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ATDEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

APPLICATION OF NUCLEIC ACID TESTING

TO BLOOD BORNE PATHOGENS AND EMERGING TECHNOLOGIES

OBRR/CBER/FDA WORKSHOP

Tuesday, December 4, 2001

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C O N T E N T S

IV. Progress Towards Single Unit NAT (Yuwen/Li)

C O N T E N T S (Continued)

P R O C E E D I N G S

Opening Remarks

DR. EPSTEIN: Good morning. Welcome to our two-day workshop on the application of a nucleic acid-based test for the blood supply. I am especially pleased to welcome here the participation of investigators from other countries who have been in the vanguard of research to evaluate the use of nucleic acid tests to screen blood and plasma for relevant viral agents. In particular, we are pleased to have several representatives from Europe and Japan at the workshop and we are looking forward to hearing about their extensive experience with NAT.

CBER began meeting with industry representatives in 1998 in order to guide the development of nucleic acid testing. At this time, nearly all the blood and plasma collected in the United States is being screened by minipool methods using NAT for hepatitis C and HIV. Some plasma is also being screened for hepatitis B by minipool NAT.

It is also, I think, a landmark that on September 18th FDA approved two NAT procedures for screening source plasma by minipool testing, those being the tests of National Genetics Institute, the so-called UltraQual HIV-1 and HCV reverse transcription polymerase chain reaction assays, and one company, Alpha Therapeutic Corporation, is

licensed for use of these procedures in screening of source plasma.

FDA is developing a draft guidance on the implementation of licensed NAT for screening minipools of source plasma, which is in the final stages of preparation and the concepts will be discussed later in the workshop by Dr. Mied. The processing of blood and plasma collected in the United States includes screening by highly sensitive, so-called conventional immunoassays which are able to eliminate units harboring infection for hepatitis C, HIV and hepatitis B. It needs to be recognized that the technology to detect these viruses already permits a very high degree of safety. Nevertheless, we know that some infectious units do escape detection by immunoassays. The nucleic acid methods permit us to eliminate many of these remaining infectious units due to their remarkable sensitivity. Indeed, it is now possible to detect, at 95 percent confidence, as few as 10 copies per milliliter of these agents using nucleic acid test methods. Indeed, the sensitivity is now limited mainly by dilutional factors such as used in the minipool format. So, we expect that the progression from the minipool to the single unit NAT, when technologically and practically feasible, will make it possible to eliminate virtually all window period donations.

We all know that plasma derivatives are subjected to procedures that would remove or inactivate all hepatitis C, HIV and hepatitis B when performed appropriately. However, FDA does see the highly sensitive screening as a necessary additional safeguard which serves to limit the viral burden in any fractionation pool, thereby helping to assure the sufficiency of inactivation procedures.

So, today's and tomorrow's agendas have been well crafted to address a spectrum of issues. I am just going to note what they are. We will review the experience to date with the implementation of NAT for hepatitis C, HIV and hepatitis B; the status of standardization of NAT testing which, you know, has been a major international cooperative effort; progress toward the development of single-unit NAT; the salient differences for NAT directed to hepatitis A and parvovirus B19 as they night be implemented in the donor and manufacturing setting; the potential for NAT to replace certain existing tests, such as the HIV-1 p24 antigen test; additional infectious agents for which NAT screening may be possible, including emerging pathogens; and the use of new technologies to make high throughput NAT even more cost effective.

Personally, I am looking forward to a highly productive exchange of information at this workshop, and I hope that this meeting will again enable FDA and the blood

industry to move quickly and effectively toward full implementation of NAT in a way that best serve the public health.

So, again, my warmest welcome and I will call on Dr. Michael Busch, from Blood Centers of the Pacific, who will speak to us on an overview of NAT and reduction of residual risk in infectious disease transmission. Mike?

Overview of NAT and Reduction of Residual Risk in Infectious Disease Transmission

DR. BUSCH: Thank you, Jay.

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It is my privilege to be able to briefly review the history of screening, the great success story that blood screening has achieved, and talk particularly about the issues around the staging of early infection, particularly focusing on HIV, HCV and HBV. Then, what I will do is briefly address the current issue. As we have moved to these highly sensitive NAT assays from minipool and considerations of individual donation, the question has arisen as to when infectivity develops in very early infection.

So, I will address some of the issues and some of the current studies that are trying to better clarify the relationship between infectivity and RNA during the very early window phases. Then I will review the international

experience with NAT yield, compiling data from around the world, particularly the whole blood sector, and then close briefly with just a little bit of discussion about cost effectiveness, not so much to necessarily move us away from the direction in which we are going, which is progressive implementation of these assays, but more to juxtapose the enormous resources we are putting here with what is actually an incredibly difficult problem in other countries where, in fact, nucleic acid testing could have a dramatic yield. Then, I will finally just mention some of the other research applications that come out of the wide-scale implementations of NAT screening.

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This slide is one that many of you have seen. The only difference is up here, where I have actually added in a series of assays as they have been implemented over the last three decades, moving from the introduction of HIV antibody to surrogate tests for hepatitis, to HTLV, progressive improved generation HCV assays, HIV-1,2 testing, p24 antigen, HCV-3.0, HTLV-1,2 and finally the NAT assays, here. Through the progressive implementation of these enhanced detection, mostly serologic and now nucleic acid assays, we have achieved dramatic reduction in the risk for HIV and HCV. We are now in the range of one in a million. There is, however, residual risk, as we all know,

particularly in the pre-NAT screening era, and we now appreciate that the vast majority of this risk comes from window phase donations. These other concerns around variant viral strains, chronic antibody negative carriers and errors in testing did exist with serologic tests but have essentially been eliminated with the introduction of NAT because NAT really is a redundant system that inherently is able to independently detect these other types of risk sources.

So, the major focus over the last few years has been on the window period, and as we have struggled to understand the dynamics of early infection and with implementation of NAT and detection of individuals both in the plasma and whole blood sector who are viremic and seronegative, we have been able to more carefully study the primary infection phases.

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Through those studies we have defined a series of evolution of phases, beginning with what is termed the eclipse phase, the period of exposure to the first detectable viremia which seems to last typically for days to weeks. During this period, we think the viruses are replicating either in liver tissue or in lymphoid tissue in the inoculation site before the virus really disseminates into the plasma. It is during this period that one cannot

detect viral nucleic acid essentially by definition, and at present most of the risk models assume infectivity exists during the eclipse period. We will come back to this because I think recent data suggests that is probably not quite the case, and this may explain why some of the current estimates of risk seem to be out of line with regard to the observed residual infections that are taking place.

One of the things both our group and several others, and Dr. Nubling in Germany, have uncovered is a phenomenon called pre-ramp-up viremia which is kind of a smoldering low-level virus with intermittent detection prior to this exponential increase in viral load to ramp-up viremia. For HCV, we know there is a long plateau phase. For most of the other viruses there is peak viremia, followed by a drop in viral load associated with seroconversion. As I will illustrate, in a number of infections we do see, around the time of seroconversion, dramatic fluctuation in viral load that we term now peri-seroconversion viremia, presumably reflecting the immune system's effort to try to resolve the infection. Then, after seroconversion individuals can become persistent carriers or, in the case of hepatitis C or hepatitis B, some do resolve the infection. There are rare cases that

have been uncovered, and I will illustrate some, of immunosilent or transient viremia.

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I will quickly buzz through these for each virus. This is HIV. As we know, there is a period typically of about ten days from exposure to ramp-up viremia. There is, in some cases, as we have uncovered in collaboration with Alpha and NGI, a transient blip phase viremia that seems to occur shortly after exposure. Then there is the eclipse period followed by the ramp-up. As we know, depending on the sensitivity of the individual minipool p24 antigen, one can detect this exponential increase in viremia, and then progressively the detection of antibody by first, improved second and finally third generation antibody assays.

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Just one illustration of an HIV pre-ramp-up blip viremia case, in this case this plasma donor was identified by minipool NAT screening at what is defined here as day zero. At that point their viral load was around 260 and they exponentially increased to ramp-up over the ensuing week. What was of interest was that as we tested back samples extending several weeks prior to this ramp-up phase, we detected a reproducible transient viremia, again, about 20 days prior to this ramp-up viremia. This was reproducible on multiple runs and seems to be a real

phenomenon. Again, by sequence analysis in one of these cases the virus is identical and highly homogeneous, consistent with exactly what evolved with ramp-up phase, which is probably consistent more with an early replication phenomenon than the inoculum itself.

In all the viruses we have done a lot of work to compile large numbers of ramp-up phase panels that allow us to derive an estimate for the exponential increase, termed the doubling time, as illustrated here for HIV. It is fairly brisk, around 21 hours. It is this kind of analysis and model that allows us to model the relative detection by minipool versus individual, as I will summarize later.

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For hepatitis C, there is a different phenomenon in that again there seems to be a pre-seroconversion blip. It is much more intermittent and tends to extend over periods of weeks and occasionally months. Then, there is this very rapid ramp-up phase, averaging about 17 hours of doubling time. So, there is a very minimal difference in days to detection between individual and minipool NAT and even the newer HCV antigen tests, as we will hear tomorrow, can detect viremia within days of nucleic acid detectability.

What is remarkable for HCV is this very prolonged, almost two-month high titer plateau phase

viremia. It is really this viremia that resulted in the high level transmission of HCV in the setting of serologic screening, and has translated into a fairly high yield of pooled NAT almost irrespective of pool size. This is followed by a brief rise in ALT about ten days before antibody conversion, which typically occurs about 70 days out with the first generation assays but the second and third generation HCV antibody tests had dramatically less sensitivity.

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This is just an illustration of an HCV ramp-up viremia. In this case, this donor was detected as ramp-up phase at day zero by, again, NGI minipool NAT and Alpha panel, followed by the early plateau phase. This is well before seroconversion. Testing back the stored plasma from several months prior to seroconversion demonstrated intermittent detection of HCV RNA. In this case we used the Gen Probe TMA assay. Replicate analysis was performed on each of these donations, and what we are showing here is the percentage of four replicates that were detected by the high input qualitative assay. You see that we went through a week here where three or four reps were detected; then the person went negative; then another week where we could detect the low-level viremia; then negative; then another blip of viremia. This was corroborated by NGI PCR well

below the limit of quantitation, so all less than 100 copies. The contribution of this blip viremia to residual risk really awaits the determination of the infectivity of this phenomenon.

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In terms of the plateau phase in a few outlier types of cases, this is data from Sue Stramer, the Red Cross experience with bona fide not only yield cases, a total of 35 cases. These are plotted here by day of the first NAT positive, antibody negative donation. The yellow portion of each bar represents the period when the donors were viremic prior to seroconversion. The red at the end of virtually all these bars represents the date when they became seropositive. You can see that these average about 50 days following NAT detection that these donors seroconverted.

I want to focus your attention on these several outlier cases towards the bottom. These represent two individuals who are what we are called immunosilent carriers. They have been viremic and seronegative. Each of these hatches represents samples that were corroborated to be viremic and seronegative for well over a year in this case and over two years in this case. So, these are bona fide immunosilent carriers that we are trying to understand the immunologic basis for. These are clearly another

contribution of NAT. These people probably were transmitting infection serially, and this has been documented in a case from France where a donor who was persistent seronegative, on look-back follow-up transmitted infection to a number of recipients.

There are also some unusual cases here where donors were viremic and then went negative for RNA and then, in fact, in one case went negative for virus by antibody and RNA and then became viremic again. So, there are some unusual intermittent phenomena that we are uncovering as we implement wide-scale NAT and enroll and follow these donors.

[Slide]

Just one example of a case of what we are terming fluctuating viremia around the time of seroconversion. This was a donor identified in Florida through the Gen Probe screening system. This donor was detected as high-level viremic by TMA. The viral loads have fluctuated around the time prior to seroconversion and, in fact, even TMA went negative right at the time of seroconversion as antibody kicked in. This is just one example of how viral load can fluctuate dramatically during the peri-seroconversion phase. [Slide]

I will just briefly touch on hepatitis B. That will be discussed in more detail by Sue tomorrow. With

HBV, we have also uncovered examples where there is intermittent pre-ramp-up viremia, in this case about two months, a month and a half prior to antigenemia. This is surface antigen coming up in yellow. That is followed by a typical ramp-up phase with viral loads increasing exponentially, a doubling time of about four days for HBV.

[Slide]

This is again a slide from Sue, which she will focus on but basically illustrating that because the doubling time is relatively slow there is a fairly long period of about 20 days when you can detect viremia by individual donation NAT before it reaches levels detected by minipool NAT, shown by the line here. Then, the improved antigen assays have substantially reduced the pre-antigenemic phase by virtue of detection of lower levels of viremia. This will be discussed in detail tomorrow in the implications for why we are not doing HBV minipool NAT.

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This slide just summarizes the data from the panels that we have studied. Again, HCV is unique in having this plateau phase. The doubling times for each virus and the frequency of detecting this pre-ramp-up viremia -- again, we see it in about a third of the HIV cases; a good half or so of the HCV cases and, again, about

half of the HBV cases seem to evidence this intermittent fluctuating viremia during the eclipse phase.

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This is just to illustrate that using the viral load ramp-up dynamics we can then model out the impact of reducing the sensitivity or enhancing the sensitivity from minipool to individual or NAT versus antigen, and by knowing the sensitivity of the assays we can derive an estimate for the window period closure one achieves by moving toward a more sensitive test. It is that window period closure which, when combined with incidence rates, can estimate the yield of that transition.

So, as Jay indicated, there continues to be interest in moving to individual donation NAT for minipool and this is just an example of what the projected impact would be of detecting ramp-up viremia, were one to do so, given the model estimates of the window period closure that would be achieved by moving from, for example, pools of 16-24 down to individual samples. So, what you can see here is that for hep C and HIV, because the viral load ramps up so quickly, less than a day, the window period closure by enhancing the sensitivity of the assay is about 20-fold. There is only about three to four days. These viruses have similar incidence rates of about 4/100,000. So, that really only translates into a projected two additional

infections detected per ten million donations, which is about the U.S. whole blood collection -- so, fairly modest improvement with respect to HCV and HIV attributable to going to individual donation. For HBV, in contrast, because the doubling time is slower, the enhanced window closure would be more dramatic, about 12 days, and the yield would be a little higher.

[Slide]

This just briefly summarizes the current estimates of risk for each virus in the pre-NAT, the minipool NAT and the single donation era. Again, these numbers, expressed both as number of cases detected per million as well as rates in 1/1.3 million, for example, for HIV, all assume that the pre-seroconversion eclipse phase may be infectious. Again, I think that is a question we will focus on now, but what you can see is that we have moved from risks in the range of one in a million for HIV and one in about 100,000 to about 200,000 for HCV and HBV. As we implement minipool NAT for HIV and HCV those risks have dropped to one in two million or so, and going to individual NAT will drop them a little bit lower, in the range of one in three million.

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Again, the question that has been posed, I think, most critically by Mico Leli is whether we are really sure

that that pre-ramp-up phase is infectious. As we have moved from serologic testing or antigen assays to various pool size sensitivity nucleic acid tests, the question has been raised as to whether infectivity, in fact, is present at all of these levels of viremia, and also whether infectivity may exist even in the pre-ramp-up phase, the eclipse phase, and are these blips infectious.

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Toward that end, there have been studies undertaken, and some others are in progress, to try to better understand this relationship between infectivity and viral load during the pre-seroconversion period. I am just going to touch on these for lack of time. The first approach is to simply take plasma stocks that have been titered out in chimps to define chimp infectivity concentrations. By correlating the NAT detection of those assays, either by doing quantitative viral load on the starting stock or by testing, by the qualitative NAT assays, serial dilutions of these pedigreed stocks, one can estimate how many genome equivalents correspond to a chimp infections dose.

In those studies, although they are limited, and the precision of the titration of these panels is limited, they suggest that for HBV and HCV as little as ten genome equivalents in an inoculum, in the total volume of plasma

introduced into the recipient, would be equivalent to one chimp infectious dose 50. So, this suggests that for window phase HBV and HCV extremely infectious and essentially all viral particles probably are capable of transmitting, and very low concentrations are able to transmit.

For HIV, the chimp model is relatively less susceptible and there are issues around potential defective virus. So, those studies suggest that 1000 to 10,000 copies may be required to transmit in the chimp model.

Other studies have been undertaken -- this well-known study by Harvey Alter, where you can infect an animal, and this can be done with chimps and HIV or HCV or with SIV models, for example. The animal can be infected and then sampled serially, plasma and cells, purified and stored away. Those samples can be characterized by NAT and antigen and antibody tests. Then one can transfuse those serial samples, stored unit volumes of material from the eclipse phase through the seroconversion phase, in secondary test animals and correlate detection of viremia with development of antibody.

This was a study by Chris Murthy, the first author, published in Transfusion a few years ago, that looked at this for HIV and demonstrated that there was no ability to transmit until the animal became viremic as

detectable by NAT. So, through the eclipse phase there did not seem to be infectivity.

One of the concerns though is that the chimp isn't highly susceptible to primary HIV. So, in order to further address that, what is going on now are studies where we are using these plasma donor panels and actually infusing material from the plasma panels into chimps.

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This is, again, a study with Harvey Alter and Chris Murthy. This is just to illustrate the design. So, we are really trying to understand the phenomenon of the blips within the eclipse phase. So, what we are doing is transfusing 50 ml of plasma from each of five donors into chimps, beginning with samples collected prior to any detectable viremia, any blips or ramp-ups, and then going to the valley between the blips and the ramp-up and following that same animal after a period where we evaluate the transition from the initial inocula, following up with inocula from the valley between the blips and the ramp-up. Then, if that does not transmit, moving to the ramp-up itself to ask is this actually infectious in a chimpanzee. If not, then we will work our way up the ramp-up phase. So, these studies are currently under way.

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The other approach to understand infectivity is to really try to milk, if you will, the human experience that is going on all the time through transfusion. One approach is to follow, in essence, donor-directed look-back strategies where a donor seroconverts to either NAT positivity or antibody and the recipients are traced through look-back.

Now, there have been a series of cases that have been studied, and I will touch on several of these, that reported recipient infection from pre-NAT or pre-seroconversion units and then went back and have studied these prior donations to ask did they have viremia that we can detect retrospectively. In a number of countries there are stored units or there may be recovered plasma off the donation. So, this allows us to really directly ask the question of the relationship between RNA levels and detectable viremia in the human context.

Once you have data from a number of these cases, you can then begin to model the duration of the infectious window period based on the inter-donation interval between the prior donation and the first positive, and compiling the whole data set. The model for this was first presented by Lyle Peterson almost a decade ago.

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Just a few examples. This is the so-called Singapore case, which was published in JAMA about a year and a half ago. In this case, a donor seroconverted to antibody. The prior donation was determined and confirmed by molecular methods to have transmitted HIV to the recipient. We obtained that material, the plasma was available, and evaluated that by both the Chiron Gen-Probe and Roche assays, serially diluting the sample to model the pool sizes that were used.

What one can see is that out of the pool sizes that are currently employed in the U.S. of 1 to 16 and 1 to 24 both assays began to detect the viremia. This unit was estimated to have about 100 genome equivalents per milliliter. So, clear documentation of an HIV. Although in this example, this was actually prior to NAT screening. This was sort of a retrospective study of an HIV antibody seroconverter where the plasma was available.

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A second case was reported from Germany, in Lancet, where there was an HICV transmission by NAT negative blood. Again, a donor seroconverted to HCV antibody. The donation was eight weeks prior. The platelet transmitted interestingly -- platelets have about 50 ml of plasma, whereas the red cells do not -- only 5 ml of plasma. The plasma was available and was able to be

tested. In the publication it was tested by an enhanced input PCR that was modeled or projected to have sensitivity down in the range of 10-30 copies and it was negative. Interestingly, we obtained that plasma and were able to run the Gen Probe and NGI assays, and Gen Probe could detect virus, qualitative high input analysis, in two of three reps. So, again, suggesting that an infectious unit for HCV could have extremely low levels of viremia, right at the limit of detection.

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This case is probably new to most of you. This was a recent documented transmission in the U.S. It represents, to our knowledge, the first case of HIV transmission prospectively identified by NAT screened blood. This was identified at the San Antonio blood center. In this case, similar to the Singapore case, the donor seroconverted to antibody two months earlier. The donation that was transfused did infect the recipient. As I will show you in a moment the genetic studies confirmed that this was a linked transmission. In the same context, we identified the plasma component and have now run the serial dilutions by the two U.S. licensed NAT assays. Essentially identical to the Singapore case, in both assays -- here is the summary -- this sample consistently detected undiluted and high rate of detection at 1 to 8 but at 1 to

16, 1 to 24 the assays began to miss this unit -- estimated copy number, again, about 100 genome equivalent. Clearly, transmissions can occur by minipool negative blood but in the cases that have been studied aggressively we can detect viremia in transmission cases.

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This just shows the linkage, the donor and the recipient, both in the envelop and gag regions -- virtually identical. So, this is an unequivocal transmission from minipool screened blood.

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A compilation of data on a larger scale though is really needed to feed a model as Lyle Peterson developed. This is probably best illustrated by what Kurt Roth has done in Germany and Austria where, in the setting where they are doing NAT screening they have rigorously followed all prior donations from seroconverting recipients, by they NAT or antibody seroconverters. Then, when they follow those recipients, they determine whether the recipients were infected and they also have a repository so they can test the stored donation samples.

Interestingly, in this analysis of a number of seroconverters for all three viruses none of the recipients were infected. This is important to emphasize. We tend to focus on these cases where transmission did occur but there

are lots of look-backs that are done where recipients aren't infected. Again, in order to get a comprehensive understanding of the infectivity of this window, we really need to generate data like this that feeds the model with both the transmitting and non-transmitting case data.

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Finally, the other approach to human evaluation is when recipients are reported to be infected potentially by transfusion, and then the donors get investigated, the so-called recipient trace back. Here one can retest aliquots of the donation plasma from all those donors if available or recall the donors, and if a transmission does occur it is important to validate that because we all know there is a higher level of background infections now than transfusion related.

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I am not going to go through this in detail, but in a study just published in Transfusion, the Japanese investigators did a very rigorous investigation of reported transfusion cases. They had 103 reported HBV, 92 reported HCV cases and no reported HIV cases. The bottom line here was that of the 103 HBV, only 16 could be determined to really be transfusion related, and most of those would have been detected by either NAT or even contemporary surface antigen assays. Of the 92 HCV recipient infections

detected, none of them proved to be real. All of the donors were negative by NAT and on follow-up. So, just again to emphasize that not every case of post-transfusion hepatitis or HIV really is transfusion determined.

[Slide]

With Sue Stramer, just over the last week, we sort of canvassed the world about the experience with NAT and this is the whole blood sector yield. I can make this available to anyone who is interested. I think there really needs to be kind of a global collaboration to keep up to date and compile the NAT yield experience.

So, what we see here again are the various countries that have implemented NAT; the pool size currently employed; the number of units screened in each region; the number of NAT positives for hep C; and then the yield. An important observation here is that we seem to see countries like the U.S., Japan and Australia that, with HCV, have NAT yields very similar, in the range of 1 in 300,000. Germany and Austria is about 1 in 600,000. Then there are a number of countries that seem to have a much lower rate of NAT detection, in the range of less than one in a million donations. Some countries, like Holland, 2.4 million without a single yield case to date. So, there is quite a variance in yield, with really the U.S. kind of driving the high end.

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Here, HIV rates again. One in about two million in several countries, but then a number of countries again that have yet to have a single detection of an HIV NAT yield case.

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In trying to understand this variance in yield, we recognize that the model predictions actually may give us the answer, and the incidence rates model allows us to predict yield. Toward this end, this is an analysis that Sue Stramer just reported at the AABB, where she looked in the U.S. at the CDC area division of the United States, and broke her donor pool up into a pattern consistent with CDC-defined healthcare regions.

What this slide compares is the HCV incidence, estimated by the anti-HCV frequency in repeat donors, relative to the HCV NAT yield. One sees the relationship that is actually clarified on this regression map which shows, again, the HCV NAT yield and the incidence rate or anti-HCV prevalence in repeat donors. One sees a nice relationship sort of on a micro level within the U.S. itself. So, we are now trying to get incidence estimates from these various countries, and we are going to extend this analysis of NAT yield relative to incidence rates to an international perspective.

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Just briefly, a quick mention of cost effectiveness and kind of issues around the relative benefits of the different NAT target agents. Importantly -- and this is some work that is going to be coming out in Annals of Internal Medicine shortly, if you look at these various agents, we tend to group them all together and say the yield is this or that, assuming that each one is equally important. But on a clinical consequence analysis, HIV, as we all know, is really a terribly important and pathogenic virus. So, for every HIV transmission we prevent we actually save the patient society seven quality life years.

Importantly, relatively for HCV one only saves 0.6 quality life years per infection prevented. One-tenth is important from a clinical prevention perspective. In HBV one-fifth is as important as HCV. Again, we know most people resolve HBV spontaneously. So, this really fits, and it is important to take this into account when we talk about adding other agent detection -- hepatitis A, parvo-B19 -- because these are even less important clinically for the patient.

This translates out into the dollars per quality life years saved. For example, with HIV we will only detect about three additional infections by adding minipool

NAT and a total of six by going to single donation NAT relative to HCV, where we are detecting 60 or so per year with minipool NAT and, yet, the quality saved is virtually identical. Again, that is because HIV is much more important and prevents much more disease by implementing NAT.

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I am not going to go through this again. This is a paper that is in press, but the bottom line is, as we all know, this is extremely expensive technology with fairly modest yields. So, the result is that we are dealing with cost effectiveness in the range of two, three million dollars per quality life year and we are not going to do any better by going from minipool NAT with or without HBV; a little bit of benefit if we can drop some other assays like antigen and anti-core to offset the cost; and even going to single donation NAT will still leave us with a technology that is extremely expensive, in the range of two million dollars per quality life year, and we are spending just in the U.S. hundreds of millions of dollars to prevent these modest numbers of infections.

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I am sure you can't see this but I just want to juxtapose that with reality. Unfortunately, in much of the world where the incidence of these agents is much greater

and, therefore, the benefits of NAT would be dramatic. This is some data developed with Anton Hanes, in South Africa, where we did what is called the detuned assay on all of the HIV positive donors detected in about a year and a half period. In South Africa, the prevalence of HIV antibody in their donor pool is about 1.2 percent. They currently stratify their donor pool into relative prevalence groups, defined by region of the country and race ethnicity parameters, and the high prevalence groups have about a 5 percent prevalence of HIV antibody, whereas low prevalence groups are at 0.7 percent, almost the same as in developed countries.

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Now, when we did the detuned assay we could project incidence rates for each of these different groups. What you can see is that the incidence rate in some settings, like the high prevalence donors, is 1.9 percent annual incidence -- just an extraordinarily high incidence rate. If we then translate that into the window period yield by adding nucleic acid testing, they would pick up 8/10,000 donations as viremic and seronegative from this high prevalence setting. As I think we will see later in a trial that Chiron has done in South Africa, they, indeed, have picked up in the range of 1/1000 NAT positive, antibody negative donations in this high prevalence

setting. So, one of the things that I hope this meeting will at least briefly address is the technology transfer of nucleic acid testing so it can become available in these resource-poor countries. [Slide]

Finally, my last slide is just to mention that I think the availability of NAT really presents us with important opportunities to contribute to the broader research agenda around these various infections. By identifying large numbers of donors in the plasma and whole blood screening sector in these very early primary infection stages, we can help characterize the epidemiology of these infections in the broad, general populations looking at incidence trends, the demographics and the risk factors associated with acquisition of these infections.

We can contribute to the molecular surveillance, characterizing the subtypes and the resistance patterns of these newly transmitting strains in the donor pool. We can help identify patients and enroll them into pathogenesis studies, particularly important for both HIV and HCV where there is enormous interest in better understanding the mechanisms of control of viremia in early infection, contributing towards development of vaccine strategies or early treatment strategies.

There, again, I think there is just a recent New England Journal paper on the benefits of early HCV

treatment -- still controversial, but through NAT screening we are identifying hundreds of people in the viremic pre-seroconversion phase who could then be enrolled into early treatment trials to evaluate the relative benefit of early versus delayed treatment, and begin to look not only at early antiviral therapies but immune enhancement strategies, such as supplemental vaccination or immunomodulatory strategies.

So, that is the conclusion of my talk. What I have tried to do is to frame the broad issues both in terms of the window period, as well as the yield of NAT and, again, tried to focus a little bit in the end on the cost effectiveness and the important challenge of bringing this technology to developing countries. Thank you.

DR. EPSTEIN: Thank you. We are running a little bit late but perhaps one or two questions if there is a burning question. Yes, Jean-Pierre?

DR. ALLAIN: Mike, I didn't remember exactly in the Schuttler paper, in Lancet, whether the individual who received platelets was significantly or not immunosuppressed. Because I think in the infectivity studies the state of the recipient is a complicating factor and that has to be taken into consideration because the same amount of viral copies in an individual

immunosuppressed is probably more infectious than in an immunocompetent individual.

DR. BUSCH: That is an excellent point. I honestly don't recall either. It also points out that in many of our populations there are immunosuppressed patients who could have more prolonged eclipse and early phenomenon. So, what we are doing with these window period models is really using plasma donors, healthy people, community acquired infections. So, as we are looking at recipients -- you know, Harvey Alter's ongoing NIH studies -- these recipients are being monitored by nucleic acid tests in addition to serologic methods to be sure we are not missing some transmissions in that setting.

DR. EPSTEIN: Thank you very much, Mike. Now I would like to call on Harvey Alter, from the NIH, who will discuss new hepatitis viruses: Do they make sense?

New "Hepatitis" Viruses: Do They Make Sense?

DR. ALTER: Well, my talk is a little bit out of order because I have to leave for another meeting.

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So, putting these emerging agents at the beginning of this session is like putting the cart before the horse. If the horse represents me, it is because when it comes to a test of these agents I am sort of a naysayer. If the cart looks empty, it is because it is hard to

interpret the data that I will be presenting. But I think it is an interesting group of viruses.

[Slide]

In any event, I will proceed by saying that my talk will prove that there is absolutely no substitute for a genuine lack of preparation.

[Laughter]

When HCV was discovered there was some evidence, that I am not going to get into because there isn't time, to suggest that there might be additional hepatitis agents, so-called non-ABC agents. And, the commercial success of hepatitis C testing led companies to pursue the potential of the hepatitis agents.

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The first discovery, if you will, was the resurrection of the GB story. GB was a surgeon who developed acute hepatitis in the 1950's. His serum was transmitted to or put into marmosets and caused hepatitis in the marmoset model. The marmoset infection could then be serial passaged to other marmosets.

Then a tremendous debate ensued as to whether this was a human hepatitis agent that had been transmitted or a marmoset agent that had been reactivated. This was never resolved, but the debate was quite vigorous. Then this story just kind of died.

In the 1990's Abbott resurrected these serial passage samples from the marmoset and, using new technology of representational difficult analysis, and having pre and post transfusion samples from the marmoset, Abbott discovered a new agent that they termed GBV-A. GB, for the surgeon's initials; virus A. It turns out that GBV-A was a marmoset agent, but as they continued to work they found GBV-B and eventually then, using the degenerate primers between these agents and hepatitis C virus, they found GBV-C which is, indeed, a human agent. And, they showed that GBV-C is transmissible to man and to the chimpanzee.

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Well, simultaneously, Gene Labs found another agent that was called the hepatitis G virus. This is the genomic map of the hepatitis G virus. As you will see, it is very similar to hepatitis C virus because these agents, GBV and HCV and HGV are flaviviruses, very similar to HCV but the one difference is that they seem to lack a core region. Otherwise, the non-structural regions and the envelope region seem very similar. One can speculate that it is the absence of this core region that might attenuate the pathogenicity of these agents, but nobody has proven that. But what was shown is that GVC and HGV are essentially the same agent, just strain variants of the same agent discovered by different companies.

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We then began to look at HGV in particular but the data are interchangeable. What you find is that in the volunteer donor population the rate of viremia was somewhere between one to two percent, and an additional relatively small number had antibody to HGV. So, the total burden in the donor population was not excessively high. But when we went to commercial donors you can see that there was a high rate of viremia, 17 percent; 57 percent had been exposed to these agents. When you went to intravenous drug use, 15 percent were carriers and 82 percent overall had been exposed. So, these were parenterally treated donors.

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We then looked at our prospective study of donors and recipients and we found that we only had 13 cases of non-ABC hepatitis in the prospective studies. Twenty-three percent of these patients developed an acute HGV infection in the wake of blood transfusion. However, this rate was not significantly different than the rate of new HGV infections, patients who had minor ALT raises that we didn't really consider post-transfusion hepatitis or patients who had no hepatitis at all. It looks like more but the numbers are small and statistically these are not different. The only difference was that these agents were

clearly much higher in the patients who were not transfused. So, this was a transmissible disease that was not clearly associated with non-ABC hepatitis.

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Overall, we calculated that since the number of hepatitis cases was small and the number of non-hepatitis cases was very large that even if HGV was a cause of hepatitis, no more than four percent of HGV infections would have transmitted hepatitis and the vast majority of people who got this infection either had no hepatitis or had coexistent hepatitis C.

[Slide]

Time is short and I am not going to show you data, but we then went on to show that we couldn't find this agent in the liver, or the techniques were not good. But we did look at different disease states. This actually is a study from Thomas and Hadzylannis, in England, and it showed that the rate of HGV in patients with acute non-AB hepatitis or chronic hepatitis was relatively low and that it didn't differ from the rate in patients who had immune hepatitis or alcoholic group of diseases or chronic HBV or HCV. So, there is no specific hepatitis association of these agents.

[Slide]

So, we kind of dropped the HGV story. It seemed like an unimportant virus. But just recently there have been two studies reported in The New England Journal of Medicine. This is the first one from Tillman, in Germany. This is Michael Monza's group. For reasons not clear, they looked at cases of HIV infection and looked at those who were co-infected with GBV-C in this case, but synonymous with HCV, and basically showed that if you were infected with HIV and then co-infected with GBV-C your survival was very significantly improved over patients who had never been exposed to GBV-C, and intermediate were patients who had been previously exposed who had antibody to the envelope GBV-C or were not viremic. A very striking difference.

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That difference was shown even in patients who had already developed AIDS, in patients who already had AIDS. If you were GBV-C positive your survival was much longer than patients who were GBV-C negative or had prior exposure to GBV.

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One could be skeptical about a finding that one doesn't expect, but simultaneously Jack Stapleton's group, in Iowa, and Dr. Xiang found exactly the same thing in another large HIV cohort. Patients who were GBV-C/HIV co-

infected have a significantly better survival than patients who were GBV-C negative.

[Slide]

Stapleton then went on to see if he could figure out why and they did in vitro studies where they used peripheral blood mononuclear cells and infected them with HIV, and then used an infectious clone for GBV-C. Basically, what this shows is the six-day production of p24 antigen in cultured mononuclear cells. This would be HIV alone, so a high level p24 antigen; a significantly lower level of p24 antigen. This is only about a third of the production in patients who were given HGV in cells that were inoculated with HIV and GBV-C simultaneously, or cells that were given HIV first, followed 24 hours later by GBV-C.

What was dramatic is that if the cells were first infected with GBV-C and then HIV inoculated 24 hours later, there was a 98 percent reduction in p24 antigen production. So, there was an in vitro inhibition, and this is the mock infection so it is almost the same as a mock infection. They speculated that somehow GBV-C/HGB was inhibiting the replication of HIV; that it wasn't a matter of cell surface receptor or entry into the cell; it was a matter of intracellular replication by mechanisms which aren't yet clear.

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So, we have been scratching our heads about this. It doesn't make a lot of sense but it probably is true. It may be the production of intracellular cytokines from one viral agent interfering with another, but we don't really know at this point.

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There are a lot of things we don't know, such as why they sterilize needles for lethal injections.

[Laughter]

[Slide]

Anyway, that is where the HGV story is right now. Subsequently, a Japanese group with Dr. Nishizawa reported a new agent called TT virus -- TT for the initials of the first patient in which this virus was found. This is three patients out of five that they studied with post-transfusion hepatitis. Shown here, in blue, are the ALT levels and, in yellow, the level of TT viremia.

What you can see is that post-transfusion there is the appearance of this new agent and a rough correlation with ALT levels, sometimes a very close correlation. They suggested that TT was a new agent of post-transfusion hepatitis accounting for the non-ABC cases.

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As it turns out, the characteristics of TTV are that it is a very small virus, about 3700 nucleotides. It is non-envelope so it has proved to be an important agent. It would not be killed by solvent detergent. It is a single-stranded, circular DNA virus. It is clearly transmitted parenterally but there is also evidence that it is transmitted fecaly/orally. And, there is evidence for this agent that it does replicate in the liver. There are now an increasing number of members in this group, including the prototype TTV virus, some later Japanese isolates named YONBAN and SANBAN, the SEN agent that I will talk about and many others. So, this family is now felt to be members of the circoviridae which are classically animal and plant viruses.

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The prevalence of TTV is higher than HTV, about 7.5 percent of the volunteer donors in the U.S. This is patients sampled prior to transfusion, so somewhere between seven and ten percent of the population is TTV infected. But this was using initial primers. The Japanese used a more inclusive primer set and found that over 19 percent of Japanese donors were TTV infected. I am sure if we had used those primers in the U.S. population these numbers would be higher. So, it is a very prevalent agent.

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In this study, as we did with HTV, we then looked at patients who had been prospectively followed post-transfusion. What we found is that among transfused patients 26 percent were TTV negative prior to transfusion and developed an acute TTV infection following transfusion. This difference was highly significant. This was highly significantly different from those who were not transfused, but almost five percent of patients who were not transfused also acquired a new TTV infection while in the hospital so this has a nosocomial route of transmission that is quite prevalent.

[Slide]

I didn't show you on a prior slide to save time that clearly this was a transfusion transmitted disease, and we could sequence donor and recipient samples and prove that there was donor-recipient transmission. But, again, the key was to look at the patients who had non-A-to-G hepatitis versus those who were transfused and had no hepatitis. There is absolutely no difference in the rate of TTV infection. The rate in patients who developed hepatitis C appeared higher but it was not significantly higher, and it is because this group received more blood transfusions. So, we could show no direct association with post-transfusion of non-ABC hepatitis.

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We looked at patients who had hepatitis C infection. There was no impact of coexistent TTV infection either on the peak of the ALT level or in the rate of chronic HCV infection. So, these agents were the same; had no apparent impact on other known hepatitis viruses.

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So, this looks like deja-G all over again and we were ready to bury the whole other viruses story when Danieli Primi approached us with still a new agent that he called SEN, again the initials of the patient, SEN. He swore this was not TTV. He had discovered this agent using the highly degenerate primers from TTV but felt this was not TTV. Well, it turns out that SEN is a highly diverse family. Shown here in pink is the total diversity of the hepatitis C virus. You can see the diversity of the SEN family is dramatically more so than HCV. Members of this same family differ from each other by 50 percent in nucleotide sequences.

[Slide]

I hate to show phylogenetic maps but just quickly to give you the idea that there seemed to be -- and this is from Dr. Tanaka in our own group -- there appear to be four major groups of these circoviruses. Here is TTV in group 2. Here is the YONBAN, the second finding of the Japanese. Here is still another Japanese agent called PMV. Here is

the SEN family which also includes the Japanese-discovered agent TUS. You can see how diverse the SEN family is and how far away it is from TTV. Yet, these are all circoviridae and they have similar physical characteristics.

[Slide]

When one goes to the same algorithms for SEN, you find it in low prevalence in the U.S. donor population, volunteer population, about two percent, but very high prevalence in the Japanese population. All of these circoviridae are more prevalent, highly more prevalent in Japan for reasons which aren't clear. When you look at parenterally exposed groups where the transfused subjects are drug users or dialyses patients, again, very high rates of transmission and prevalence.

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The same procedures with SEN came up with a different outcome. Again, when we looked at transfused subjects versus non-transfused subjects 30 percent of transfused subjects developed a new SEN infection, again highly different than the non-transfused subjects but, again, there was a background transmission, a nosocomial transmission even in those not transfused.

[Slide]

But the difference was in this slide. When we looked at 12 of those same 13 cases of post-transfusion non-ABC hepatitis, 11 out of 12, or 92 percent of them, developed a new SEN infection post transfusion. We were looking at only two of the SEN agents, SEN-D and SEN-H, because we had previously shown that these agents were in relatively low prevalence of the donor supply and seemed to associate with the hepatitis cases.

So, we selected the agents that might be most likely to pay off, and it seemed to pay off in that there was a high rate of positivity in the hepatitis cases; a significantly lower rate in the non-hepatitis cases, but the rate was still high in patients who didn't get hepatitis. Again, because this group was large and this group was small, one could calculate that no more than five percent of all SEN infections would have been associated with hepatitis. It was co-associated with hepatitis C virus, and here is the rate of non-transfused people. So, we are stuck with a significant association with post-transfusion non-ABC hepatitis, but that doesn't necessarily mean that it is a causal association.

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When we looked at people with other liver diseases the rate of SEN infection, the same two agents, was identical in patients who had non-AB hepatitis. We

looked at patients with acute liver failure because most of these cases are unexplained. It doesn't look like SEN is the answer to these, and the rates in hepatitis B and C and even non-viral liver disease are all the same. So, the only association of these SEN agents with hepatitis is that they were found in a very high rate in this particular group of post-transfusion cases. We are waiting for analysis of the TTV study that Blaine Hollinger is doing to see if a similar finding will be forthcoming in that study.

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In conclusion, we don't really know what these agents mean. Despite the fact that we found these cases, I don't really think this was the cause of their hepatitis. I think it is somehow an artifact of our selection process, but it is very hard to rule that out. We are going to discover molecular agents. The sensitivity of our techniques is so high now that we will continue to discover new agents, and there are three possibilities for these new agents.

One is that they are not pathogenic; that these really represent commensal agents or, if you will, a normal viral flora. Perhaps these agents have other purposes. Perhaps they are there for evolutionary purposes. So, that is one possibility.

Secondly, it is possible that most members of these families are benign; that there may be some variants that cause disease of the liver, as perhaps in DNH, but also of other organs. There are many unexplained diseases. Are these an agent of some unexplained disease? There is no evidence for that as yet. But it is difficult to study this because the prevalence is so high. You have to find this enormously high association with a disease to work on causality.

Lastly, they may cause disease only in persons who are uniquely susceptible, either genetically susceptible or by immune deficiency or by some other susceptibility factor. Thus far there is no evidence for this and they don't appear to cause more disease in patients, like HIV-infected patients. But it is very difficult to prove any one of these three hypothesis.

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But we are working on it and, as a government worker, I want you to know that I give a hundred percent effort to whatever I do.

[Laughter]

We have distributed funding and today happens to be a 23 percent day so you are getting a pretty good effort. Thank you very much.

DR. EPSTEIN: Harvey, thank you. That was both informative and entertaining. Again, one or two questions. Steve?

DR. KLEINMAN: Steve Kleinman. Harvey, I know some years ago you reported CMV hepatitis in your patients. You know, maybe that is an analogy for SENV where it is a virus that really doesn't cause hepatitis in most people and, if it does, it can just cause mild hepatitis and, in fact, you don't see any chronic sequelae because it is a rare event and it is really clinically not a very significant event.

DR. ALTER: Yes. When I grew up, that was a $64 dollar question; now it is a 64 million dollar question. It is hard to rule out the possibility that it only causes disease in an occasional patient or even that it causes diseases that we are not looking for right now. There is no evidence now that we should be testing. But the counter argument is always, "well, what if?" What if it might do something.

DR. KLEINMAN: But not even going there, just to the extent that you have a clinical finding that you need to explain and you can explain it as an unusual manifestation of this viral infection that isn't very clinically significant. I mean, that is why you get CMV

hepatitis; it happens but nobody really thinks of CMV as being a hepatitis agent.

DR. ALTER: Well, you do know that hepatitis is part of the CMV story and in some people it can predominate. So, there is a little difference but I can't rule it out. That is why we have been reluctant to give up on these viruses, because there may be something there. They could be a very interesting class of viruses, and why do we have all these viruses in our body? That is what intrigues me.

DR. EPSTEIN: I don't think we should give up on viruses that haven't given up on us. Any other questions? No? Thank you again. We are going to move on to our next set of speakers on the topic of regulatory issues and perspectives, and Dr. Paul Mied will first talk about the FDA agenda on implementation of NAT for hepatitis C and HIV. Paul?

I. Regulatory Issues and Perspectives

US FDA: Implementation of NAT for HCV and HIV

DR. MIED: Thank you, Dr. Epstein.

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This morning I would like to briefly outline for you FDA's current thinking on implementation of NAT on pooled source plasma and whole blood samples, HIV RNA and HCV RNA.

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By way of background, let me discuss some FDA perspectives that were summarized, the recent history and the current regulatory status of pooled sample NAT. Back in September of 1994, we held a workshop to discuss the potential application of nucleic acid based methods to the screening of blood and plasma donations for HIV. It was felt at that time that although these methods were clearly sensitive, they were not ready for implementation on a large scale. But the industry actively pursued the development of NAT for screening blood and plasma donors. Due to the cost and labor intensiveness of NAT, there was much interest in testing pools of plasma samples, or minipools by NAT.

By 1997, some manufacturers in Europe had voluntarily instituted NAT on minipools. About that time, the European Union issued a directive, and this directive stated that by July 1, 1999 HCV RNA testing would be required in Europe for all plasma for fractionation, and that the requirement for HIV-1 RNA testing would follow at a later date. In the United States NAT or minipools were first introduced as an in-process control test for plasma for fractionation.

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However, the European directive and our own position at that time that pooled sample testing by NAT for HIV or HCV constituted a form of donor screening provided impetus for the rapid development of NAT for all blood and plasma donations. We have taken the position that NAT tests used to screen blood and plasma for HIV RNA and HCV RNA are subject to regulation as biological products under the licensing mechanism.

NAT screening of blood and plasma donations was expected to improve blood safety while not interfering with current measures of safety. Therefore, we permitted the clinical study of this investigational technology on a large scale under IND. Such large-scale studies were necessary to demonstrate the efficacy of NAT in further reducing the residual risk of infectious disease transmission primarily because of the low frequency of window period donations. Testing of pooled specimens rather than single donations was identified as a preferred format due to the technical state of the development of NAT. Test kit manufacturers and testing laboratories submitted INDs describing their test method and validation at the preclinical level. Blood organizations and establishments intending to use the assay for donor screening also filed INDs to describe their clinical trial protocol for validation of pooled sample NAT. So, donor

screening by NAT for HIV and HCV on pooled specimens was implemented nationwide under the IND mechanism.

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On November 26, 1999 FDA published a draft guidance for industry application of current statutory authority to nucleic acid testing of pooled plasma. In this guidance document we provided industry with alternative pathways for regulatory approval of pooled sample NAT tests for HIV and HCV to screen blood and plasma donations.

On December 14, 1999 we published a guidance for industry on the validation of NAT methods to screen plasma donors in the manufacture and clinical evaluation of in vitro tests to detect nucleic acid sequences of HIV-1 and 2. This document provided guidance on manufacturing and clinical trials to obtain licensure of the test method for use in donor screening for transfusion-transmitted viruses. In this document FDA also discussed standards for NAT methods used to test pooled plasma.

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As Dr. Epstein mentioned, on September 18, 2001 FDA licensed the first NAT system. This is the NGI UltraQual HIV-1 and HCV RT-PCR assays. These assays are performed in-house by NGI. And, Alpha Therapeutic Corporation was licensed to use the NGI assays for

qualitative testing of pooled samples from donors of source plasma. I should mention that data collected by NGI under IND and submitted in their license application demonstrated that the licensed NGI HIV NAT method is sufficiently sensitive to replace p24 antigen screening of pooled samples of source plasma.

Also as Dr. Epstein mentioned, at the present time nearly all source plasma and whole blood collected in the U.S. are tested for HIV-1 RNA and HCV RNA, and this testing is being done using the licensed NGI pooled sample NAT method or under an approved IND for pooled sample NAT.

Now that a NAT method has been licensed, I am going to outline FDA's current thinking on NAT testing, product management and donor management using a licensed method for NAT on pooled source plasma or whole blood samples for HIV RNA and HCV RNA. FDA intends that these elements of our current thinking form the basis for a draft guidance document in the near future that provides recommendations to blood and plasma establishments, manufacturers and testing laboratories that are implementing a licensed NAT method.

At the March, 2001 meeting of the Blood Products Advisory Committee FDA proposed uniform algorithms for management of whole blood and source plasma donations tested by pooled sample NAT and a multiplex test for both

HIV RNA and HCV RNA. If a reactive net result is obtained for a master pool, it is appropriate to perform subsequent testing to identify the individual unit that is positive as the basis for the reactive result on the pool. The focus of the FDA proposed algorithms was the action that should be taken in the event of discrepant testing results, such as when the master pool was reactive but individual donations are then found to be non-reactive.

Data generated using NAT under IND showed that in each discrepant case it was the master pool that was falsely reactive. This was due to contamination either during specimen handling or during the assay run. False negatives on individual donations have not been seen in the studies performed using various NAT methods under IND.

The Blood Products Advisory Committee felt that in each case in which there was a discrepancy between the master pool reactive NAT result and the negative NAT results on individual donations, the negative NAT result on individual donations should be considered the definitive test result. Therefore, units could be released on the basis of the individual donation test results.

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This first algorithm is likely to be used by establishments testing smaller pools, for example, pools of 16 or 24 samples from whole blood or source plasma

donations. Those establishments would most likely prefer to directly test all individual donations from which samples were originally pooled to make the NAT reactive master pool.

Now, if you are one of those blood establishments, you should test the individual donations using the same NAT method -- primers, probes, etc. -- that was used in the original NAT on the original master pool. If one or more of those individual donations is reactive you may release the non-reactive donations provided serologic tests on those donations are negative. You should discard the reactive donation, defer the donor indefinitely, and test the reactive donation using a discriminatory NAT. That is essentially the same NAT test for the RNA of the individual viruses.

If the discriminatory NAT is positive for HIV RNA or HCV RNA, you should notify and counsel the donor regarding the meaning of the test result and the need for medical referral so that follow-up testing may be performed. You should also perform look-back for HIV or HCV to identify prior donations from that donor made within a certain time period. False-positive NAT results have been known to occur. Therefore, the donor may be eligible for reentry depending upon serological test results and other criteria.

If the donation is negative for both HIV RNA and HCV RNA, you should test the donation using an additional NAT. That is a NAT that uses an amplification technology and/or primers that are different from those that were used in the original NAT on the master pool. If the additional NAT is positive, you should defer the donor permanently; notify or refer the donor for medical follow-up and perform look-back for HIV or HCV. If the additional NAT is negative, you should notify and appropriately counsel the donor, and that donor may be eligible for reentry.

On the other hand, if all individual donations are non-reactive, our current thinking is to permit the release of all donations provided, of course, serologic tests for HIV and HCV on those donations are negative. However, as part of an overall quality assurance program, we encourage you to conduct additional testing to determine the cause of the initial reactivity of the master pool.

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The second alternative algorithm is more likely to be used by source plasma establishments that prefer to perform a deconstruction of the master pool that contains a larger number of donations by testing archived or freshly pooled subpools to identify the reactive individual donation. You should test the archived or freshly made subpools using the same NAT method -- primers, probes, etc.

-- that was used in the original NAT on the master pool. This deconstruction of the master pool to determine the basis for the reactivity may actually involve several layers of testing using archived or freshly pooled subpools. If one or more of the subpools is reactive, you may release the donations that comprise the non-reactive subpools provided, of course, serologic tests on those donations are negative.

You should test the individual donations that comprise the reactive subpool using the same NAT method that was used in the original NAT on the master pool. If one or more of the individual donations is reactive, you may release the non-reactive donations provided, of course, that serologic tests on those donations are negative.

You should discard the reactive donation; defer the donor indefinitely; and test the reactive donation using a discriminatory NAT. The rest of the algorithm is the same as the first algorithm that went directly from the master pool to testing of individual donations.

On the other hand, if all subpools are non-reactive, our current thinking is to permit the release of all donations that comprise the non-reactive subpools provided, of course, that serologic tests for HIV and HCV on all donations in those subpools are negative. However, as before, as part of an overall quality assurance program,

we encourage you to conduct additional testing to determine the cause of the initial reactivity of the master pool.

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FDA's current thinking is to include recommendations for donor reentry into a draft guidance document on NAT implementation. We are also considering using that draft guidance to update the reentry algorithms for donors deferred because of serologic HIV or HCV test results since the reentry procedures for those donors should also integrate both NAT and serology test results.

We are considering recommending that three groups of donors, deferred because of HIV test results, be considered for reentry. The first group consists of donors who had NAT reactive results but were seronegative, those we just talked about. The HIV discriminatory NAT may have been either positive or negative but if an additional NAT was performed, a NAT that uses amplification technology and/or primers that are different from those that were used in the original NAT method on the master pool, that test must have been negative.

The second and third groups consist of donors with negative NAT who have a repeatedly reactive screening test for HIV antibody and negative or indeterminate HIV-1 Western Blot or IFA results on the index sample, or who have a repeatedly reactive EIA for HIV-1 p24 antigen with

an indeterminant, that is, an invalid or non-neutralized result on the neutralization test.

For all three groups of donors deferred because of HIV NAT or HIV antibody or antigen test results, we are considering recommending that a follow-up sample be taken after a minimum time period of eight weeks for follow-up testing of the donor by both a licensed HIV NAT and a licensed HIV antibody EIA.

Data that we saw presented at the June, 2001 Blood Products Advisory Committee meeting demonstrated that an eight-week follow-up period encompasses the pre-seroconversion window period for HIV with sufficient confidence that negative serology tests after at least eight weeks have passed rule out HIV infection.

If you wish to perform follow-up testing on the donor prior to the end of this eight-week period, you may do so for donor notification purposes or for medical reasons. However, if you again obtain a NAT reactive or repeatedly reactive antigen or antibody test result during this eight-week deferral period you should permanently defer the donor. You may use negative results on the HIV tests prior to the end of this eight-week period and donor counseling, however, only a negative screening test result, obtained at least eight weeks after the repeatedly reactive

result on the index donation, qualifies as the test of record for purposes of donor reentry.

I should also add that to alleviate the concern about the inappropriate entry of a donor infected with HIV-1 group O or an HIV-1 variant, the testing on the follow-up sample from the donor should include a licensed HIV NAT method that is labeled sensitive for HIV-1 group O and HIV group M variants.

In addition, the anti-HIV-1,2 EIA test performed on the follow-up sample should be the original EIA test for HIV-1 and HIV-2 that was run on the index donation, or there could be an alternate EIA that also an HIV-2 test and is labeled sensitive for HIV-1 group O.

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In the absence of evidence for seroconversion, that is, if the anti-HIV EIA is negative, a negative NAT on follow-up testing at least eight weeks later may be taken as evidence that any previous reactive NAT result on the index donation that was not confirmed by a reactive result on additional NAT was, in fact, an error. If both the NAT and EIA tests on the follow-up sample are negative, the donor may be reentered, that is, becomes eligible for future donation. A donation taken at a later date would then be tested using the usual battery of screening tests. Thus, two NAT tests and two EIA tests would be performed

and must be negative before a unit from that donor could be used.

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FDA's current thinking is to also include in the future draft guidance document a recommendation that two groups of donors, deferred because of HCV test results, be considered for reentry.

The first group consists of donors who had a NAT reactive result but were seronegative, those we already talked about. The HIV, HCV discriminatory NAT may have been either positive or negative, but if an additional NAT was performed it must have been negative.

The second group of donors consists of donors with a negative NAT who have repeatedly reactive screening tests for anti-HCV antibody, with negative or indeterminate label results on the index sample.

As we have already heard this morning from Dr. Busch, our current research indicates that detectable viremia may be intermittent and may also be resolved in about 20 percent of cases of HCV infection. For purposes of NAT possible HCV viremia, optionally you may follow-up the deferred donor with an HCV NAT and an anti-HCV EIA test at any time up to six months after the index donation, for example, eight weeks later. A reactive HCV NAT or positive HCV antibody test result would exclude the possibility of

reentry for that donor. If that NAT test is non-reactive and HCV antibody testing is not positive, the donor should be follow-up with another HCV NAT and an anti-HCV EIA at least six months after the index donation to qualify the donor for reentry.

The data that we saw presented at the June, 2001 Blood Products Advisory Committee meeting demonstrated that a six-month follow-up period encompasses the pre-seroconversion window period with sufficient confidence that a negative HCV serology test, after at least six months have passed, rules out HCV infection.

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Therefore, for purposes of reentry we are considering recommending that a follow-up sample be taken after a minimum time period of six months for follow-up testing of the donor by both a licensed HCV NAT and a licensed anti-HCV EIA. If both the NAT and EIA tests on the follow-up sample are negative, the donor may be reentered, that is, would be eligible for future donation.

So, I think I will stop there and take any questions you might have. Yes, Dr. Schochetman?

DR. SCHOCHETMAN: Gerry Schochetman. I just wanted to mention a case we came across of somebody who was HIV EI positive and full-blown Western Blot positive but RNA negative by multiple tests. The only way we were able

to confirm that was by collecting some PBMCs and showing that there was a low level of proviral DNA, which we could sequence and show that the individual was a subtype B. So, there may be another kind of case where somebody may be continuously NAT RNA negative but could actually be infected.

DR. MEID: Right. Of course, those donors would not be reenterable because of the persistent antibody.

DR. EPSTEIN: Now we are going to expand the perspective to look across the pond and hear a talk from Micha Nubling, from the Paul Ehrlich Institute, also on regulatory issues and perspectives. Thank you very much.

Paul Ehrlich Institute and European Union Views

[Slide]

DR. NUBLING: First of all, I would like to thank the organizers for the opportunity to speak here and to present the views of PEI. I will also present the views of the EU on plasma derivatives. Glenda Sylvester was invited as well but couldn't come and, therefore, I took over her presentation as well.

[Slide]

Blood components are estimated as drugs according to the German Drug Rule and, therefore, respective regulations are possible and the responsible party is the PEI. Several years ago, or two years ago, HCV-NAT was

introduced. I will cover this shortly. Then I will go to comparative studies concerning HCV co-antigen. The question was if this assay could replace or substitute at least HCV NAT. Then I will come shortly to HIV and our plans to introduce this also as screening for whole blood components in Germany.

[Slide]

This is already history. The regulation for blood components HCV NAT was introduced as a screening test for erythrocytes and thrombocytes and later also for seropoeitic plasma by PEI, already in April of 1999. At this time we defined a sensitivity limit which was to be fulfilled of 5000 international units per milliliter and this was to be controlled by run controls. Pooling, of course, was possible with this requirement and, at this time, there was no commercial assay available so also in-house tests were to be accepted as long as they fulfilled validation requirements, which we defined also at PEI.

The meantime experience with this requirement is that it is feasible, but we also have to say that the experience benefit is somewhat lower than expectations based on incidence data which I provided two years ago. But I also have to say that the basis for the incidence data is quite different in some blood banks where repeat

reactive donations go into incidence calculations. This might also be a reason for this discrepancy.

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In numbers, we have had a recent presentation from Prof. Seifried and Hitzler at the IABS meeting with the most recent data, and we see some discrepancy between different organizations which collect blood in Germany. The smaller donations and less associated with universities or small hospitals have a higher frequency of NAT only positive donations, approximately 7.3 per million, while the Red Cross blood donations have quite a lower frequency, approximately a factor of ten. This is probably due to different populations, donor populations because these donor populations came mainly from cities while the Red Cross blood donations cover mainly rural areas in Germany.

We have also addressed incidence. Viral transmissions have to be reported to EPI by an installed reporting system, and since this NAT regulation has been in place there has been no adverse incidence reported of HCV transmission by blood.

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So, this is the experience and the benefit which is obtained in the meantime. Approximately three years ago the antibody to HCV for antigen ELISA became available and immediately the question was if this test could be used

also for screening in addition to HCV NAT or even replacing HCV NAT, and I just want to summarize some studies we have performed in this respect.

We had access to 52 follow-ups of the very early infection phase, similar to the follow-ups Michael Busch already reported, covering in total nearly 500 anti-HCV negative window phase samples. We analyzed all these samples by different NAT methods and also by the core antigen and also we genotyped the different panels. I would like to shortly summarize the data.

[Slide]

In blue you always see the core antigen ELISA. Here is the cut-off value. In red is the HCV RNA. So, here is the run-up phase, as was already introduced by Michael Busch. We see it as parallel HCV detection in 10 out of the 52 panels where there is no difference between these two assays. So, the first PCR positive assay sample is also antigen positive. This was for 10 of 52 panels.

[Slide]

For 21 of 52 panels we see the following pattern, also represented by only one panel, of course. There is a delay of antigen detection, approximately one week. Later on there is a consistent detection of HCV RNA and core antigen. We also see these blips or these low titer viremia samples in many of these panels.

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The last pattern which I want to share with you is fluctuating antigen detection. Again, there is a steep increase in HCV RNA and the second sample is also core antigen positive. But later on there is again a decrease in HCV RNA titer and this is followed by core antigen negative results and borderline results. This pattern we see in 9 out of 52 panels.

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So, summarizing these data again, the red columns represent the severe RNA. The blue columns represent core antigen. We have grouped the different columns in respect to increasing HCV RNA titers. We see immediately that the high titer RNA samples are also detected by the core antigen test very consistently, but approximately at 10^5 units per milliliter and lower the core antigen test becomes negative while NAT remains positive.

I have to admit that with the requirement which is in place in Germany there would also be certain low titer samples missed. This are the blip samples again. A few low titer samples would be missed and, therefore, it is under discussion if this sensitivity requirement should be changed to higher sensitivity. We can also calculate the HCV RNA doubling time. It is a slightly different figure compared to Michael Busch's but maybe the analysis did not

use the same panels and not the same algorithm. With this HCV RNA doubling time during the virus burst we can also calculate effects. If the sensitivity requirement is, for example, changed to 2500 we would approximately get ten or less of the diagnostic window phase.

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Infectivity of low titer HCV RNA transfusion has already been mentioned by Michael Busch. There are different applications compared to ours, and Schutler was also recognized before. There are some examples which at least could be the basis for the concern that blip donations or low titer donations are, indeed, infectious.

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Summarizing this study, our conclusions are that the HCV core assay protects, indeed, the majority of the window phase donations but not all, and for a country which already has NAT regulations in place with a higher sensitivity, it is not easy to substitute HCV NAT by such an assay. So, the sensitivity of the core assay is lower compared to our regulation at the moment. We know that there is a second version of the core antigen assay available which is much more sensitive and the question, of course, reappears. For low titer viremic samples, like the blip samples, RNA concentration on a single donation NAT would detect these samples.

[Slide]

I come to the second topic, HIV-NAT. We had the recent experience in the last three years of transmission of HIV to recipients of blood transfusions in four documented cases. So, window phase donations were the cause for transmission of the virus, and we analyzed these cases retrospectively and three of them were p24 antigen negative. One was p24 antigen positive. But all of these donations had virus titers which would be easily detected by even minipool or pooled NAT. Three other donations have already been identified during this period by NAT, which is applied on a voluntary basis already by many of the blood donation centers in Germany.

[Slide]

The further background concerning HIV-NAT in Germany, the plasma industry already requires plasma tests for HIV RNA. Therefore, at least the major blood donations have already introduced HIV RNA testing. So, 75 percent of the donations are already covered. The reason for transmissions would have been prevented by HIV-NAT on minipools.

[Slide]

As a consequence of this, we are discussing now introduction of HIV NAT for the next year. This should cover the cellular blood components as well plasma. The

proposed sensitivity in this case is 10,000 international units per milliliter which corresponds approximately to 5000 copies per milliliter. This should come in place during the next year. Again, we will accept validated in-house methods or commercial NAT methods which have been approved by EPI. In the time being we are performing a collaborative study on in-house NAT assays, which will be finished in December of this year, or during this month. We have now 19 different in-house assays, similar in-house assays which participate in this study which are performed in blood donation centers and for HCV, 22. So, there is quite a high range of those assays already performed.

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Again, the RNA titers are increased during the window phase of HIV. We see that if we would rely on the p24 antigen assay only at the high titer, RNA donations would be detected by p24, and by this proposed sensitivity requirement we would close the window by approximately three to four days compared to the situation if we would introduce p24. So, this is the current situation. There is some delay. The RNA doubling time for HIV is 21 hours and one can calculate the effect of increasing sensitivity on this basis.

[Slide]

There are many discussions in Germany concerning royalties for screening markers of NAT and these discussions also are the basis for certain delays in the introduction of NATs in different countries. First of all, the royalties are to be paid for single donation. This is in principle also supported by EPI because with this requirement we can get rid of the bigger pool sizes because it is not much more cost effective compared to single donation or small pool sizes.

On the other hand, in our estimation the size of the royalties is difficult to justify. Nobody has any questions about royalties in principle but if you compare the size of the royalties to the royalties which have to be paid for anti-HCV assays, there is a lot of benefit compared to the introduction of HCV NAT and there are not very good arguments, at least if this is compared.

Furthermore, there are some feelings in Europe that NAT manufacturers didn't contribute too much to the introduction of HCV NAT and the major work was done by blood donation centers and plasma manufacturers in the past, and now that the feasibility has been shown these manufacturers jumped on and tried to get what they can get.

Also, I have to say that from a regulatory point of view royalties which are not expectable or at least the sizes are not clearly expectable provide less room for

future regulatory decisions because we also have to keep in mind cost and benefit.

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The current situation in Europe, and this is not the EU only, is that HCV NAT has been introduced in different countries like France, Germany, Switzerland, Austria, United Kingdom, Norway and Finland but in many of these countries only on a study basis. So, it is not a permanent regulatory requirement. Several countries also allow HCV NAT or HCV antigen testing, like in Italy or Spain and in Poland, for example, only HCV antigen is used for screening of blood donations. So, there is quite a diffuse picture around Europe, and this picture will probably change also based on the outcome of these royalty discussions.

Concerning HIV NAT, this has already been introduced in France, Austria and The Netherlands in the past and, as I already said, in Germany we expect an introduction during the next year.

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I come now to viral safety of plasma-derived medicinal products, and this is the presentation which I currently got from Glenda Silvester, from the European Medicines Evaluation Agency, in London, which is responsible for respective regulations.

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The current situation for plasma-derived medicinal products is effective treatment and prophylaxis, prophylaxis of conditions causing morbidity and mortality possible with these products. The viral safety has been achieved at a high level for blood-borne viruses, HIV, HCV and HBV, and for other safety problems they have been minimized. For example, formation of inhibitors which has been a topic two years ago approximately. There is also a sufficient supply of these products so, in total, there is a favorable benefit to risk balance.

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You know all the different points with the current level of safety of plasma-derived medicinal products has been achieved. It starts with donor selection, with testing of donations and, therefore, the different viruses, testing for HCV RNA and inactivation procedures, GMP and also antibodies present for some of the viruses which have a certain impact on safety. So, in total we can conclude inactivation and removal procedures, together with GMP, provide better protection for unknown and new viruses as well and, once identified, testing can target the specific viruses and minimize the load on the manufacturing process.

[Slide]

This specific testing has already been introduced for HCV. The requirement for pool testing for HCV RNA is now in place since July, '99. And, pools must have to be non-reactive and a run control of 100 international units must be detected in a parallel test.

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This has also found place in the European monograph for human plasma for fractionation. So, validation of assays is mentioned there. The internal control to test for inhibitors is prescribed there as the representing state-of-the-art, and guidelines on validation of NAT have been defined.

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I would just like to share with you our experience with this regulation at EPI. We still use the Amplicor HCV in a validated version for retesting also plasma pools which have been pretested by the manufacturer and are now, by definition, negative for HCV RNA. In the meantime, since this regulation was in place we have tested more than 5000 pools and in this big size of pools there was no repeatedly HCV NAT positive pool identified. I am sure others who have performed similar retesting have a similar experience. So, this requirement is now the basis for, let's say, many discrepant results.

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Last September, September of last year the EMEA workshop on viral safety of plasma-derived medicinal products was held with a particular focus on non-envelope viruses. I would just like to share with you the conclusions of this workshop.

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First of all, it was again stated that there is an excellent safety for envelope viruses and no need has been seen for a regulatory approach for HIV or HBV NAT to be introduced for plasma testing. Development of improved inactivation and removal steps for a number of envelope viruses, on the other hand, remains an objective for all plasma-derived medicinal products and effective steps need to be implemented for all coagulation factors.

Concerning hepatitis A virus, this was also discussed at this workshop. The conclusion was that NAT testing of plasma pools should not be introduced for HAV RNA because of low titer to be expected in plasma pools and because of the experience that screening or testing of plasma pools for hepatitis A virus RNA did not prevent transmission of this virus by Factor VIII preparation in the past. This was approximately two years ago.

[Slide]

A further topic was parvovirus B19, and there are two aspects of parvovirus B19 transmission itself and also vulnerability to unknown envelope viruses.

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First of all, consequences of parvovirus B19 transmission are normally harmless. It is a common infection and we very seldom see problems associated with it, but there are certain risk groups where problems can occur and these are patients with hemolytic disorders, immunocompromised patients and also pregnant women where the fetus can be infected and cause harm.

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Concerning safety approaches for parvovirus B19, heat treatment of lyophilized products has been shown not to be very effective for this virus. The same is true for nanofiltration. This has some effect but not a very efficient effect and new techniques are still in development to inactivate or remove parvovirus B19.

On the other hand, we have neutralizing antibodies in immunoglobulins and S/D plasma, nevertheless, high titer parvoviruses may not be neutralized any longer by the presence of these antibodies as a certain incidence has shown. NAT for parvovirus B19 may reduce the virus load during the process and complement other safety measures.

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The conclusions on parvovirus were inactivation and removal is currently different. Some approach can give limited inactivation or removal depending on product and process, and NAT testing of minipools can be estimated as a complementary measure for inactivation and removal, but the impact of this measure has to be proven with respect to virus safety. There is no final proof, at least up till now.

[Slide]

The introduction of inactivation or removal steps such as NAT -- there are complementary measures such as NAT should be prioritized for coagulation factors and solvent/detergent-treated plasma because of reports of proven transmissions by these products, and for NAT immunoglobulin because of use in high risk patient groups and pregnant women.

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There is already a draft now out for discussion in the Pharmaeuropa which proposes that the production of NAT immunoglobulin, that plasma pools should be tested for parvovirus and the maximum limit has been proposed for 10^4 international units per milliliter of or RNA in plasma pools. So, any concentration lower than this is accepted but higher concentration should be avoided. This is still

out for comments but this is the first approach in Europe concerning parvovirus B19.

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Finally, I would like to thank Glenda Sylvester for these slides and you for your attention.

DR. EPSTEIN: Thanks to you, Dr. Nubling. If there are one or two pointed questions for Dr. Nubling? Dr. Tabor?

DR. TABOR: Yes, I want to ask a couple of questions about the HCV core antigen information, and I apologize if I misunderstood what you said. Are there countries in Europe where it is being used in place of NAT, or is it being used in addition, or do the individual centers have a choice as to which to use?

DR. NUBLING: In Spain and Italy it is up to the blood donation centers to use NAT or core antigen. So, they can use either of these.

DR. TABOR: And this is for blood?

DR. NUBLING: This is for blood components, not for plasma.

DR. TABOR: And is the technology that is used all the same or are there several different manufacturers?

DR. NUBLING: To my knowledge, there is only one manufacturer. It is Ortho which is present at least on the European market, and this test is also used in these

countries. Poland is the one country which introduced for the time being, on a study basis, HCV core antigen only; not NAT because of the high costs. But it is not clear at the moment if they will continue with this or if they will change the current requirement. But for the time being they have identified quite a huge number of antigen-positive donations which would not have been detected.

DR. TABOR: One last question, is there a reference standard for the core antigen test in the European Union?

DR. NUBLING: No.

DR. TABOR: Thank you.

DR. EPSTEIN: Dr. Bianco?

DR. BIANCO: Celso Bianco. I was a little bit confused about your discussion on B19. On one side, you said there is a lack of well-defined description of the benefits that would come from screening for B19. On the other hand, you mentioned a guideline that comes from the EMEA that would regulate that screening. So, you predict that this would be a mandated assay for plasma derivatives in Europe?

DR. NUBLING: If this is followed it will be mandatory for NAT immunoglobulin only for the time being because this is defined for a risk group for parvovirus B infection and, therefore, this is at least the first

regulation which will be in place probably in the near future.

DR. EPSTEIN: Dr. Allain?

DR. ALLAIN: I still have some trouble with the difference, massive difference, eight times difference in HCV RNA positive between the Red Cross and the non-Red Cross centers, and I wanted to make sure of several elements. First, the pool size is the same?

DR. NUBLING: No. In Germany we rely on the sensitivity requirement, 5000 international units have to be detected and there are different approaches to detect this. So if you have a high sensitive assay, the pool size may be higher or bigger compared to an assay which has lower sensitivity. So, it is sure that the 5000 international units is fulfilled for all places, for the German Red Cross and for the other blood conation centers.

DR. ALLAIN: Second, I understand that at least a couple of years ago maybe until now some centers use ultra centrifugation prior to testing. Is that still in place particularly with the Red Cross or only the other centers? Or, what is the status of that?

DR. NUBLING: It is still in place for at least one big Red Cross center, as far as I know and, of course, this centrifugation also has to be controlled by a run

control which reflects the 5000 international units. It is still in place.

DR. ALLAIN: Finally, is there any difference in the percentage of first-time donors between the two populations?

DR. NUBLING: It is a question I cannot answer immediately.

DR. ALLAIN: Because the difference of eight times is just about what you would expect between first-time and repeat donors.

DR. NUBLING: Okay, but, of course, the number of the smaller centers are not only first-time donors. But I cannot say what the percentage of first-time donors is in the two different settings.

DR. EPSTEIN: Thank you. We have a few more minutes for just general discussion. I assume that any of the earlier speakers will be pleased to respond if there are questions to be raised. Perhaps I will start off with a question for Dr. Alter.

You made a point that hepatitis G has not been found in the liver. But then you showed us that it readily affects PBMCs. So, I think one question we might ask ourselves is whether we should be looking for an immunological disease if there is a disease to be found.

DR. ALTER: I think one has to decide whether to make the effort to look for these infections as a group in all kinds of diseases, and certainly diseases where lymphocytes or cells involved would be a good starting point. The data on the mononuclear cells came from infectious clone that Dr. Stapleton has developed. I didn't realize that was available until his paper came out.

DR. EPSTEIN: Thank you. Could I ask anyone who speaks up to please use the microphone and please identify yourself for the sake of the transcription? Any other comments or questions?

[No response]

Well, we will take advantage of a little extra time. Let me ask you to be back at 10:55 so that we can stay on time.

[Brief recess]

DR. YU: Let me introduce the first speaker, Dr. John Saldanha. He really doesn't need to be introduced for this audience. He is going to talk about the NIBSC/WHO program. Let's welcome Dr. John Saldanha.

II. Standardization/Quality Assurance

NIBSC/WHO Program

DR. SALDANHA: Thank you.

[Slide]

I would like to thank the organizers for inviting me to this meeting. What I hope to do in the next 20 minutes is to go through very briefly on the ways that we established the WHO standards for NAT testing and the working reagents. Then, at the end I would like to talk about some quality assurance programs that we run.

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I will start off with some definitions. NAT assays -- what are we trying to do with NAT assays? We are trying to measure the amount of nucleic acid in a sample. And, in the past, before we had standardization, these were measured in a variety of units, either genome equivalents or copy numbers or not detectable units. A program of standardization was started in order to assure the quality of data coming out of testing laboratories.

In order to be able to standardize assays, we need to provide standards or working reagents and these are reference materials which are used in assays to look at the genome equivalents, in this case the variable being measured. To be able to do this, we established WHO's international standards.

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The different systems that we know for measuring nucleic acids in samples -- we have got RT-PCR systems, TMA and B-DNA assays. These are used in some form or another,

either as qualitative or quantitative assays. If you use any of these assays, I can guarantee you would get different answers for the amount of viral RNA or nucleic acid in the system. This is because nucleic acids are not equivalent in all sorts of preparations. You have incomplete genomes; secondary structures which might affect the quantitation, etc., etc. I think it is very difficult, using these systems, to be able to compare results.

[Slide]

With the WHO standards we use a common unitage which is the international unit, and this is an arbitrary unit. This is to get away from nucleic acid molecules or to use the SI systems which is nanomoles per liter which, in my opinion, are very difficult to accurately determine. It is, I think, virtually impossible to work out accurately the number of genome equivalence. So, to get around this problem we defined an arbitrary unit which is the international unit.

[Slide]

International standards are used as a gold standard. These are standards that are established by the WHO. In turn, they are used to calibrate secondary standards and then working reagents or run controls which are used to control each assay run.

There are several requirements for a WHO international standard. They should ideally be a lyophilized preparation so that they have long-term stability, and we do assays to prove this which I will show you later on. We should have adequate supplies of the reagent to last ideally five to ten years. And, and this is important, we normally do a collaborative study using laboratories from as many different countries as possible to establish these standards.

[Slide]

The establishment takes a very sort of set form. We identify candidate materials and assess the suitability such as stability; organize a collaborative study and assign a unitage -- a concentration in terms of international units per milliliter. We then send a report to the WHO expert committee on biological standardization which meets once a year, and this committee then establishes the standard.

[Slide]

With the studies that we have done so far, we have done several studies. We have looked at hepatitis C right through to hepatitis A, and we normally have a variety of candidate materials. In the first study we had two materials from NIBSC and a liquid preparation from CLB, in Holland. With HIV, we had a material from CBER which

was lyophilized, and with B19, the same thing, and with HAV, which is our latest standard, we had materials from ISS, in Italy. The ones that are colored, the candidate samples, went on to become the eventual WHO standards.

[Slide]

These are the standards which we have established. The first WHO standard for NAT testing was established in 1997 for hepatitis C virus RNA. This was followed by two standards established in 1999 for HIV-1 RNA and hepatitis B DNA. The parvovirus standard was established last year, and we are completing the study on the hepatitis A standard. So, these are the four major blood-borne viruses which have WHO standards established.

[Slide]

What do these standards consist of? These standards are wild type viruses diluted in plasma or cryosupernatant. The hepatitis C is genotype 1A and has 5 times 10^4 international units per vile. The B is a genotype A, surface antigen subtype ADW2. The HIV-1, which is the material from CBER, is genotype B material, and these have all been assigned concentrations in international units per milliliter.

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We choose the concentration in international units per milliliter to be as close to the determined value

in NAT units per milliliter. This is to save a bit of confusion. Of course, the quantitation, as I mentioned earlier, will differ with different viruses. So, the ratio of international units to NAT detectable units per milliliter will also differ for each virus. What I think I am trying to get away from is to encourage people to use international units and not try to convert NAT detectable units into international units because this ratio is very dependent on the type of assay that is used.

[Slide]

Having said that, I can give you some figures and you can see, for example, that for hepatitis C virus the ratio differs between 3.3 to 8.3. This is basically because the assays differ in their sensitivity. With hepatitis B it is about 2.0 to 7.0 With HIV it is about 0.7 and B19 is about 0.628. Ideally, we should have a ratio of 1.0 to 1.0. This doesn't work out.

[Slide]

The other aspect of the study is stability of the international standards. There are two ways of doing these studies. We can do real time stability studies and accelerated degradation studies. In accelerated degradation studies vials of the sample are incubated at different temperatures and then the quantity of virus is

measured to look for the degradation. You can make some assumptions to account for the stability of the material.

[Slide]

There are limitations to the stability studies. We real time studies you can get changes in sensitivity of the assays over time so that what we are measuring may not reflect degradation but reflect the increase in sensitivity of the assay over a period of years. There is low individual assay precision and the time scale is probably longer than the shelf life of the sample.

Accelerated degradation studies also have their problems. At high temperatures of storage, where you get reliable data for working out the degradation, you can often get difficulties in reconstitution of the material, and reliable estimates are difficult to work out.

[Slide]

If we look at the hepatitis C international standard, we did some accelerated degradation studies on this and showed that there was no loss of titer for samples stored at 20 degrees for about 200 days. Working through some calculations, which I won't go through, this equates to stability of the material for over two years at zero degrees and below, and we store all international standards at minus 20 degrees.

[Slide]

I also have real-time data on the international standard and this is because we have done three studies, in 1997, to establish the international standard, and two calibration studies for working reagents and genotypes, in 1998 and 2000. I think it is obvious that the titers of the sample in not detectable units per milliliter for qualitative assays really hasn't changed very time over four years, and the same for the assays, which are the quantitative assays given genome equivalents per milliliter, are around 5.8. So, I think these are real-time data on the stability of the hepatitis C international standard.

[Slide]

The other thing that we need to consider is replacing the international standard because although the standard is supposed to last five to ten years, we have practically run out of the standard already, and this is because of the high usage because of the regulations in Europe, and we have very few vials remaining of the standard.

In the original study we had a second material, B, which was also calibrated. At the SOGAT meeting, which is a WHO working group, we decided to replace the existing standard with this material, B. We are going to do that by looking at the stability of the second material against the

existing standard in a series of degradation experiments, basically to show that the titer of the second material which has been stored at minus 20 is equivalent to the first material.

[Slide]

The other thing that we have done is to calibrate working reagents against the international standard. In an ideal world we should first establish an international standard and then make secondary reagents from this. But, in fact, with hepatitis C and HIV-1 working reagents were established long before the international standards and it was necessary to calibrate these working reagents against the international standards.

[Slide]

With hepatitis C, we have calibrated four genotype-1 reagents and one genotype-3 reagents against the standard, and that has been published. With HIV-1, we have calibrated working reagents, 7 type B reagents against the international standard. The preliminary results are being completed, and I think the study is about to be published. So, we have all the commonly used working reagents at the moment which are calibrated in international units per milliliter, which makes comparison of results and any sort of legislation that is brought in much easier I think.

The other study we have done is to calibrate genotypes or subtypes of HCV and HIV against the international standards. The C international standard is a genotype 1A and the HIV-1 international standard is a genotype B. There is an argument saying that is it possible to calibrate genotypes 2 to 6 against the international standard and these subtypes A to H, N and O against the HIV-1 international standard?

We have completed the analysis of this study and this study is currently under way. I will show you some results for this study which has been completed in the next few slides.

[Slide]

This is to illustrate the way that we analyze the results. We send out panels to laboratories and then work out the titer of the samples, and have this sort of chart. This gives the number of the lab and the titer of the virus determined by the lab. These are just the different types of assays, Amplicor, TMA, in-house single loop, in-house nested. I think it is obvious that most of them cluster around. There are no sort of outliers, which I think is good news.

[Slide]

These are examples of the Amplicor assay, which is the quantitative Amplicor assay calibrated in international units per milliliter.

[Slide]

These are the results of the qualitative assays in not detectable units per milliliter, and the monitor assays in international units per milliliter. Again there is very little difference between these figures.

[Slide]

This, again, is an illustration of the genotype 2 sample. Again, most of the laboratories get the same sort of figure.

[Slide]

In this table I will show you the calibration of the genotypes against the international standards. What we have done is we have compared the titers of these materials against the international standard and got a figure in international units per milliliter. So, for genotype 2, which is that figure, it has approximately 10^4 international units per milliliter and genotype 6 has approximately $10^{4.65}$ international units per milliliter.

[Slide]

There are several conclusions we can draw from this study. The majority of participants in this study used the Amplicor assay and I am afraid there was

insufficient representation of other assay types to allow full assessment. So, I guess the overall means are biased towards the Amplicor assay.

[Slide]

We now have a panel of genotypes which are available for use. Although we haven't got an official calibration yet, we have calibrated them in international units per milliliter and the data, I hope, will be published fully.

[Slide]

We have got valuable information on the performance of assays across the genotypes and the definitive assessment really needs to be done by using more assays with the B DNA and TMA. We have had only one laboratory doing the B DNA assay and three doing the TMA in the study.

[Slide]

I will briefly go on to quality assurance. Quality assurance is defined as plans and systematic activities implemented within a quality system. Basically, in quality we are looking for degree of excellence, reliability, fitness of purpose, freedom from effects and conformance to requirements.

[Slide]

Within NAT, in a NAT quality assurance program we need to have access to good calibrated international standards and working reagents which can be used in our assays. We need to calibrate genotype panels, which we have for HCV and HIV-1 for looking at the specificity of the assays. We also need to take part, at least once or twice a year, in proficiency studies to monitor the overall performance of the laboratory. So, these are the sort of quality issues that a NAT lab faces, I think, to be able to work well.

[Slide]

This is a list of the NAT working reagents, which I am sure most of you have seen before, and these are the reagents which were available from NIBSC for hepatitis C, HIV-1 and 2. I give these figures in international units per milliliter if you divide this by about half. B19, hepatitis B, hepatitis A, we haven't got an international standard yet, and a multiplex standard which has all five viruses of these concentrations.

There are also other working reagents, C, HIV-1, HBV and B19, available from other laboratories and I think Mei-ying Yu will describe the CBER reagents, and these have all be calibrated again to respective international standards.

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Going on to proficiency studies, these are blind panels which are tested once or twice a year, and what they do is look at performance of a validated assay and show the reproducibility of the assay, confirm the skill of the laboratory and compare the results of one laboratory to another.

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In the U.K. I run a group called the U.K. NAT testing laboratories. We, so far, have sent out three proficiency panels over the years. The first two panels have dilutions of genotypes 1 and 3, ranging from 50,000 IU to 0.2 IU. The third panel had dilutions of genotypes 1 to 6 and negative controls. Over the years the participants have increased from six to nine in these panels.

[Slide]

My final slide is really the results of the last panel, which I hope you can see. These are the six genotypes of hepatitis C, ranging from 100 IU down to 5, and 120 for the other four, and five diluent samples. All the labs to start with could pick up 100 IU/ml of all the genotypes and this fulfills the European requirement which Micha Nubling mentioned of a validated assay being able to pick up 100 IU/ml. There was only one lab that picked up false positive, and the majority of laboratories could go down to 20 IU/ml of all the genotypes.

So, in some ways this is a very good illustration of how the laboratories in the U.K. are actually getting closer together in terms of sensitivity and specificity of the assays.

I will conclude by saying that for the standardization and quality assurance of NAT we now have available the standards which are calibrated in international units, which can be used throughout the world, and we have panels such as genotype panels and proficiency panels for looking at the performance of laboratories. Thank you.

DR. YU: Thank you, John Saldanha. Let's have some short questions and comments. Later on in this session we are going to have a discussion session. Please?

DR. BIANCO: Celso Bianco. John, you were very emphatic in trying to encourage the use of international units. However, all the regulatory agencies around the world continue to use -- or most of them -- copies or genome equivalence, at least in some of the regulations. How are we going to agree on something like that?

DR. SALDANHA: Well, actually the majority of regulatory authorities use international units. CPMP, as Micha Nubling pointed out earlier, define HCV in terms of international units per milliliter. The German regulatory authorities have 5000 IU/ml as well. In the U.K. we use

100 IU/ml. With HIV the problem is slightly more difficult because the HIV-1 assays have been well established and it is only recently that we have had an international standard and the calibration studies have only just been completed. But what I hope is that once these materials are available and have been calibrated most laboratories will switch over to IU because it makes things so much easier. Although people talk about copies and genome equivalence, as far as I can see, they are not strictly equivalent and you can't compare one with the other.

DR. YU: I would just like to comment that CBER now is going to use IU versus copies. Okay?

DR. BISWAS: Thank you very much, John. Our next speaker is Dr. Micha Nubling, from the Paul Ehrlich Institute, who will talk about the European standardization and quality assurance program.

Europe

[Slide]

DR. NUBLING: John already mentioned standardization through common reference preparations.

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I would like to focus on two related but, nevertheless, similar topics, different topics. This is standardization of NAT tests and I would like to introduce you to the European IVD directive, which is the regulatory

framework for future IVD regulation in all of Europe, and especially come to the so-called common technical specifications which have been defined for at least some of the NAT assays.

The second part of my presentation will also cover quality assurance in laboratory and here I would like to come to the proficiency testing system as it is now installed for official medicine products controlled laboratories network, which is organized by EDQM in Strasbourg.

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First coming to the European IVD directive, just a very short introduction, it is a regulation which defines the conditions of placing IVDs on the European market. Once these conditions are fulfilled the IVD can be CE marked and this allows them free movement of IVDs in the common market in all of Europe. The IVD directive defines quite generally so-called essential requirements which cover mainly reliability of assays and protection of users and third parties.

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This is just a picture to make it a little bit clearer. In order for a product to become CE marked, the manufacturer has to install so-called process specific steps, for example, by showing conformity with certain

standards, like the ISO 9000 standard and EN 46001. In addition to this, product specific requirements have to be fulfilled which are later on in so-called European harmonized written standards and in the essential requirements in the IV directive and, last but not least, let's say the most important products, the so-called high risk IVDs, which are laid down in the so-called common technical specifications, CTS.

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The IV directive is a very new regulation and currently we are just in the middle of the first transition phase, meaning that CE marking is already possible, CE marking of IVDs is already possible since June, 2000 but, nevertheless, also national approvals can be applied and are still valid. Then there comes the second transition phase. After the end of 2005 all IVDs in Europe have to be CE marked and the national regulations, which are quite different in different countries in Europe, will be replaced totally by this IVD regulation.

[Slide]

I mentioned already the common technical specifications. These are product specific requirements, and the question is why they have been defined. With think they are a flexible, fast and updatable process and this is important for several reasons. Flexible and fast and

updatable because there is a working group, which is a steady working group, which has defined now the first version of the CTS but if there is a need, they can convene again and improve or update the current version of the CTS. This may be possible if, for example, test improvement occur and there is a need for a new requirement. Epidemiological changes have occurred in the past and the respective requirements need to have all these changes. Also, availability of samples, sample use and diagnostic evaluations change. For example, yesterday evening we discussed HIV-2 seroconversion panels which are not available at the moment but, for example, once these panels will be available they would be included in the CTS. Last, not least, state-of-the-art of IVDs changes quite rapidly with time and this also has to be reflected by a fast process.

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The CTS consists of general principles and of a number of qualifications of standards to be used for diagnostic evaluations. I just want to briefly introduce some of the general principles which lead, to a certain extent, also to standardization of NAT assays which are concepted for the European market in the future.

One example is that the detection limit of assays has to be expressed as a 95 percent positive cut-off value

based on the international standard, as John already introduced and as he made reference to the EPI validation guideline and how this value for the different assays should be established.

Genome and subtype detection efficiency has to be investigated and the respective information has to be given to the user. Quantification limits should also be expressed by reference to the national standards, and that is also an approach for how the system failure rate should be calculated.

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An important statement in the common technical specifications for NAT assays is that the so-called functionality control or internal control is a check for the whole procedure, including extraction/amplification detection estimated as the current state-of-the-art. So, any test without such a control will have problems in Europe.

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There are also certain statements made about how to investigate robustness, including pre-seroconversion samples, high titer samples. Carryover has to be investigated and also the whole system failure rate has to be established.

[Slide]

The number and qualification of samples have been defined for the tests which could be used, at least for blood banks. So, certain numbers of subtypes and genome types have been defined, and also how these numbers could be achieved. I already mentioned the detection limit. This is just to give you an impression. There are many other tables. Also, for specificity certain numbers have been agreed to for the different NATs for different viruses, how specificity should be expressed.

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At the moment, we still have national approvals and different manufacturers contacted PEI also in order to get approval. At the moment we have some problems or at least discussions with tests, why the whole procedure is controlled. An NAT procedure may start with a concentration of viruses, for example, by centrifugation, then extraction, precipitation and capture of nucleic acid amplification and detection of amplification products. Assays which have internal controls or positive controls which consist of in vitro transcripts cover only these later steps of the whole procedure and, for example, centrifugation and detection of viral genomes is, by definition, not controlled. For the time being, therefore, at least it is discussed at the moment if a parallel run control should be required for such tests, which contains

real virus and which should be performed once per run in order to control at least once per run the whole process. Of course, this run control should contain the targets in suitable concentrations in order to make sense.

So far the so-called common technical specifications are still in a draft version at the moment, but they will be accepted by the European Commission in due course very probably.

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The second topic of quality assurance and official medicine control laboratories, or MCLs, as already mentioned several times, there is a requirement for pool testing for HCV RNA and pools have to be non-reactive and run controls have to be used and methods have to be validated by internal controls to test for inhibitors. This is the requirement as it is stated in Europe, and there is also testing of plasma derivatives, the so-called OMCLs have to retest the manufacturing pools, and a future target is that there could also be a mutual recognition between OMCLs in the future.

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So OMCL retests these manufacturing pools and there were efforts made by the European department for quality of medicines, in Strasbourg, to implement and maintain excellence among the different OMCLs in order to

allow potentially in the future mutual recognition. This consists of exchange of expertise and experience between different OMCLs between, let's say, more experienced OMCLs and OMCLs which just start with NAT testing. In this network, also a validation guideline has been defined and testing modalities -- how many samples should be tested; the algorithm for getting final results -- have been agreed upon.

Also, EDQM also defined or prepared the so-called biologic reference preparation which is estimated as a kind of European standard for HCV RNA, and last but not least, established a proficiency testing system for the different OMCLs.

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I would like to summarize shortly what has been obtained in this field so far. The whole proficiency testing system started in 1998. At this time, before the respective regulation came into place, it was a kind of trial phase and OMCLs exchanged materials and performed a kind of self-assessment with common samples. At this time there were eight OMCLs through Europe which volunteered.

Since 1999, since this regulation has been in place, there has been a certain system established for how this proficiency testing has to be performed. First of all, there is an external scientific advisor who designs

the protocols. Two studies are performed per year. A set of 20 coded samples is sent out to the different OMCLs containing different dilutions of HCV RNA positive samples and also different genotypes. The main focus is not to obtain maximal sensitivity but to focus on the consistent detection of the 100 international units, which is the sensitivity limit for this plasma pool requirement.

In the meantime, since 1999, six studies have been performed. The missing study is just being finished at the moment. The number of participants increased from eight in the beginning to between 12 and 14, and these OMCLs come from different European countries as well as from Australia.

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I would just like to summarize in very few words the results. In the first two years this was still a start-up phase for new NAT labs for several of the OMCLs. So, a first failures had to be recognized in some labs. These 100 international units were not detected consistently, but over the different studies there was a steady overall improvement. The last study where the results have been obtained there was no failure at all, and all the OMCLs which participated had 100 percent detection of this concentration, which is important.

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This are just the results of the last study which I just mentioned. It is not very good to read but here are the 100 international units in this sample. Here are higher concentrations, and they all got positive results. It was 100 percent positive, and with 32 international units still 86 percent of OMCLs get positive results. What is at least as important is that for negative samples there were no false-positive results reported.

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So, this was a short overview about the proficiency testing system as it is performed for control laboratories in Europe, and I have to mention EDQM who provides the umbrella under which the studies are performed, especially Guy Rautman and Jean Mark Spieser who put much effort into this. As I mentioned also, there are external scientific advisors and two of them are present here today. Thanks a lot.

DR. BISWAS: Thank you very much. Are there any questions for Dr. Nubling? Yes?

DR. GALLARDA: Jim Gallarda. Dr. Nubling, can you describe the relationship between the OMCL guidelines for an external run control which specifies 100 IU/ml as compared to the European Pharmacopeia supplement 2000, which says a run control should be some multiple of the 95 percent limit of detection of the technology? I am just

wondering if one supersedes the other, or if there is some harmony between the European Pharmacopeia and OMCL.

DR. NUBLING: From an acoustic point of view, I didn't understand everything, but I think you asked for the 95 percent positive cut-off value in relation to the 100 international units?

DR. GALLARDA: Right, the European Pharmacopeia supplement 2000 specifies that an external run control for NAT should run at some multiple of the 95 percent limit of detection of the assay.

DR. NUBLING: Yes.

DR. GALLARDA: Whereas, the OMCL specifies 100 IU/ml for that external run control. I was wondering about the difference in the two organizations.

DR. NUBLING: Maybe I do not understand your question but I will try to answer to the extent that I understood it. This 95 percent cut-off value defines the concentration, a method to detect in 95 percent of the assays. So, if I would have an assay which would detect consistently this 100 international units, the 95 percent sensitivity of this assay would be approximately 30 international units. This is a prerequisite because a factor of three gives you a certainty of nearly 100 percent detection in three times higher concentration. This is

what we need to achieve in order to take consistently these 100 international units.

DR. BISWAS: Micha, I have a quick question for you. You had mentioned something about a whole system failure rate. What do you mean by that? What would be a partial system failure rate? What does that mean?

DR. NUBLING: A whole system failure rate is defined for a complete system which is installed, for example, by a manufacturer in a laboratory, including extraction and everything. And, one parameter which should be investigated is the complete procedure to be, let's say, challenged by low titer samples in order to get independently which step makes the failure to get the whole failure rate of the system.

DR. BISWAS: Thank you very much, Micha. Our next speaker is my colleague, Mei-ying Yu, from CBER, FDA.

CBER/FDA

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DR. YU: In my talk I will cover standardization for donor-screen NAT and in-process control NAT, and also I will address the other quality assurance issues.

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As you all know, standardization of NAT methods is very essential because we have diverse NAT methods, and they have varied sensitivity, specificity and

reproducibility. We also have varied minipool size for HIV, HCV NAT. We have 96 to 1200 for source plasma, and for whole blood we have from 16 to 24 pool size.

We also need analytical standards. This is so that we can have a standardized assay and also standardized reporting so that we can report it either as copies or, preferably, IU/ml. Analytical standards will monitor laboratory proficiency and will be used for lot release of test kits.

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Donor screen NAT, this is in BPAC, Blood Products Advisory Committee, held in March, 1997 and it says the pool test for HIV, HCV and HBV is a form of donor screening, requiring IND and licensure. Dr. Paul Mied already mentioned that this morning. So, we need a donor identification notification deferral and follow-up. We need to identify and notify recipients of implicated products. We need to quarantine or destroy the positive units and components, and we need to perform look-back to retrieve, destroy and unpooled or untransfused units.

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We do have CBER NAT standards for donor screened NAT. There are not standards available for HIV and HCV. Currently, the NIS standard for HBV is under development. The current required analytical sensitivity is 100 IU/ml

for pool tests and 5000 IU/ml for original donation. Note that this is IU/ml, not copies/ml. The current guidance document, the CBER guidance document has yet to be updated.

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The CBER HCV panels were formulated from window period units. This is negative for anti-HCV, of course, and is anti-HIV and HBsAg and not detectable for quite a few other viral agents. It is HCV genotype 1B. The entire HCV sequence is known. This is in collaboration with Dr. Steve Feinstone that we determined the entire sequence. At that time, five laboratories participated in small-scale studies, and it was determined to contain 5 times 10^7 copies per milliliter.

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So, current CBER HCV RNA panels is a ten-member panel and was derived from the HCV stock, diluted with a defibrinated pool plasma. We have eight positive, ranging from 5 to 10^5 copies per milliliter. It contains two negatives and each was filled 0.57 ml per vial and stored at minus 70 degrees Celsius. At that time it was tested by nine laboratories and seems to be very much in the target levels that we wanted to have.

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Panel member number one, that contains 1000 copies/ml, participated in the collaborative studies. John

Saldanha just mentioned it. That particular collaborative study a total of five international HCV reference reagents were calibrated against the international standard. In that particular collaborative study CBER member number one was assigned to have 250 IU/ml.

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We also have HIV-1 RNA panels in CBER. Panel A, subtype B has five members, and this is a window period of plasma units diluted with the antibody negative defibrinated pool plasma. It has four positives, ranging from 100 to 2.5 times 10^5 copies/ml, and it has one negative and is 1.1 ml per vial. Actually, the current WHO standard for HIV is originating from this window period unit.

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We also have panel B that is also subtype B that has ten members. This is a cultured patient isolate diluted again with defibrinated plasma. It has eight positive, ranging from 10 to 2.5 times 10^5 copies/ml. It does have 105,000 copies in the panels. There are two negatives and the fill size is 1.25 ml/vial.

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The third panel is HIV subtype panel. This is under development. It has eight subtype panels, A, C, D, E, F, G, N and O. Each panel has seven members, except that N has four and O has 18 members. Right now, currently

it is under pilot-scale studies. It is being characterized and titered by several NAT manufacturers. Full-scale panels will be formulated if data obtained from pilot-scale panels are as expected. I would like to mention that all these HIV panels are formulated in Dr. Hewlett's laboratory.

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In BPAC, held in September, 1999, they agreed that -- this is the exact wording that I put in here on the slide -- pending a policy on screening whole blood donations, FDA may not require studies to validate the clinical effectiveness of NAT for B19 DNA under IND for plasma for further manufacturing. So, this only applies for recovered plasma and source plasma, not for whole blood. So parvovirus B19 is an in-process control test. So, it is unlike HIV, HCV and HBV NAT. However, in that BPAC it was mentioned that we should quarantine and destroy in-date when possible.

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Parvovirus B19 NAT, we need the validation as an analytical test, and approve it under relevant product license. Now, the proposed limit, which we mentioned in the FDA NAT workshop in 1999 and also the subsequent workshop held by the NHLBI, we said that the proposed limit is 10^4 genome equivalent of B19 DNA per milliliter in all

manufacturing pools. At that time we didn't have an international standard. So, we would like to proposed that as 10^4 IU of B19 DNA per milliliter in all manufacturing pools.

As you know, this particular level was set because of the B19 transmission associated with the SV-treated pooled plasma in the Phase IV study in healthy donors. In that particular instance, less than 10^4 genome equivalent/ml, none of the lots transmitted to the recipients. So, for such high titer screening, whatever the residual B19, it will be neutralized by the anti-B19 present in all large pools, and then also whatever the residual B19 is will be cleared by the manufacturing procedure.

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So, for standardization of B19 NAT from plasma for further manufacturing we proposed that, for instance, in preclinical studies, like for specificity studies, we proposed to have 500 healthy donors monitored and also 100,000 donations in the form of minipools. Then for sensitivity we would like to have about 20 known positives, and for analytical specificity, like interference or analytical sensitivity and precision, it will be very similar to the guidance documents for HIV and HCV. As you know, this is high titer screening for B19. So, the

analytical sensitivity had to be greater, much greater than 100 IU/ml. And, no clinical studies, and we only would like to make sure that the manufacturing pool will be within less than 10^4 IU of the B19 DNA per milliliter so that the viral load in the starting pool would be very low to begin with.

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This is for the B19 DNA standard. This is derived from a window period plasma unit that has 10^{12} equivalent/ml, and we dilute it. This is with cryopooled plasma. It is not defibrinated plasma. This one is anti-B19 negative plasma. So, the formulated standards contain 10^6 IU/ml. This is 1 ml per vial. The consensus titer was determined by collaborative studies because the CBER preparation is a liquid formulation targeted to contain 10^6 genome equivalents. It was a candidate preparation for establishing an international standard for B19 DNA. Of course, AA, which is the freeze-dried preparation, was selected as an international standard but the CBER standard was determined to contain 10^6 IU/ml.

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So, we questioned the BPAC in June, 2000 should the FDA recommend that if a plasma pool or minipool is found to be HAV positive the individual HAV NAT positive

donors should be identified and notified of the test results.

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The BPAC decision then was that data provided did not demonstrate risks significant enough to warrant donor notification and look-back measures. So, HAV NAT, by default, for plasma for further manufacturing is an in-process test and is not a donor screened test. That now needs to be validated by a clinical trial.

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We have not yet set any standards for HAV NAT. However, based on the viral load, the HAV NAT will be very similar to HIV and HCV NAT validation.

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So, we formulated a CBER HAV RNA standard, and this is derived from a window period plasma unit that contained 10^6 copies/ml, and diluted with the cryopooled plasma. It is estimated to contain 10^4 copies/ml. However, the consensus level is still being determined by the WHO collaborative studies because this was one of the candidate preparations to establish the WHO standards for HAV NAT. And, it is 1 ml/vial, stored at minus 70 degrees Celsius.

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Let me talk about quality assurance. As you know, for quality assurance we have assay validation that

includes the laboratory tests and well as clinical testing, specificity, sensitivity and precision. Quality assurance also includes quality control testing of components and final test kits, and acceptance criteria specification has to be set to ensure lot-by-lot consistency and also Good Manufacturing and Good Laboratory Practices.

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The quality assurance issues we already talked about. We really need to standardize the assay using the well characterized panels and reference standards. We need to ensure the quality control of components and, finally, test kits using, again, well characterized panels and reference standards. Then, we also need to monitor operator proficiency using proficiency panels and training programs.

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Other quality assurance issues, for instance, the sample preparation includes collection, storage and extraction and, actually, improved transportation as well. The manufacturing consistency of primers, probes and enzymes is very crucial. Performance of controls, calibrators and quantitation standards, specimen and kit stability, instrument and software validation.

Here I mention some controls and quantitation standards, like potency of specimen controls, purity,

identity and potency of synthetic oligo, internal controls and quantitative standards. Then, the stability and you also need to make sure you have the control set at, you know, low, medium copy numbers and so forth. Acceptance criteria and specifications need to be set.

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Finally, in the interest of time, I am just going to mention the issues for pool specimens, for instance, for the QA issues. Demonstrate enhanced sensitivity or equivalence of testing pool, the current methods, evaluate the matrix effect due to pooling, and validate logging and tracking procedures for the inventory of specimens in a given pool. You need quality assurance in computing and recording of results. We also need to validate instruments, these different instruments and software used to monitor pool specimens.

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We have a guidance document. We have an FDA guidance document for HIV-1 and 2, and this can apply for HCV and other viruses in general. Then, we also have another guidance document, a draft guidance document that Dr. Mied already mentioned, and there is a guidance document that is specific for NAT for HCV. It is a very good document. Dr. Micha Nubling already mentioned it. I list some of the analytical procedures. Some are FDA and

ICH documents to validate some of the NAT test as an analytical procedure. This can be a reference document.

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In summary, NAT is currently being implemented in the U.S. as either donor screening or in-process control. For standardization, CBER currently has reference panels for HIV and working reagents for B19 and HAV. So, we need quality assurance for NAT-based tests used to screen blood and plasma donations. Thank you very much for your attention.

DR. BISWAS: Are there any questions for Mei-ying? No? In that case, we will break for lunch.

DR. YU: Is there general discussion for Dr. Saldanha, Dr. Nubling and myself?

DR. BISWAS: They want lunch!

[Whereupon, at 12:05 the proceedings were recessed to reconvene at 1:05 p.m.]

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

DR. YUWEN: We are going to start the afternoon session. I would like to make a short announcement for this afternoon's schedule. This afternoon we are going to have three sessions. Session number three is going to be experience with the minipool NAT for donor screening, and we have numerous industry presentations. After section three, we will have section four, which is progress towards single unit NAT. Then there is going to be experience with minipool NAT screening for HAV/parvovirus B19. We have a tight schedule and each presenter is allowed to have no more than ten minutes. What I would prefer is for everybody to present about eight minutes or so and leave a minute or two for a short discussion.

Once our computer starts, I will first introduce Dr. Any Conrad, representing NGI. I have a clock with me. This will force everybody, and the clock is set for eight minutes so when the clock clicks you know you have no more than two minutes left. Thank you.

III. Experience with Minipool NAT for Donor Screening (HIV/HBV/HCV) Plasma for Further Manufacturing NGI

DR. CONRAD: Hold on a moment. Don't start my clock yet! If any fire alarms go off, I get extra time!

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I am sure many of us have heard this, we are going to do a summary of the HCV, HIV and HBV trials conducted with many of my associates, partners and collaborators who you will hear from in a moment. I will briefly give the results of the HCV and HIV trials that resulted in our approval, and the status of the HBV. I thought I would do one other thing. Since everyone else who comes after me is going to break down their individual things, I thought I would group all of the testing that we have done into one massive group and see how those numbers compare to the individual companies that you will hear from later.

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At NGI we use a modified PCR method technology that uses Southern Blot detection. I am sure many of you have heard this before. The HIV and HCV tests are now approved and HBV is being run under an IND. We use a robotic pooling device, called the T-can. It makes pools of 512 -- I apologize to all of you who have seen this 2000 times, but we will get this clear one last time.

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That is the T-can.

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The way we make pools is our pools are eight by eight by eight pools, consisting of 512 members. So, the pools have 512. If you test a pool and the pool is negative, we make the inference then that all of its component members are negative. If it is positive, all that you need to do is test a row of eight, a column of eight and a layer of eight and --

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-- lo and behold, where the row, layer and column intersect you have the positive sample. So, essentially what it does is -- this is an economy measure; it makes things faster, easier and less expensive.

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The sensitivity assays, as determined in the analytical sensitivity components of our submissions were, for the HBV, we use 2 ml which comes out with a 95 percent detection limit of 4 copies or 1 international unit; for the HCV-1 ml assay we have a 95 percent detection cut-off of 20 copies with 5 international units; and the HIV is 1.4 and 5.

Listening to Mike Busch's talk this morning, I was struck by an interesting point and I will diverge for a minute from my talk, and that is that when we talk about single unit testing, the other alternative to that would be to drive the sensitivity of assays lower and lower and

lower. Theoretically, by increasing the amount of material that we test we can actually get the detection cut-offs to be sub single unit. We can make them 0.3 IU/ml. That may, in fact, turn out to be a more economic mechanism than going to single unit donations. I think you can achieve the same sensitivity. If we were to take all 512 members of the pool and test the entire pool instead of an aliquot of the pool, theoretically it would be the equivalent of single unit testing, but that would necessitate us getting better and better mechanisms to purify viruses from high volumes.

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The clinical trials were conducted on 343,729 donations from approximately 48,000 randomly selected donors. The samples were also tested for the standard immunologic tests, but an important distinction needs to be made here, that samples from new donors were tested by RT-PCR only after they had passed their serologic testing, but all donors that were already in the system or not new to the system were tested simultaneously with the RT-PCR reaction. So, there will be numbers that I give you that have people who were both serologically positive as well as serologically negative.

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The results for the HCV were 0.031 percent of the donations, or 105 out of the 342,000 that were found to be positive for HCV, and 85 of these donations were EIA negative. In other words, they were in the window period. But since other serologic tests also occur, like ALT, we took those 85 samples and we looked at how many of them had normal ALTs. So, 75 of those units would not have been interdicted had NAT testing not been employed.

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Just some interesting numbers to keep in mind. The mean time to seroconversion for the samples we detected in the study was 57 days, plus/minus 14. The range went from 22 to 120 days. The average viral load of these antibody-negative ALT normal samples was 1.36 times 10^7 copies, so very high titer. Obviously, that supports all the data that showed this rapid and high increase in viral load, with a low of 7500 and a high of 56 million copies/ml.

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The HIV results show that 18 out of the 342,000 donations were found positive for HIV. Ten were both antibody and antigen negative; eight were positive for either p24 or antibody by the time we encountered them in the pooling system. Of the 18 donations, they came from

four donors, for a frequency of approximately 1/12,000 donors.

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An important component, which is going to be discussed I think in further detail later, is that we made supplemental comparisons of the HIV pool testing using NAT compared to both Colter and Abbott's p24 antigen kit. I think, again, the most important message and summary message here is to notice that the antigen positive, PCR negative samples -- there were none, and this led to our ability to submit in the license the statement that PCR is a substitute for the p24 antigen test, much to the delight of some and chagrin of others.

[Slide]

The conclusions are that 75 of the donations for HCV wouldn't have been intervened. A total of 8.5 times 10_{11} copies were interdicted and not placed into plasma because of NAT testing, and the HCV plasma testing was actually sort of simple and functional to do.

[Slide]

For HIV, six HIV units were interdicted that would not have been detected, for a total viral load of about 7.15 times 10^{8} and, again, an important thing is that the safety of the units was actually enhanced by using PCR and antibody screening versus p24 and antibody screening.

[Slide]

HBV, just briefly a summary because you are going to see a lot of detail about this, we have actually identified two different classes of individuals. We found people who were heading towards an acute stage of HBV, where they have a rapid escalation in viremia, followed by S-antigen positive, and those people then get brought out and those are the acutes. We have also found a second and more common class of people whom we call the chronics, who have these sort of low, smoldering viral loads. They are also sometimes S-antigen positive but we have also found a group in here who have S-antibody, core antibody. So, they are S-antigen negative but they still have some DNA. So, HBV adds a more complicated series of issues that have to be addressed, and I think that you will hear more about that in a bit from my colleagues.

[Slide]

But there are four classes of people that you can encounter, the HBV core antibody negative, positive and different antigen statuses. I think that that is going to be important.

[Slide]

Then, for the last slide I thought I would just give you the summary of the number of donations that we have tested and some of the frequency rates. We have

looked at 11 million, 600-something thousand HBVs for a total of 1136 serologically negative. We have looked at 18 million HCVs for a total of 1653 serologically negative, and we have looked at 14 million HIVs and found 101 donations that were serologically negative. And, those are the frequencies for the serologically negative in those three large studies. So, 1 in 10,000, 1 in 11,000 and 1 in 142,000 for HIV. That is all.

DR. YUWEN: Any short questions or comments? Yes?

DR. ALLAIN: You mentioned you have 85 HCV positive and you said there were window periods. Did they also convert? How many of them seroconverted?

DR. CONRAD: We attempted to follow-up on all patients in the clinical trial, some of the patients that were donors. But of all the people who came into the clinical trial that made up those 82, all of them seroconverted. So, all the patients whom we were able to follow through time seroconverted.

DR. YUWEN: Our next speaker is Dr. Lorraine Peddada, representing Alpha Therapeutic Corporation.

Alpha Therapeutic Corporation

[Slide]

DR. PEDDADA: Today I am just going to have a brief overview of what we did with the HIV and HCV

screening program, and give an update on our progress with HBV PCR.

[Slide]

We heard the history of PCR already today several times, but we actually started in 1997, working with NGI as the principal investigator for the HIV and HCV INDs. In 1999 we filed an IND for HBV, and in September of 2001 we received the first license to test source plasma using PCR.

[Slide]

We have already gone over our three-dimensional pooling scheme.

[Slide]

This is the sensitivity of PCR screening source plasma at our pool size, which is 512 donations per pool, and it is about 2000 copies/ml for HIV and HBV and 10,000 copies/ml for HCV.

[Slide]

During our clinical trial we submitted data on the enrolled subjects for HIV and HCV, and we did a retrospective analysis of samples that we had in inventory from positive donors. With those panels we were able to calculate a window period reduction of four days for HIV and 57 days for HCV.

[Slide]

The HBV clinical study was initiated in August of 2000, and our basis for enrolling would be that the donor is PCR positive with minipool testing or hepatitis B surface antigen positive, but not both. We had 26 PCR positive eligible donors and we enrolled five. We had nine HBV surface antigen positive donors and we were able to enroll four. Our plan was to follow the donors six months or to seroconversion. We were monitoring the donors weekly with PCR surface antigen testing, and later we added anti-core, anti-HBE and E antigen because the donors presented a very complex pattern.

[Slide]

With the HBV trial we have only enrolled nine subjects. So, what we did was to look in our inventory for panels of donors that would qualify. We found 65 HBsAg positive donors and we analyzed the look-back donations from all of the donors. From 65 donors we were able to look at 512 donations, and we screened each one undiluted by PCR, using the NGI UltraQual test, and 315 were positive undiluted. Then we prepared simulated master pools of 512 and tested again. We were able to detect 142 of the donations, which gives us a sensitivity of 45 percent.

[Slide]

This is just to give you an idea of how we calculate window period. What we are trying to do is

determine the amount of time it takes for a donor to go from being HBV DNA positive to surface antigen positive.

[Slide]

Because we have the panel of donors, the 65 donors, all of the samples and the bleed dates, we were able to make these calculations. So, with a pool size of 512 we were able to analyze samples from 43 donors and we achieved a reduction of 15 days in the window period. Because we were not sure how the master pool of 512 we would work, we also analyzed primary pools of 64 and we were able to reduce the window period by 23 days. And, the total window period as measured by NAT in undiluted samples was 36 days in our hands.

[Slide]

This is just to illustrate that in the plasma industry we do have a 60-day inventory hold and by closing the window by 15 days we are pushing back our inventory hold 15 days. Then you add onto that the 60-day inventory hold.

[Slide]

This is a summary of our screening to date. We have been screening HIV and HCV for four years -- four years for HIV and HCV. One year, actually, we did not screen using PCR. So, that is three years for HIV. The total donations tested is six million for HIV and the total

number of positive donations was 472 and this includes all positives, not PCR only. This gives us a frequency of 8 donations per 100,000.

For HCV we screened ten million donations and had more than 3000 positives, for a frequency of 32 donations per 100,000. Since we have just started HBV, we have screened 2.5 million donations, with a frequency of 11 donations per 100,000.

[Slide]

What I did next was to look at all of the PCR positive donations out of the six million screened, and look at PCR only to see how many we would pick up just with PCR. We picked up 84 out of six million, which gives a 50 percent increase over what we would have gotten with antigen alone.

I also looked at PCR plus antibody. We were able to detect 33, and we are calling these true positives because we got confirmation that the donor was actually positive.

Over here, what I have labeled as false positives would be the donations that were p24 antigen positive only. I am very pleased to hear that we may be able to reenter these donors that were deferred. They may be reentered into our program. We detected 837 antibody positive

donations, and we really don't know if they are true positives or not.

[Slide]

In conclusion, I just want to say that with minipool testing we have been able to detect donations that were positive and not positive by antigen and antibody, and we have reduced the window period donations of our manufacturing pools and, hopefully, eliminated many high titer units. Any questions?

Dr. YUWEN: Any questions or comments? Keep it short, please.

DR. GALLARDA: What was your test of record for surface antigen?

DR. PEDDADA: What was what?

DR. GALLARDA: What was your test of record for the surface antigen EIA?

DR. PEDDADA: Genetic Systems.

DR. YUWEN: I would like to remind anybody from the audience to please identify themselves when they ask questions. Thank you, Lorraine. Our next speaker is Dr. Donald Baker, from Baxter.

Baxter

[Slide]

DR. BAKER: This may seem like deja vu to you because you are going to see two Baxter presentations, but

this is the Baxter on this side of the Atlantic as opposed to the Baxter on the other side of the Atlantic, which is going to be the next one.

[Slide]

I am going to be looking at this from the perspective of a manufacturer. I am not going to talk about PCR test details because I am, frankly, not competent to speak about that.

In the integration of the PCR, we integrated in two places in the manufacturing process, one is the donor screening and the second is the production pool. Now, from a manufacturer's perspective, which is not necessarily shared by regulatory agencies, our objective is to get a low defined level of viral burden in the manufacturing pool. That is not considered primarily a donor screening test.

[Slide]

In the resolution of the results, the information flow is, of course, critical and this is something that we won't get into in this presentation but I just wanted to illustrate the complexity of the flow of the results. We use NGI as our NAT testing lab and you can see between the centers, many of these which are contract centers to us, there is a tremendously complicated flow of information.

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I am going to present our integrate summary which, for HCV and HIV, will be from 1998 to June of 2001. It will include both the qualified, that is donors who have donated at least twice successfully, as well as the applicant donors. In the first half of this year applicant donors represented about 11 percent of the PCR positives, just to give you some sense of their contribution. So, for HCV in a little over 5.2 million donations we found 894 positive and that represented 313 donors. Obviously, because of the short time between donations, an individual donor might contribute two or three positive donations before they are deferred.

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For HIV, again we started HIV and HCV testing simultaneously, we turned up 14 positive donors. During that time period and still to date we are continuing to do p24 testing. Had we not done p24 testing in those five million donations, we would have had approximately another 30 individuals who were only p24 positive who would have come into the PCR testing. So, that gives you some sense of the number of individuals that the p24 test is excluding for us at present.

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For HBV, there are times when it is better to be lucky than good. Initially, when we were looking at HBV we

considered that it might be possible or more economical not to do HBV testing and just throw out positive pools. I am very glad that we did not take that approach because, as it turned out, our HBV positivity in PCR was much higher than we had initially anticipated and it would have been very uneconomical not to do HBV testing.

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Finally, here are our PCR positives with time. I wouldn't read a lot of meaning into the apparent increase with time with some. A lot depends on how fast your PCR turnaround is, and our PCR turnaround has become more rapid and, consequently, since we exclude our EIA positive results, we have had less chance with EIA testing to exclude individuals so we have had more individuals enter the system, which is why the PCR positivity has seemed to increase in some circumstances. It also is predicated on changes in collections practices and addition of new centers and the increasing number of donations.

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Finally, the system works. It is robust and reproducible. We have never had a positive manufacturing pool, touch wood. It is timely. We have been able to, of course, complete all the testing and donation resolution within our 60-day inventory hold. And, it has had a minimal impact, at least in terms of deferred plasma. We

have approximately 1000 donations or so that have been discarded in this period of time. Thank you.

DR. YUWEN: Thank you. Are there any questions for Donald? Michael?

DR. BUSCH: Mike Busch. It was interesting to hear that you do test your applicant donors and you sort of separate your yield in applicant versus registered. On a per donation basis, what is the rate of NAT only in the applicant versus the registered donors? This would give you a sense of the relevant incidence in these first-time applicant donors versus your regular repeat donors.

DR. BAKER: You know, Mike, I know where you are coming from but I don't think I can address the question because, don't forget, we do our EIA and antigen screening first so they all get kicked out. So, of the PCR positives, for the first half of this year there was only about 11 percent that were applicants. But I don't know what the EIA positivity among the applicants is.

DR. BUSCH: What percentage of all accepted donations are from applicant versus regular registered? Do you have a sense of that?

DR. BAKER: We don't accept applicant donations. Right? So, they have to donate twice before we accept them. I can't give you the answer you want. We don't collect our data that way.

DR. BUSCH: Thank you, Donald.

DR. YUWEN: Our next speaker is Dr. Gerald Zerlauth, representing BaxterHylandImmuno.

BaxterHylandImmuno

DR. ZERLAUTH: Thank you.

[Slide]

This is the other part of Baxter's activities, comprising actually both sides of the world. We are collecting plasma in the United States, pooling it in the United States and sending it over to Europe. So, this HIQ PCR IND approach is a truly global activity.

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As I said, we are collecting our plasma in the U.S. in community bioresources plasma centers, 23 centers which bring about one million liters of plasma per year. These donations and serology samples are sent to Hoover, Alabama for serology testing, while the PCR vials are going to the pooling lab in Round Lake, north of Chicago. These samples are pooled upon the arrival of the serology data. So, we are only pooling serology non-reactive sample into pools of 512, according to the scheme already seen in pools of 512 individual donations.

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These the PCR pools, the master pools are then sent to Vienna in a daily approach by airplane and are

tested in Vienna with a test system which we call HIQ PCR. Most of you know it. It is the same for HIV, HCV and HBV RT or PCR, depending on the virus. It is a laser-induced fluorescence detection system based on size-dependant separation on polyacrilomide gels. We have an internal standard which we termed the internal quality marker. The tests are validated as qualitative tests, and these tests, of course, are tested against all available genotype panels and are validated according to the guidelines that have been cited several times this morning, in Europe and, of course, against the FDA guideline and, of course, against all the panels that have been released by the FDA.

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So, overall, the sensitivities are now in IU and not calculated but tested against WHO standards, calibrated, as given here, HBV, 11 IU/ml; HCV, 24; and HIV-1, 65. Just to complete, we also include parvo B19 which has 34 IU/ml, which is a quantitative assay; and HAV, with 157 what we call Baxter units because we are lacking an international standard at the moment. Just to avoid genome or other equivalence we have Baxter units which will be replaced as soon as we have a WHO international standard.

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The results, as we are a newcomer in this IND business, we only started in April and we have done 540,000

donations so far in about 1000 pools, and we have seen 13 positive pools for HCV, one for HBV and none for HIV. In terms of donations, we have 13 donations but these 13 donations were from three individual donors that we could identify in this process and, obviously, one donor a non-HIV. So, I think we have to carry on until we get the necessary number to calculate the numbers as we have seen them previously. Of course, we are going to use depository samples to increase the number of positive pools and show that the system works.

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So, this is actually what I have to show at the moment, and I hope next time I have more conclusive results. Thank you for your attention.

DR. KLEINMAN: Steve Klienman. Just a very quick question, is this a triplex assay or three individual PCR assays?

DR. ZERLAUTH: These are three individual PCR assays and extraction as well for two.

DR. BUSCH: Mike Busch. A clarification, so the plasma that you are testing is a separate collection system, separate centers from Baxter U.S. is collecting?

DR. ZERLAUTH: It is a Baxter owned group of plasma centers. We just used those because we have those in our European files and we can use that plasma in Europe

as well. So, we get the million liters for testing and most of it for use in Europe.

DR. BUSCH: It is completely separate collection from what Don Baker was talking about?

DR. ZERLAUTH: Don Baker's is contained in this to some degree.

DR. YUWEN: Our next speaker is Barbara Masecar, representing Bayer.

Bayer

MS. MASECAR: Thank you for the opportunity to present this afternoon.

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I would like to briefly give you a summary of the NAT donor screening at Bayer Corporation.

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This is a recap of where we are right now. All of our donations are currently screened for the big three, HCV, HIV and HBV and we are also doing in-process testing for parvovirus B19 by NAT. Currently, HCV, HIV and parvo testing is Bayer's Raleigh test laboratory in North Carolina and that is on a 96-sample minipool format. The HBV testing is performed at NGI on master pools, if you will, of 480 samples that are prepared at the Raleigh test lab and then sent to NGI, in Los Angeles.

Today I will be giving you summaries of the HCV, HIV and HBV, and my colleague, Todd Gierman, will be presenting the parvovirus data later on today.

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As far as HCV goes, we began testing in September of 1997 and we have been using the Roche AmpliScreen microwell plate method. We began enrolling follow-up study donors that were found to be NAT positive and antibody negative in 1997, and we continued that enrollment until January of this year. During that time frame we screened 6.5 million samples representing 385,000 donors approximately.

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During that time we discovered and identified 383 NAT positive, antibody negative donors. I want to underscore here that this was as judged by the Abbott EIA version 2.0 test. Of these 283, 289 were qualified; 94 were applicant. We were able to enroll 152 into the follow-up studies, which is 24 weeks in our program. We found that compared to EIA 2.0 the reduction in the window period was 66 days.

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We provided 71 of the sample panels from follow-up to Leslie Tobler, Blood Centers of the Pacific, for further study, and she, among other things, retested these

panels with the Chiron version 3.0 EIA test. What she found was that in 42 of the 71 panels seroconversion did not occur simultaneously with the version 2.0 and 3.0. As you might expect, in those cases where there were discordant results seroconversion with the 3.0 preceded seroconversion by 2.0 by a median of 17 days.

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In 55 of the 71 panels that Dr. Tobler tested the index donation showed concordant negative results with both the version 2.0 and 3.0. So, the index donation being the first NAT positive donation was, indeed, negative with both versions of the EIA test in 77 percent of these panels. However, in 16 of these panels, or 22 percent, the index donation was actually positive with version 3.0. Interestingly, two of the 16 we followed for about six months, and with version 2.0 there was never a seroconversion. One of these was RIBA positive, one was RIBA indeterminate, but in our program we don't test RIBA unless the EIA is positive. So, we didn't have an indication prior to this work that the RIBA was positive.

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Of 383 of those donors that were identified as NAT positive and antibody negative, 23 of the study participants were negative for all tests upon follow-up. This could be assumed to be due to false-positive NAT, but

the more likely reason is low level contamination during collection, processing or testing. In 1997, when that screening began at Bayer, at about that same time we changed to an open tube drain and drip method for sample collection.

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So, we got wise to that; that wasn't the way to go. So, we reinstituted closed sample collection in November of last year. Also, early on we changed lab processes so that multiple positives from a single minipool are always confirmed from a backup sample. So, since February of '99 all HCV and HIV positives are confirmed by backup samples if they are not the sole positive in the minipool of '96. So, four of the 93 were collected prior to February of '99 and one, perhaps two, were collected after the implementation of closed sampling. There is some conflicting information from the plasma centers on that. Unfortunately, we stopped enrolling -- well, fortunately or unfortunately, we stopped enrolling donors in the following January. So, in the time frame to collect it -- there is no data -- is quite short.

[Slide]

Switching to HIV now, we also use the Roche AmpliScreen HIV microwell plate method. The testing began in September of 1999, and we ended follow-up study

enrollment in April of this year. During that time we screened about four million samples, representing 200,000 donors.

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During that follow-up study period we identified 23 donors that were positive by HIV NAT only and seven donors that were positive for both NAT and p24. This is how it falls out as far as applicant and qualified as defined by ABRA, three of the NAT positive only donors were applicant and 20 were qualified, and all of the donors that were positive for NAT and antigen were qualified donors.

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We enrolled nine of the NAT positive, antibody negative donors into the follow-up study and what we found was that the reduction in the window period relative to p24 antigen was six days, with an N of 14, and nine days relative to antibody, with an N of eight. So, you can see by these numbers that we also had some additional data from subsequent donations from donors that were not enrolled in follow-up. Indeed, all of these nine did confirm upon follow-up.

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Just a slide about assay performance in our hands, the AmpliScreen HCV assay has about a 1.5 percent positive control failure rate and a 0.5 percent negative

control failure rate. For HIV, it is somewhat better. It is 0.8 percent positive control failure rate, with less than 0.1 percent negative control failure rate. This compares quite favorably to some data that we have from our own viromarker test laboratory when it was in operation, in 1997, of 2.4 for positive control failures and 1.1 percent for negative control failures.

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HBV by NAT -- as I mentioned, the testing is performed at NGI on pooled samples that are prepared by Bayer. We began testing in July of 2000. We ended follow-up study enrollment the following August. During that time we tested 349,000 samples, representing 45,000 donors.

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We identified 19 donors as NAT positive and surface antigen negative. We enrolled ten in the follow-up study and the average reduction in the window period was 11 days relative to surface antigen. Again, the N is 7. That is comprised of four participants of these ten, and three that we got data from subsequent donations.

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So, of those ten that were enrolled in the follow-up study, as I said, four were subsequently surface antigen positive. However, six did not test surface antigen positive during the follow-up study. Four were

anti-core positive, and that was after following them for 12 to 14 follow-up samples. All those were positive the first time we tested them for core but it was already when they were in follow-up. Two were negative for all subsequent tests, and that was HBV NAT, surface antigen, anti-surface antigen and anti-core. For one of these donors we only had one follow-up study and the other one we followed for 29 weeks basically, but they were negative. Interestingly, both of these were from the same plasma center, collected a day apart. So, I am not sure what that means but it is coincidental.

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Follow-up HBV NAT data for three of the four surface antigen positive, anti-core negative donors was intermittent during follow-up period, and it may be indicative of a preexisting low-level HBV infection, as was already presented. Further characterization of follow-up samples for these HBV follow-up donors is in progress at NGI.

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In conclusion, I would like to acknowledge some folks that helped me with the data presented here, Eleanor Davis, Sue Banazek, Matthew Nicosia from Bayer, Leslie Tobler from Blood Centers of the Pacific and Rich Smith from NGI. Thank you for your attention.