DR. TABOR: What are your regulatory plans

and what stage of investigation have you reached in using NAT testing for screening for hepatitis B?

DR. NAKAJIMA: When we include the hepatitis B in our minimum requirement, we have to shut down the import from the overseas of the imported plasma products from overseas, so we cannot do that at this moment. Unfortunately, in our country, JRC is the only one, collecting organization, and they do the HBV NAT system already. So we do not have the actual problem concerning the transfusion products, so if the foreign manufacturers are ready to implement HBV, we can proceed to the minimum requirement including in the minimum requirement of the HBV. Until that, we have to collect our data.

DR. TABOR: Thank you. That's very interesting. How about Germany and --

DR. CHUDY: Of course, we are both thinking about increasing HBV safety regarding to NAT and opposite to the NTHBC testing. And my view is it is now difficult to define individualized QHQ compared to HC because the detection unit must be so low as they can do it. What is so low? I think maybe 300 or 400 copies and that's as far as practice and other strategy -- HBV testing for HC testing and also another kind of

extraction, of something.

I think at the moment you cannot take the same extraction procedure for HBV you can't transmit to extract HBV. That's my personal view. I think there is no generic extraction procedure now that gives the same efficacy for all nucleic acid. I think this problem is to be resolved. It is helpful, I think, now we have a WHO standard, we can start some collaboration studies to see what is the detection unit of the different viral copies and then we get an impression to come to a decision.

DR. TABOR: Yes?

DR. DODD: I think that we need to be rather careful not to walk in lockstep with every one of the agents for which we might be able to test. I have some concerns about assuming that we need to be testing for hepatitis B. I'm not saying we don't need to be testing, but I think we need to remember, for example, that this is probably one of the agents that is expressed most variably in terms of incidence and prevalence around the world. We've actually seen some quite interesting data today already about a relatively high yield for HBV detection, in some populations of donors that have generally considered to be at somewhat

higher risk than those for single blood donations. I think we've also seen some interesting data from Japan which has a very high prevalence and a significant incidence of hepatitis B. I think we need to recognize that there is not apparently a major demonstrated risk of HBV transmission in this country, perhaps in part because we do do cor testing and I think any one of us would be hard put to identify a recent case of transmission of hepatitis B through a pooled plasma product.

So my plea is let's be a little bit careful and let's not regard it as an Everest and do it just because it's there. I think we need a really good rationale before we make our lives even more complex. You heard what Dr. Chudy said about the technical difficulties inherent in looking for hepatitis B tests in this environment.

DR. TABOR: Dr. Stramer and then we'll take the questions from the floor.

DR. STRAMER: I have a question for the FDA. Along the lines of hepatitis B, even without NAT testing using single donation testing we today have the ability to close the window by the implementation of more sensitive HBS Ag antigen tests or if the FDA chose

to evaluate those that are currently licensed, perhaps the elimination of those with lesser sensitivity would perhaps do more as far as yield for hepatitis B than implementing a whole new technology.

DR. TABOR: I'm not sure I have the full answer to that. My knowledge of the tests that are licensed is that even though there is some variability, even though they all meet the minimum FDA standards, there is some variability from manufacturer to manufacturer. None of them would close the window period.

DR. STRAMER: Of course not, but there's probably enough variability between the test which would be greater in the amount of window period reduction than by going to minipool HBV DNA testing.

DR. TABOR: That's an interesting perspective. And the other question I guess would be if somebody had an even better HBS Ag test than what's on the market now, would we be pushed to implement that? That's very interesting.

Yes, on the right hand.

MR. SNAPE: Terry Snape, Bio Products Lab in the U.K. Just following up your question on hepatitis B testing, Chairman, it's worth remembering

that in Europe there is no uniform mechanism for mandating the testing of blood donations for, for example, hepatitis B DNA. HCV RNA testing came about because of the CPMP requirement for testing of plasma pools. With the specific exception of Germany, it's hard to see that HBV DNA testing would be mandated generally in other member states.

DR. TABOR: The other microphone?

MR. MacPHERSON: Yes, Jim MacPherson. A lot of people know that the ABC members send about 600,000 liters a year of plasma to the Swiss Red Cross and they have indicated just about a month ago that they want us to implement HBV NAT by the end of 2000. Now they've put that request on hold because they are aware that the data on pools that they're talking about using the same size pools that we do now, that there are data that would show that pools of 16 or 24 can also be matched by some of the, for example, chemiluminescence technology that's used in the Prism system. But I suspect that certainly within the next few weeks we're going to be hearing back from them in terms of what the requirement is actually going to be and how and that we have to figure out how we would do that.

DR. TABOR: And this would be for plasma?

MR. MACPHERSON: Well, that's how we started out doing what we're doing now was for plasma, for recovered plasma.

DR. TABOR: Dr. Heldebrant?

DR. HELDEBRANT: Yes, again, one thing that this emphasizes and really shows is that we have three regions of the world that are all struggling with how to implement NAT testing and they've all taken different approaches. Europe has taken an approach of saying manufacturing pools for HCV have a manufacturing pool limit. The Kosasho in Japan has a requirement for hepatitis B testing which is understood, given the situation that they have in Japan. The FDA has yet another mechanism. As a manufacturer's representative, we have a situation where like it or not, we have to comply with all three and so our plea, at least from the plasma side is that please use the mechanisms for harmonization and please harmonize where we're going with NAT implementation and NAT regulation. It will make it easier for all of us and we'll have a much more rational basis to apply it.

DR. TABOR: Dr. Allain?

DR. ALLAIN: I wanted to mention about the HBV that the situation is much more difficult in my view

than for HIV and HCV. In particular, we have shown recently some data indicating that you can have NTHBC present, an infectious unit which is negative by PCR or reasonably sensitive PCR and we are not the only ones who have shown that.

In addition, also in the literature, there are some cases of HBS Ag positive sampled, confirmed positive, which are DNA negative, whether or not it's infectious is not clear, but presumably if you can produce the protein that the likelihood of infectivity is fairly high. In addition to that as has been mentioned today, the very low level in the pre-seroconversion period is a problem, so I think it's really a case of its own different -- or different from the other that has to be looked at. But in my view, I think relying on genomic detection is not a good idea and I think we are going to stay, we should stay probably with the HBS Ag and NTHBC until things are clarified.

DR. TABOR: Any further comments from the panel? Dr. Stramer?

DR. STRAMER: I have another question for the FDA.

(Laughter.)

Hearing what you've heard over these several meetings on NAT that FDA has held or Blood Products Advisory Committee has held or even your other meetings with IND sponsors, what time line in a perfect world would you design, starting perhaps from product release and then licensure, could you outline something for us that perhaps the Agency would like to see?

DR. TABOR: Well, I don't know if I'm answering you directly, but you know the licensure of any product is governed not by what FDA wants, but by what the sponsor wants because it depends on when it's submitted and when the data is complete and when the deficiencies are corrected. And in addition, as you know, we're not allowed to talk about -- actually, it's forbidden by law to talk about products under consideration.

DR. STRAMER: I'm not asking for specifics. I'm saying if you could create a perfect world with CGMP compliance, no deficiencies in batch records, we're living in an ideal world.

DR. TABOR: Okay, well, in an ideal world, under -- actually Dr. Epstein took a daring step, but a very good one in permitting or encouraging FDA to permit the use of NAT screening for HCV under IND and I believe

that began by most manufacturers in -- pardon me?

DR. STRAMER: Early 1999 to mid-1999.

DR. TABOR: And some were beginning earlier than that, really.

DR. STRAMER: For plasma.

DR. TABOR: Right. 1998 for plasma, probably. So for plasma you've got -- you've had two years to collect data, you know, I would have expected applications to have flooded our office previously. So it's really up to the sponsors.

DR. STRAMER: I would think it would also be up to the FDA to allow a nationwide IND, which is really unprecedented to be able to say at what point do we come to closure and I think at the last BPAC meeting two members have already asked that, at what point do we look at licensure of pool testing.

Clearly, the industry, as we talked about in a previous session needs to get to the point where all cellular components are released based on pooled NAT. However, we're going to all get there, but then how do we proceed with licensure?

DR. TABOR: Well, as I said, you can't have a license until you have an application and an application is complete. It does create a dilemma

because in some senses, the IND process should not be used as a substitute for delay in licensure and I'm glad to hear that single donor NAT testing is not going to delay things further because there were rumors it would.

On the other hand, we're all in agreement that blood and plasma are safer, certainly blood is safer because of NAT testing under IND and not even any even hint that it's hurting the blood supply. There's no question in my mind and that of everybody else here in this room, probably, that it's helping. So we can't discontinue the INDs, but we can't approve an application until the application is there and the data is there. And it's a common misconception that FDA controls this.

DR. STRAMER: I wasn't implying that in any way, but I think given what we heard today from Gen-Probe if they file a single donation test, BLA at the same time they file a pool, perhaps when one doesn't have a crystal ball either one could get delayed. So an application could be delayed because both are not ready in tandem. So I was just curious about regulatory guidance, perhaps that the Agency could provide the manufacturers.

DR. TABOR: Well, I'll ask for comments

from other FDA people who are here also, but my answer to that would be that we encourage the early submission of applications, the early completion of the data sets and we'll review them as quickly as we possibly can.

Dr. Jacobs, would you like to comment?

DR. JACOBS: We've really been asked the question primarily of what will happen to one IND as another one goes forward and the answers that we have given have been that all the INDs will go forward in parallel, that once one technology has been approved that we will ask for some comparisons between the two. Those would probably not be done on clinical ones, on clinical trials, but they will continue to go forward. But there will then be a requirement that there will be some comparison once one is licensed.

The other questions that we've been asked is following licensure, what would FDA consider in terms of making a recommendation for testing and I think that recommendation would first there would have to be a licensed test and then we would have to look at the applicability of that test, how applicable would it be for a universal situation, what is the throughput, etcetera. So that would be a separate consideration from licensing and we certainly encourage people to send

in applications. So I hope that's responsive to your question.

DR. STRAMER: Well, yes, the bar certainly changes for any of the manufacturers once there is a licensed comparator because then one could easily say go back and do your trials against the licensed test.

DR. TABOR: But it's very possible that there could be one licensed test and other INDs that were still running and I think just, you know, a practical basis there would be no way to avoid that, no one of the current IND holders could handle the entire market even for a short period of time.

Are there any other questions?

DR. BIANCO: Not a question, but just a comment. I think we've all heard your encouragements for submissions for licensure. That's, I think, what Sue wanted to hear from you.

DR. TABOR: Thank you. Was there another comment? Okay, any comments from the floor?

Well, thank you very much. I want to thank all the speakers. I want to especially thank those who came from Europe and Japan, speakers from Germany, the U.K., elsewhere and thank all of you for helping make this a successful meeting. The meeting is adjourned.

(Whereupon, at 4:59 p.m., the meeting was concluded.)