DR. ALLAIN: There is some confusion, at least in my mind, regarding the anti-HCV third generation. I thought until probably two or three years ago that third generation meant addition of NS5 antigen. But if you do that, you don't gain 17 days in the window period. So, does that mean that the test you used was, in fact, an antigen sandwich that detects IgM in addition to IgG?

DR. TOBLER: I am Leslie Tobler. The only reason that we did it was because we were trying to compare the data that we would extract from these seroconversion panels that we had gotten from Alpha, and Alpha screened with HCV 3.0. Therefore, before we could do anything, I needed to get these panels screened by HCV 3.0. The results were surprising to me but, actually, I tend to be an HCV 3.0 fan and they were quite pleasing.

DR. ALLAIN: But you don't answer my question, what is the format of your 3.0? Is it an antigen sandwich or an indirect antibody assay?

DR. TOBLER: I am actually not quite sure but I do not believe it is a sandwich. I simply know that it is the licensed version of HCV 3.0 in this country.

DR. ALLAIN: Then it doesn't make sense how you can gain 17 days in the window period just by adding a different antigen mixture.

DR. TOBLER: But we did. Trust me, we did.

DR. MIMMS: This is Larry Mimms, Gen-Probe. It is an indirect assay. I don't want to speak for the Ortho people but it is an indirect assay, and what they have done, they have looked at a different confirmation of the 33C antigen, which I think is either reduction sensitive or not. I can't remember if it is reduced or non-reduced form but there is a structural component of 33C that is recognized early on, but it is indirect.

DR. MASECAR: Yes, and the two donors that were negative throughout follow-up but were both positive with version 3.0, their RIBA reactivity was in the 33C antigen.

DR. TOBLER: Barbara, I need to correct you. One of them was surprisingly RIBA negative for the entire six-month period.

DR. MASECAR: Well, it was plus/minus in C33. It was RIBA negative but it was plus/minus --

DR. TOBLER: And the other one was RIBA positive for the entire six months.

DR. YUWEN: Thank you, everybody and thank you, Barbara. Our last speaker in this section is Dr. Chuck Watson, from Aventis.

Aventis

[Slide]

DR. WATSON: I would like to explain our process of testing. We will only test -- well, we test for all

five viruses. We have been doing that since -- well, we have ben doing the big three viruses, HCV, HBV and HIV, since April of '98 and we added parvovirus B19 and HAV in March of 2000. All our samples are previously tested by the licensed test and they have to be negative. We pool the two dimensions and we will test down to the individual donor when we find something positive.

[Slide]

Is the virus load in international units for our test? Basically 400 for HBV, about 4000 for HCV and about 25,000 for HIV.

[Slide]

This is how we make our pools. We start with the individual donations. We pool 12 of them together. Then we take ten of these groups of 12 and pool them together until a group of 120 to make what we call a midipool. Then we take these midipools and we pipette ten of them together to make a maxipool. We pipette them in two dimensions, in the horizontal and the vertical, and then they are tested in duplicate for each of the dimensions. That is our test process.

[Slide]

What is our yield? You can see. While this yield excludes the serology positive in the unit itself, it does not exclude subsequent serology positive where the

donor seroconverted after the sample entered into our pooling process. So, there are some serology positives there. It is also a combination of both qualified donor and applicant donor information.

[Slide]

These next two slides show a little different picture of PCR testing. What it really talks about is what is the effect of PCR testing. What does it do? From this, in reality, I am coming from a manufacturer's point of view. The theoretical load or burden that would be missed in serology tests is in the second column. The maximum load that would be missed by PCR, the maximum burden for a negative PCR is in this column. So, the benefit of screening in logs is approximately two logs for HIV and HBV but almost five logs for HCV. So, for every unit that is found positive you are removing six to eight logs for HBV; you are removing seven to nine logs for HIV; and you are removing approximately seven to twelve logs of HCV. That is just by finding one positive unit.

If you take that then and take those burdens, the serology burden, the PCR burden, theoretical burden -- this is given our sensitivities -- and you look at different methods of manufacturing, you can see that you have taken down the burden going into manufacturing so that you have enabled your manufacturing process as your inactivation and

your virus removal methods to become much more effective. We believe that that does increase the safety margin of the plasma entering manufacturing. Thank you.

DR. YUWEN: Thank you. That completes our first session. In the next 50 minutes of so we are going to have five speakers representing the blood banking industry. The first one will be Dr. Larry Mimms, representing Gen-Probe.

Blood Bank Experience

Gen-Probe

[Slide]

DR. MIMMS: I would like to thank the organizers for the opportunity to talk about the Procleix HIV-1, HCV assay, which is the product of a strategic partnership between Chiron and Gen-Probe.

[Slide]

We actually started thinking about this in 1994, shortly after the NAT workshop in that year. It was a very discouraging workshop where everybody went away with the feeling that it really wasn't practical, but with the insight of the National Heart, Lung and Blood who funded a contract in 1996, we began in earnest refining and developing a blood screening strategy using some of the unique tools that we had developed over the previous ten years at Gen-Probe.

[Slide]

Our overall strategy was to develop and manufacture an in vitro nucleic acid amplification test for the viral detection in blood and plasma. We had set out to develop both a semi-automated and a high throughput fully automated system. Those have been termed the Procleix and the automated Procleix systems respectively.

Our goals were to hit 100 copies/ml with very good sensitivity, to include an internal control. We also developed discriminatory assays for HIV and HCV to deconvolute our algorithm and, of course, the formulations would have to be compatible with both formats, both platforms.

[Slide]

Since we started on the project under contract with National Heart in 1996-99 when we implemented under IND testing, along with the American Red Cross, the ABC, and the AIBC a number of things have happened from '99 to 2001, namely, that 100 percent or close to 100 percent of the blood in the United States is now being tested by nucleic acid. About 70 percent of the blood in the U.S. is tested with the Procleix system. The remaining 30 percent is with the Roche system.

We started with a pool size of 128 and migrated down to a pool size of 16. We have now implemented nine sites across the United States. We started with one small

laboratory in San Diego with the American Red Cross. We now have five American Red Cross centers, and I am sure Sue will talk a little bit more about that later. We have implemented individual donor testing at three military blood centers.

[Slide]

To make a long story short, in two years of screening -- and this is the total data; this is pirated from Sue Stramer concerning the IND yield samples, over 20 million donations tested. About 113 HCV and six HIV viremic donors were identified who otherwise would have been transfused. So, the test has done admirably well in the field to date.

[Slide]

Internationally, I think we, as manufacturers, have made a contribution, perhaps not so much in Germany but certainly in Australia, Portugal, Singapore, France, New Zealand, Spain, Italy and soon Ireland.

[Slide]

We designed this assay from the ground up for high throughput screening. There is a lot of technology. We have over 20 patents associated with this co-amplification/ detection system with HIV, HCV. It is fairly complicated, as the FDA can tell you, in terms of its constituents, but it is very simple for the operator to

use. We made it as compatible as we could with the current immunoassay system.

The co-amplification and detection that we use, we have a single tube in which we have target capture for the sample prep method. We have a transcription based amplification method, TMA, for amplification, and a truly homogeneous detection system, using chemiluminescent-labeled probes. We have also used modified oligonucleotides that have very high affinity binding for RNA that make it all happen. That also adds complications in terms of sequencing those oligos. So, we had to develop all new sequencing methodology as well.

We designed the assay from the ground up to detect every subtype of HIV and HCV. We chose conserved regions for HIV and HCV. We have redundancy of primers and probes, and we actually have targeted two separate regions within HIV. We have implemented an internal control which is an RNA transcript added at the beginning of the run. It has unique properties in this assay for controlling of all three steps of the assay, including the target capture system.

As I have mentioned before, we use a discriminatory assay. We have built into the system positive identification from pipetting in the T-can all the way through the assay results and matching with pools.

We have designed high throughput and automation, and we invested quite a bit of money. We built a unique, state-of-the-art 93,000 square foot manufacturing facility dedicated, at this point in time, to one product and we have the capability of producing over 100 million tests per year in that facility.

[Slide]

In terms of the assay protocol, I won't dwell on this in any great detail but I am serving as kind of an introducer to the technology because following me will be Sherrol McDonough, describing some of the individual donor testing results. She will be describing the Tigrus system and Christina Giachetti will be talking about the Triplex system, all of which are entirely compatible with this protocol. We have the T-can pipetter, a target capture system for washing away the sample, and a luminometer for plates, Weaver HC, for reading the chemiluminescence. All in all, a technician can run 200 samples in six hours with this system.

[Slide]

In our clinical trials -- these are the pivotal clinical trials that were conducted in the United States from August to November of 2000. We have a number of sites, including the American Red Cross, ABC and AIBC. We used a one-dimensional pooling scheme. It is very simple.

You make a pool of 16 and any positive pool is then run. All the individual constituents of that pool are run in the Multiplex assay and any Multiplex individual positive member is then discriminated by HIV-1 and HCV discriminatory assay -- a very simple deconvolution.

In this study we tested 191,000 donations in 16-member pools, so almost 12,000 pools with the Multiplex assay. Then, we also tested 2400 individual donor specimens with the two discriminatory assays. We had five blood bank testing sites around the United States and three master lots of reagents. We compared the results to licensed DIA. Supplemental and discordance were tested with an alternate NAT.

[Slide]

All in all, you can look at this slide, our overall specificity was 99.67 percent based on an initial reactor rate of an adjusted reactor rate of 0.31 percent.

[Slide]

We also did a clinical sensitivity study. We did analytical sensitivity studies in-house, which Christina will describe in more detail, in which we have sensitivity for HCV of around 2-3 IU/ml, and for HIV 15 IU/ml.

[Slide]

Basically, Sherrol McDonough will describe much of the sensitivity data but I will focus on the 2100 known

HIV-1 and HCV infected positives. You can see that at a pool size of 16 -- we diluted these known positives 16-fold and we have 99 percent sensitivity compared to samples run neat. For HCV only reactive samples, a 99.6 percent sensitivity. For samples which were co-infected, and there were quite a few co-infected samples in this population, we had between 9 and 8.9 and 100 percent sensitivity.

[Slide]

The discriminatory assays showed comparable, statistically similar sensitivity to the Multiplex assay. We have also, over the last two years, spent a lot of time analyzing invalid run rates, and we have been monitoring these rates worldwide. Our analysis of the data has led to opportunities for improvement over the last two years. We have analyzed the contributing factors and we have, in fact, implemented changes in our customer training, labeling, hardware and software.

[Slide]

Those changes have improved the initial invalid run rate and we now find that our rates are very comparable to those of enzyme immunoassays which, according to the data we have seen, can range, depending on site and serology assay, from 0.5 to 4.5 percent.

[Slide]

Let me just show graphically what has happened. In the yellow triangles is Australia, all five sites from Australia. The American Red Cross data and all of the United States data combined with Australia. You can see that our invalid run rate now is hovering below three percent. I think what we were gratified by is that during crisis when certain sites were running three to four times in volume we did not see a significant increase in the invalid run rate.

[Slide]

Finally, we believe that the sensitivity and specificity of the HIV-1, HCV assay has met our design goals. The same is true with the discriminatory assays. Christina will later show some data on our ability to detect all of the subtypes, including O, and we believe that all of these data taken together indicate that this assay has the performance needed to be licensed --

[Laughter]

Thank you. That is all I have.

DR. YUWEN: Any questions? Thank you, Larry. Our next speaker is Dr. James Gallarda, from Roche Molecular System.

Roche Molecular System

DR. GALLARDA: I want to point out that the agency asked that we streamline the number of presenters we

bring to the workshop. So, we have submitted to the agency the presentation that we are going to present for HIV and HCV for the public record. But today, for the sake of the eight minutes that I have, I will be giving you an update on the HBV assay that we have in development.

[Slide]

So, the main reason for doing HBV nucleic acid testing is this sense that in the donor population there is a relatively high number of potential window period units that are released for transfusion. Based on an early report by Schriver in '96, I think it was, at an incidence of 1/63,000 this would translate to about a couple of hundred transmission events per year. The funny thing is that I understand that there are no reported incidents of post-transfusion hepatitis B since relatively sensitive surface antigen assays have been implemented.

So, we are going to be doing an IND next year, a large-scale IND, to look at the contribution that minipool HBV NAT might have in reducing the window period and interdicting these units.

[Slide]

One of the first things that we have done is to look at the AmpliScreen HBV assay, its analytical sensitivity, and one way to do that is to do serial dilutions of the WHO international standard. So, what I

show on this slide is a histogram showing the percent detection rate at different inputs of HBV with the two sample prep methods that the AmpliScreen system uses. We use multiprep when we are doing pools of 24. When we get down to resolution of the individual donor we do standard prep.

This graph shows that somewhere between six international units and three we would expect to see a 95 percent hit rate for the Multiprep method which would be used on pools, and for the standard prep somewhere between 13 and 16 IU/ml. We, obviously, will need to expand the study to have a higher degree of confidence in the final sensitivity of the two methods.

[Slide]

Another way that we look at analytical sensitivity is to take highly defined, highly characterized plasma molecules for various genotypes. This shows quite nicely that for HBV we are pretty much reaching the theoretical limit of detection based on Poisson statistics, which means that if you have one copy of plasma DNA in a test you would, at best, expect to detect that roughly two-thirds of the time. So, with highly defined input molecules for genotypes A and C, in this graph, we are reaching our theoretical limit of close to one copy per PCR 66 percent of the time. We have replicated this study for

all the other genotypes as well and have seen the same phenomenon.

[Slide]

If we look at our clinical samples and the genotype inclusivity of the AmpliScreen assay, we took a panel of HBV genotypes and dropped them down to 50 copies/ml for each of them and tested all of them in replicates of 24, and we are at 100 percent detection rate at 50 copies/ml. We will be expanding this study as well for the non-clinical performance part of the IND study.

[Slide]

One of the most interesting topics to discuss about HBV is what will the contribution be for doing minipool NAT testing with a virus that has a very mediocre viremia for a relatively long period post-infection.

So, what I would like to do is show you a few slides of a collaborative study that we did with CBER to compare the performance, in a blind study, of the AmpliScreen assay with seven surface antigen assays that were coded and tested in parallel single-unit testing as compared to the minipool NAT testing.

[Slide]

The panel that was selected was comprised of ten seroconversion panels, each panel having ten individual members. Those were chosen for their surface antigen and

HBV NAT profiles. They represented samples that were in the pre-viral ramp-up or the blip phase that both Mike Busch and Micha Nubling talked about earlier this morning, as well as sample that are closely spaced together during the ramp-up period. In addition, there were 28 control samples that were included in this bind study.

[Slide]

If we look at the detectability of the WHO international standard, there were duplicate replicates of each of three levels for the WHO international standard. There were two replicates at 400 IU/ml, two at 400 and two at 40. The AmpliScreen assay detected all those replicates in a blind study. Then, these were the seven surface antigen assays, four of which were currently licensed and three which are currently unlicensed. You can see that various surface antigen assays had the ability to detect down to 400 IU/ml. After that all surface antigen assays were negative. The AmpliScreen assay still was positive at 40 IU/ml.

Comparing to the previous slides I showed on the titration studies that we have done, the AmpliScreen assay has a 95 percent limit of detection we would expect to be below 6 IU/ml. Its 50 percent detection rate is going to be less than 3 IU/ml. This is compared to the most sensitive surface antigen assay, assay A, which was

reported earlier by Dr. Biswas to have an estimated sensitivity at one S to CO of 88 IU/ml.

[Slide]

This is a summary of the ten serial conversion panels that were evaluated in the blind study. For all of the surface antigen assays, with the exception of assay A, all ten panels were informative. Assay A had an 18 percent procedural error rate so two of the seroconversion panels did not yield data.

So, if we compare the performance of AmpliScreen in a minipool configuration -- again, these are pools of 24, consistent with the pooling strategy that we are using for HCV and HIV -- we are detecting anywhere from two to three days earlier in the minipool format, for surface antigen assay A, up to 21 days or so for the licensed surface antigen assays. So we expect that minipool testing with AmpliScreen, relative to the current licensed assays, will close the window by one to two weeks.

If you look at each of the individual seroconversion panels themselves, then the greatest number of days of earlier detection by AmpliScreen in a minipool format ranged from 11 when compared to the most sensitive assay, assay A, to nearly 80 days for one of the currently licensed surface antigen assays.

When you compare what is the least number of days of earlier detection by AmpliScreen in the minipool configuration, there is parity we several of the unlicensed assays to seven days earlier.

[Slide]

Here are a few examples of the seroconversion panels. This shows the profiles that we saw. Here is an example where a particular panel tested by AmpliScreen and minipools showed parity with two of the unlicensed assays and one of the licensed assay. So, at day minus seven, relative to the index, these four assays showed equivalent detectability.

[Slide]

There was a seroconversion panel, 6283, that AmpliScreen detected what we believe to be one of the blip samples. So, intermittent viremia, which is certainly low level. There were no surface antigen assays that detected that, and I think Micha discussed that earlier this morning.

Where there was consistent detection across consistent bleeds, AmpliScreen and minipools detected at minus day 21. Surface antigen assay A was just at the one S-CO cut-off three days later and the other assays fell off. There was later detection at various time points relative to he index bleed.

[Slide]

Here is another panel where assay A had a procedural error. So, this is an example of a panel that was uninformative for that assay. However, for all the other assays, either the unlicensed assay or the four licensed assay, AmpliScreen detected the sample almost 20 days earlier than the other surface antigen assays.

[Slide]

The conclusions for HBV testing here for the minipool configuration is that this prototype HBV assay can detect less than 6 IU/ml at a 95 percent LOD. We have yet to confirm that accurately. We are less than 3 IU/ml, about 50 percent limit of detection using the WHO international standard. The test can reliably detect genotypes A through F. The specificity, that I didn't show, on a small seronegative population was 100 percent. The sensitivity, at this point, seems to exceed that of both licensed and unlicensed assays for surface antigen. Thus, we would expect that the minipool configuration should close the seroconversion window. How much? We will have to wait until we do the clinical trial to confirm.

I think that is it. Thank you.

DR. YUWEN: Any questions for James? Thank you, James. Our next speaker is Dr. Sue Stramer, from American Red Cross.

DR. STRAMER: Good afternoon.

[Slide]

My goal was to show slides that haven't been shown before --

[Laughter]

-- but at this point it is going to be difficult.

[Slide]

I would like to first thank my numerous collaborators at the American Red Cross.

[Slide]

Larry showed the composite slide of this but I will just use the slide to remind you that there are two major programs for NAT screening of whole blood donations in the United States, one that Larry Mimms presented, from Gen-Probe, which is the TME assay, and the Roche program.

This summarizes the programs from North America for the first two years of screening, including the Canadian Blood Service, who kindly provided data.

[Slide]

The summary slide, which Larry did show, shows that over this two-year period, in close to 30 million donations for HCV, we have a yield of 113 or 1/260,000, close to the 1/300 that we have been talking about.

For HIV we have had a yield of eight. If we remove two that were p24 antigen, we have had a total yield of six, over 26 million-plus donations, for a frequency of 1 in 4.4 million.

[Slide]

Specifically to move to the Red Cross Program, we have been screening since March of 1999, initially in pools of 128, followed by a conversion to pools of 16. So, you can see that the number of donations screened now has exceeded 17.5 million donations, which is probably close to 15 million donors -- so, there are quite a few human bodies associated with this testing.

Our unresolved pool rate which, compared to serology, is much lower for NAT compared to serology. Overall it has been 0.17 for our pools of 16. The yield for the two programs is shown for HCV in gold, at about 1 in 300,000 overall. HIV, we have been consistent with the total U.S. experience of 1 in 4.4 million. If you remove those positives from the number of donations that tested NAT positive individually, that yield is the number that are false positive, which is 574.

So, if a donor comes in and asks the question what is the likelihood of me testing false positive by this test, it is about 1 in 26,000 which is, again, far better than any FDA licensed test we do today.

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[Slide.]

Looking at the performance of all of our laboratories, and, hopefully, this is clear, we start with the first lab at pools of 128 and then migrate from that laboratory to a pool of 16 to the addition of the four other Red Cross laboratories, and the dates are listed.

What I want to point out, and to go into a little bit of discussion, as Larry did, is the performance characteristics of the test. I mentioned the unresolved pool rate or, as Paul Mied mentioned this morning, the number of pools that are reactive that don't resolve which would be a rather large inconvenience since you have to test 16 individual donations. That rate is relatively low.

The rates that kill us or that hurt us are the invalid rates. Here you can see the range of invalid rates for our five laboratories, 2 to 5 percent, with a mean over this entire time period of over 17.5 million donations for 3.5 percent.

We also run four external run controls in each run. If they don't provide the expected test results, those runs are considered suspect and negatives have to be retested. So here is another chance for us to fail runs for a grand total of about 5.2 which is very high.

Another opportunity for retesting of samples is if the internal control doesn't perform as expected per

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sample. So those rates are relatively low but have increased since we went from pools of 128 to pools of 16.

We have been working with the manufacturer, with both equity and training for our staffs to make sure that we can minimize invalid run rates and have been very successful at doing that. So I don't want to leave that with a negative note, but I want to let you know with a positive note that, if you look at invalid rates by laboratory, they are all going down.

This one, I don't know if that is going down, but it will over time, I'm sure. We are optimistic. But, certainly, for the labs, the other four labs, we see a decrease so our total invalid rates for the system have decreased and, for the most recent master lot we have been running, instead of sorting these by time, they were already sorted by master lot.

So I just used the master-lot sorts. But they are running much lower now than we have over the two-and-a-half-year period of time.

[Slide.]

Just to compare it to serology, our rates for serological markers are less than 1 percent for invalid rates. This shows you, for October in the American Red Cross, three-quarters of a million donations tested, the

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rates for well-established markers that we test for, HCV antibody and HIV antibody, are very, very low.

We just implemented a new p24 antigen test and we have been having problems with PPC aberrance. We have had instrument problems plus there is a learning curve associated with the test. So the same types of issues I mentioned for NAT are now occurring for the new serological tests. You can see the invalid rates are relatively comparable. So invalid rates are determined by both equipment and the staff performing the tests.

[Slide.]

To go into the specifics for our yield for NAT, we have had 632 individual donations reactive when you sort through how many were confirmed by an independent test for NAT, which is PCR, by an independent sample, which is plasma, or by follow up. I mentioned, we had 54 yield for HCV, four yield for HIV. The difference here breaks out into these different false-positive categories.

[Slide.]

Breaking down the 61 total from both the 54 in pools of 16 and the seven in pools of 128, this tells you how many of 61 we have confirmed by PCR in the index sample, how many we had an independent sample from the plasma unit to confirm, how many confirmed by follow up. 27 of these 35 seroconverted in a mean of 39 days.

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This is a little smaller number than for the source plasma reported but it is probably because the frequency with which we see donors in follow up may be less than it is for plasma. So it may be biased by sampling times.

Sex is pretty much split between males and females. First-time repeat donors, mean age is 36 for HCV-positives. 29 of 61, or less than half percent, are elevated for ALT. So, ALT, like p24 antigen, are two tests that have outlived their usefulness.

[Slide.]

If you look at where our HCV-NAT-positives come from, whether we look at it by absolute number or you look at it by frequency, what we see is the highest concentration in both incidence and prevalence, as Mike Busch said earlier today, for HCV as well as NAT frequency for the Mid-Atlantic and South-Atlantic, the Southern part of the United States. So that is where our NAT-positives concentrate as well as all the other HCV-positives we have.

[Slide.]

If we look at the viral load of our NAT-positives relative to viral load for seropositives, random seropositives, they are fairly comparable. The range of viremia is comparable. Genotype distribution for the GenProbe test, you can see that we have detected various

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genotypes so their genotype detection is good. Actually, we have detected more diverse non-1A/1B genotypes than we have with serology.

[Slide.]

I wanted to talk about an immunosilent donor we had that Mike mentioned earlier this morning. You can see this donor who I will show two years of data for was flat on ALT over the two-year period of time. The slide goes to one year. This is their multiplexed and discriminatory S to COs on the GenProbe test, as one would expect for a positive.

Viral loads; this was genotype 2B. Going to the second year, you see the same data. No ALT elevation, consistent TMA levels and high viral loads.

[Slide.]

Putting this donor with the rest of our follow-up donors, who we have had 35, brings this donor down to the two-year mark. This is a genotype 2A. We have had another 3B donor who fails to seroconvert. We have had a couple of resolved infections, one abortive infection. Here is the resolved infection--one abortive infection, one donor who cleared virus prior to seroconversion. So we have had some interesting findings.

[Slide.]

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Lastly, what do we do with our NAT-positive donors? We enroll them in a study to look at recent risk factors. So, of all of our eligible donors for the recent risk study, 35 percent have enrolled. We do concentrate, or try to concentrate, on those that are confirmed NAT-positive. So we have had a 55 percent success rate here and a 32 percent for our NAT-only-positives that are false positives and we follow those up to serve as controls.

[Slide.]

So, in conclusion, our NAT performance has been excellent over all. The yield is consistent over time at about 1 in 300,000 in HCV. The highest numbers are in the Mid- and South-Atlantic regions. We have had three cases of resolved infection. I didn't mention one following interferon treatment. One case of abortive infection. Our seroconversion, meantime, is 39 days.

The yield for HIV has been 1 in 4.4 million with one p24-antigen-confirmed positive. So that gives you an indication of the relative sensitivity of the p24-antigen test. Our false-positive rates are much lower than serology rates and no one has reported any biological false positives.

[Slide.]

Lastly, our major issue has been our invalid rates, but we are addressing these with the vendor with

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more frequent replacement of equipment and hopefully with more durable laboratory equipment in the future. It also decreases with staff experience with the assay--that is, the learning curve.

Thank you.

DR. YUWEN: Any questions, comments? Thank you, Sue.

Our next speaker is Dr. Celso Bianco from America's Blood Centers.

ABC

DR. BIANCO: Thank you very much.

[Slide.]

I have to acknowledge that the source of everything that I am going to say is Sally Cagliati and Mike Strong.

[Slide.]

America's Blood Centers is a network of 35 independent blood centers that collects about 6.7 million units and generates over a million unit liters of plasma, recovered plasma, every year. We have established sixteen laboratories for screening in 1999.

[Slide.]

They are spread throughout the country. There are three laboratories, the ones in red, that are with Chiron-GenProbe and they screen about 4.5 million units of

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blood. And then there are thirteen labs that are with the Roche molecular systems and screen about 2 million units a year.

[Slide.]

This is an aggregate set of results, the NAT yield between March '99 and June 2001, and the numbers are very consistent across the two systems and with the numbers that have been presented before by the other speakers. But we identified a total of 55 HCV and a total of five HIV, one of them having a p24-antigen-positive.

[Slide.]

What is interesting that we can take from this set of data that we have seen is that a number of our labs still use the EIA 2.0 for HCV. Our laboratories and other centers use the 3.0 from Ortho and from Abbott. I want to note the difference in yield from the two different systems, really documenting the higher sensitivity of the EIA 3.0.

[Slide.]

Again, in terms of RIBA, when we compare the RIBA 3 to the HCV EIA 2.0, again a higher number of units that are identified as positive. The same with a lower one with the EIA 3.0.

[Slide.]

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So there is a significant difference between the yield of antibody-negative, NAT-positive, specimens tested by EIA 2.0 and EIA 3.0. HCV EIA 3.0 is more sensitive than the EIA 2.0. The yield is lower in terms of NAT.

Also, we should note that the contribution of confirmatory tests, and, in this case, the RIBA, is limited. Actually, we do not see much value in the performance of RIBA on sample specimens that are positive on HCV NAT and, obviously, they were positive also on the HCV ELISA test.

[Slide.]

In terms of HIV-1 p24-antigen testing by ABC centers, between March, '96 and August 2000, we have identified only four donors that were positive on NAT with a much larger number or nonspecific test results, individuals that were repeatedly reactive, again showing that a very positive site in terms of p24 antigen being replaced by the NAT testing.

[Slide.]

These are some of the characteristics of this HIV p24-antigen-test-positive donors. They were all NAT-positive when they were tested.

[Slide.]

So, in terms of HIV-1 p24 antigen, the assay has made limited contribution to the identification of donors

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in the window of seroconversion. All specimens that are positive on HIV-1 p24 antigen are positive on NAT and p24-antigen is redundant and it should be removed, eliminated, after licensure of NAT.

[Slide.]

When we look even at the other supplemental test results, and we heard, actually, a scheme for confirmation from Dr. Mied today, that recognized the limited value of indeterminate test results in Western Blot. So the question that we ask is does confirmation contribute to donor notification in the post-NAT era.

In fact, most EIA-positive, NAT-negative, specimens are Western-Blot- or IFA-negative for HIV. Donors who are EIA-positive, NAT-positive, are positive for HIV regardless of the confirmatory test that we use and the status of donors who are EIA-positive, NAT-negative, can only be determined really on follow up regardless of Western Blot of IFA results, unless those are all positive.

Since the introduction of NAT, the contribution of Western Blot and IFA to resolution of positive EIA results for HIV became extremely limited.

[Slide.]

Again, a point that Dr. Stramer made very well, is the failed runs in both assays. NAT has performed

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extremely well and with failed runs that are close to that that we observe in our serological assays.

[Slide.]

Again, in terms of nonspecific test results, the numbers have been rather small.

[Slide.]

So, in order to summarize the NAT performance, the NAT assay showed remarkable specificity, much more than what we expected. I remember that that was a great discussion that we had in the early days of NAT. The resolution of false-positive NAT results is complex and we still don't have very clear--but we have help from FDA in that sense and we heard about it today, and in recent months, in the definition of a clinically negative individual.

We would like to be able to tell somebody, "This was a false-positive test result. You are clinically negative." NAT assays have failure rates in the same order of magnitude as ELISA assays.

[Slide.]

So, in summary, NAT performed under FDA-approved INDs has effectively contributed to the safety of the blood supply by identifying several individuals who were in the window of seroconversion for HIV. Although in smaller numbers, NAT has identified individuals in the window for

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HIV. HIV-1 p24 has not contributed to the safety of the blood supply after the introduction of NAT screening.

Supplemental tests lost much of their significance after the introduction of NAT and NAT assays under IND are highly specific and have rates of failure that are similar to those of serological assays.

[Slide.]

I have still another minute that I would like to use to touch on another subject that I did not have an opportunity to get into the program and that is in the screening of recovered plasma for parvovirus B19 and HAV that we are going to discuss a little bit later today.

ABC has been working with assay manufacturers for the development of in-process testing of plasma. I was very pleased to see the presentation by Mei-Ying classifying and giving us very good guidance on what is called in-process testing. The assay would be able to detect 10^4 International Units per ml. Essentially, pools of approximately 250 specimens would be screened after expiration of cellular products.

If the pools test negative, plasma would be used for further manufacture. Positive pools would be broken down into minipools of 16 to 24 specimens and retested. Plasmas associated with negative minipools would be used for further manufacture and all plasmas associated with

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positive minipools would be discarded. No attempt to identify the positive specimen in the pool would be made since this would have been classified as in-process testing.

Thank you very much.

DR. YUWEN: Any questions?

Thank you. I'm sorry we left ABC out of the--

DR. BIANCO: That's okay.

DR. YUWEN: Our last speaker in this section is Dr. Rich Gammon from AIBC.

AIBC

DR. GAMMON: Thank you.

[Slide.]

This afternoon, I just want to talk a little bit about AIBC's nucleic-acid-testing experience.

[Slide.]

Just a brief history about AIBC. It was established in 1983 as a purchasing group for small to moderate-sized blood centers. It has about 980,000 donations, over 30 blood centers and consists of both a reference laboratory and a NAT laboratory.

[Slide.]

When we looked into the testing in 1998, we wanted the widest available testing. The clients wanted both HCV and HIV testing by NAT. It needed to be done at a

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reasonable cost. That is why we used pools for 24 to achieve the goal. And we needed a rapid turnaround time. We wanted testing completed within 24 hours.

[Slide.]

We wanted testing to be available to anybody that wanted it and that included AIBC members, AIBC nonmembers and we really service all kinds of sizes of blood centers from as small as 2000 units to over 100,000 annual units a year.

[Slide.]

The participating sites are predominantly located east of the Mississippi. You can see we have the largest concentrations in New Jersey and Florida. Our NAT lab is in Lakeland, Florida. If you go along I4 east and west, it is about halfway between Orlando, which has a Roche site, and Florida Blood Services which has an ABC-GenProbe site. The reference laboratory is in West Palm Beach, Florida on the southeastern portion of Florida. There are 23 sites total.

[Slide.]

AIBC had control of the clinical protocol from April of '99 to August of 2000. We initially started off in 24 specimen pools. That was later reduced to 16 specimen pools in June of 2000. This was implemented at the request of the test manufacturer. We did not have any

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request from our clients to make the pool size smaller. This was done primarily for standardization of pool size and for the data that would be collected in the fall for licensure.

[Slide.]

The data that was collected during the portion of the study showed that there were about 525,000 specimens tested in pools, 23,286 multiplexed pools. About 3 percent of those were multiplex reactive and a little less than 6 percent were reactive pools which were nonreactive upon individual multiplex testing.

The specificity of the pooled testing was 99.8 percent, fairly close to what Dr. Mimms presented earlier.

[Slide.]

Looking at some of the testing data, there were 64 donors that were Western-Blot-positive out of 62 that were discriminatory HIV positive, 99.9 percent concordance. One of the donors that was NAT-negative, Western-Blot-positive came back and was tested for donor-counseling purposes and ended up being a false-positive Western Blot and, potentially, if the other donor was a false-positive Western Blot, that would fit with studies published elsewhere which show Western Blots can have a false-positive rate of about 1 in 250,000.

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All seven HIV-antigen-positive donors were also NAT-reactive for HIV. You can see the serology of them. Five of them were Western-Blot-positive. Two of them were Western-Blot-negative. And there were 339 donors that were RIBA-positive out of which approximately three-quarters were NAT-HCV-positive. This also fits with other published studies that show that about 25 percent of individuals infected with HCV will clear the virus and, therefore, will be antibody-positive, NAT-negative.

[Slide.]

Follow-up studies from the first portion of the study; we had 39 donors in the study. The donors were not compensated to return. Thirteen donors came back, or about a third. All were potential HCV yields. Two actually turned out to be HCV yields. For instance, of 1 in 262,000, 11 were nonreactive on follow up. This may indicate false reactivity at the index.

There was zero HIV yield. Unfortunately, five donors that were--we only had on category for follow up at this portion of the study; NAT-reactive, serology-negative. There were five donors that fit this category for HIV, but they were all lost-to-follow-up.

[Slide.]

Moving on to the second portion, the GenProbe-Chiron clinical protocol was implemented on August 20,

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2000. We tested approximately the same number of specimens as we did in the first portion of the study. Approximately 550,000 specimens were tested, 34,000 pools. Approximately the same number of pools, 2.6 percent, were TMA-multiplex-reactive. Of those pools that were reactive, approximately 8 percent of them were nonreactive upon individual multiplex testing.

The specificity of the pool testing was the same, 99.8 percent here under the GenProbe protocol as it was under the AIBC IND.

[Slide.]

The second follow-up study here, these donors were compensated for their time to come back and the number increased from a third to almost a half returned. We did have three HCV yields, about 1 in 183,000, caught in the preseroconversion window period. Two donors seroconverted at three and four months, respectively. One donor was HCV-positive by TMA and PCR but was lost-to-follow-up. We did have one HIV yield that was TMA-reactive and PRC-reactive. This donor was lost-to-follow-up.

[Slide.]

A little bit about the employees of the NAT lab. We had eleven medical technologists that were trained over about a two-and-a-half-year period. The majority of them, 64 percent, that were trained are currently employed.

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Three have been there since the origination date, so we have very little turnover in the NAT laboratory.

[Slide.]

During the period of the GenProbe protocol, we have had twenty problem runs. Out of the invalid runs, we had four in which greater than 10 percent of the calibrators plus the specimen results were invalid giving us an invalid run, two in which there were invalid calibrators greater than 2 of 9. Three were a result of tech error. One was a possible contamination of a calibrator. And in one, the leader jammed on the first TTU.

[Slide.]

Suspect runs; fitting the definition of a suspect run, the controls did not meet acceptance criteria in seven, for the reactives remains the test of record. The nonreactives to be repeated. Equipment issues; there were two issues where a leader bar code failed to read a TTU for ten master pools and, in one instance, the leader lost memory.

Donor reentry; there were five substudy donors eligible for reentry from two sites. One donor has been reentered. One is pending, getting the paperwork together. The donor that has been reentered has not returned to

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donate so we don't have any subsequent data from this donor.

[Slide.]

In summary, there was a low invalid-run rate. We tested 30 percent more specimens in September of 2001 versus September of 2000. The invalid-run rate did not really change during that time period. Also, too, we feel that employee retention may help contribute to this. A quick turnaround time for NAT for HIV and HCV. All blood components have been released, fully tested and that includes even during the increase in testing during September of 2001.

[Slide.]

During the two-and-a-half-years of study, six donors were identified in the preseroconversion window period, five for HCV, giving us an instance of about 1 in 214,000. This seemed fairly comparable to what we have seen the Red Cross and ABC. And one for HIV, or about 1 in a million.

That completes my talk. Does anyone have any questions?

DR. YUWEN: One question for Dr. Cannon. You mentioned you have twenty problem runs; right?

DR. GAMMON: That's correct.

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DR. YUWEN: How does that convert percentagewise? What is the percent overall?

DR. GAMMON: I don't have that. I had asked GenProbe about this late last week, but we just didn't have time to get it together. I can tell, in perspective, we do about two runs a day and we are talking about a 15-month period here. So it is not a very big number.

DR. YUWEN: About 700 runs?

DR. GAMMON: Yes.

DR. YUWEN: Thank you.

DR. GAMMON: You're welcome.

DR. YUWEN: Now I am going to hand over the podium to Dr. Li. He is going to chair the next session.

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

DR. LI: In this session, we are going to primarily focus on the single-unit testing except for the last one which is still going to be on the minipool testing. We have two other modifications to the agenda. One is that we are going to have an additional speaker on single-unit testing from Roche. I believe it is Jim Gallarda. Also, if you look at the time, you see it varies from ten to fifteen minutes, but we are still going to stick to ten minutes each talk.

So, our first speaker is Sherrol McDonough from GenProbe.

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Duplex(HIV/HCV) TMA, GenProbe

DR. McDONOUGH: Thank you and good afternoon.

[Slide.]

I will be talking today about implementation of individual donor testing. I think it was pretty amazing the things that were done when this group got together with a common view, a common decision, of what to do in terms of minipool. Now we are starting to talk about individual and I am not sure that our common goal, common vision, is there yet.

But what I would like to share is some of the work that we are doing at GenProbe to help gather the data needed to make the decision to move forward.

[Slide.]

What we do in the multiplex assay, I just want to remind you, the initial screen is done with the multiplex HIV-HCV assay. All the nonreactive samples, you can go ahead and release the product. Only reactive samples are then tested in the discriminatory assays.

[Slide.]

As you know, when you go from testing a pool to an individual sample, there is an inherent increase in the analytical sensitivity. What we have been doing is looking at what happens in terms of clinical sensitivity. We have looked at that in terms of seroconversion panels, known

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positive samples in a high-risk population. I will also show some specificity data.

[Slide.]

This shows the results from the testing of ten seroconversion panels in the multiplex assay. Each panel member was tested either directly, neat or after 1-to-16 dilution. In the case of testing the samples in a pool-diluted situation, the RNA was detected, on average, ten days prior to antibody. When the samples were tested directly, without dilution, RNA was detected, on average, twelve days prior to antibody.

Likewise, if you compare to p14 antigen, RNA was detected, on average, three days prior to antigen and, undiluted, seven days prior to antigen detection.

[Slide.]

In terms of looking at known NAT-positive samples, we have looked at over 2,000 known HIV or HCV samples. Sensitivity, when the samples were detected following a 1-to-16 dilution was 99.3 percent. In this same population, the sensitivity went to 99.8 percent when the samples were detected directly.

The lower results are for discriminatory assays and I will move on.

[Slide.]

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In terms of the high-risk population, we looked at 539 subjects. The primary risk factor for these individuals was in vitro drug use. Samples were tested neat or following a 1-to-16 dilution. In the case of the true-positive HIV samples in this population, all were detected neat or at a 1-to-16 dilution.

In terms of HCV, you can see there is a very high level of HCV positivity. Virtually half of this population was HCV-positive. The sensitivity was 98.1 percent following at 1-to-16 dilution or 99.3 percent when tested neat.

[Slide.]

The specificity study was done at eight different volunteer blood-donor sites collected from 103 donor sites in the Continental U.S. as well as five U.S. Military sites; Hawaii, Japan, Germany and Guam are include. You have already seen the results from the specificity for the pools. I have just shown it here again, a specificity of 99.67 percent.

We have tested 27,880 individual donations, almost three times the number required in the FDA Guidance Document, and the specificity is 99.85 percent in this population.

[Slide.]

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We had an opportunity to look at some of the demographics of this population because now we are testing individual donations instead of pools. This slide shows the results of specificity when the samples are analyzed by gender. So 4,460 samples from female donors and the specificity was 99.8 percent. Over 22,000 samples from male donors were tested and the specificity was 99 percent. You can see there are overlapping confidence intervals.

[Slide.]

We could also look at the donors by age. We stratified by approximately ten-year periods, those donors less than 21 years old, 21 to 30, 31 to 40, et cetera. The specificity in these different populations ranged from 99.7 to 99.9 percent. Again, there are overlapping confidence intervals as you can see on the right side of the screen.

[Slide.]

Finally, we were taking a look at the data here when analyzed by race of the donor. The categories shown are black nonHispanic, white Hispanic, white nonHispanic, other. There was a category for unknown and also information not available. Again, the specificity in these different populations varied from 99.7 to 99.9 percent with overlapping confidence intervals, again.

[Slide.]

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Now, I would like to move on and talk about the Procleix Automated System. We believe that this is something that will make implementation of either smaller pool sizes or individual donations more acceptable in the laboratory. You saw all the pieces of equipment that Larry Mimms showed. All of those pieces of equipment are now consolidated into this one instrument. It is currently called the Procleix Automated System. You may have heard it called TIGRIS in the past.

[Slide.]

The goal of this instrument is to fully automate all the assay steps and to fully integrate the information from the sample ID through to the results with the run report and everything is LIS-compatible.

[Slide.]

This just shows the steps required for the semiautomated system. Most of the clinical results that you have seen so far are from the semiautomated system. As Larry mentioned, it takes an individual about six hours to perform 200 TMA tests. There are two one-hour walkaway periods, but it still takes the individual that amount of time.

[Slide.]

The projected system productivity for the TIGRIS is 500 dual for the HIV-HCV or triple, as you are going to

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hear about with Dr. Giachetti, a triple analyte detection test per eight-hour shift, or a thousand dual or triple analyte detection test per twelve-hour shift.

[Slide.]

This just shows some analytical sensitivity data that we have generated with in-house sensitivity panels. As you have heard, the goal for the assay is 95 percent positivity at 100 copies per ml. The analytical sensitivity certainly exceeds those goals for both HIV, in which the positive is 99.5 percent, or HCV, in which the positivity is 99.9 percent.

[Slide.]

When we have looked at a negative panel member, we have looked at 22,000 different replicates of this panel member, there were only four reactive replicates for a specificity of 99.82 percent. A series of panels has been tested over three months with six different instruments. Out of 13,340 replicates in this study, there were 32 invalid replicates for an invalid-replicate rate of 0.24 percent.

[Slide.]

This is a carryover study showing that, when we did a run on the TIGRIS with over 200 samples, the green arrow refers to the assay cutoff mark. We are looking at signal-to-cutoff versus sample. The HIV-positive

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calibrator, HCV-positive calibrator, are positive and, above the signal cutoff. All the other samples are negative with the exception of these four marked by the white arrows at the top. Those were samples spiked with 10^9 copies of HIV RNA per ml.

What you can see is adjacent samples are nonreactive as are all the others throughout the run. So carryover has been addressed in this instrument.

[Slide.]

So, to conclude, the Procleix Assay System is designed for high-throughput use in blood-bank testing facilities. Testing of smaller pool sizes or individual samples does result in an inherent analytical increase, an increase in analytical sensitivity.

We have shown you studies and you have heard throughout the day that there are examples of samples that are missed at a pool size of 1-to-16 or 1-to-24 which will be detected at a smaller pool size or when tested individually. The specificity in individual samples is equivalent or better than the pooled specificity.

[Slide.]

The automated system gives results consistent with the semi-automated system. We are meeting or exceeding sensitivity goals, specificity goals. The internal-control performance shows a very low sample

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invalidity rate and there are no problems identified with putative interfering substances although I did not show that data.

[Slide.]

We believe the Procleix Automated System will reduce personnel time requirements in the lab and increase adherence to GMP. So we will continue to work on addressing implementation of smaller pool sizes or individual donations in the future.

Thank you.

DR. LI: Any questions?

DR. GALLARDA: Jim Gallarda, Roche. Sherrol, on your high-risk population, you had picked roughly 500-some-odd samples half of which were positive in the assay, but you reported the sensitivity at 99 percent. How were you defining sensitivity there?

DR. McDONOUGH: The second number you were talking about came from a population of known positives. In the high-risk population, we looked at--we had 100 percent sensitivity in the high-risk population for HIV and I think it was 98 percent for HCV. I don't think I am answering your question.

DR. GALLARDA: I just remembered the numbers were like 500-and-some-odd in the total population and then, in

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one of the rows you showed, about half of them were positive.

DR. McDONOUGH: By serology.

DR. GALLARDA: Oh; by serology. Got you.

DR. McDONOUGH: And/or NAT.

DR. GALLARDA: Okay.

DR. LI: The next speaker is Dr. Cristina Giachetti.

Triplex(HIV/HCV/HBV) TMA GenProbe

DR. GIACHETTI: Good morning.

[Slide.]

My talk today will be on the Procleix HIV/HCV/HBV assay or triplex assay that is currently under development in our lab.

[Slide.]

The assay goals are shown on this slide. Our sensitivity goals are at least 45 copies per ml or 15 International Units per ml for hepatitis B virus, 100 copies per ml or 60 International Units per ml for HIV, 100 copies per ml or 30 International Units per ml for hepatitis C virus.

We would like the test to be able to detect HIV, HCV and hepatitis B genetic variance and to be able to close the serodeletion window. The analytical specificity

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goal is at least 99.5 percent. All the assays have an internal control for assay monitoring in each sample.

At the same time as we developed the triplex assay, we also developed discriminatory assays for resolution of the repeatedly reactives. The same assay formulations would be applicable to Procleix automated or a fully automated system, or TIGRIS.

[Slide.]

The assay protocol is the same as you are familiar with from the HIV/HCV assay, so our assay protocol is completely integrated. We do sample processing using the a capture system followed by amplification with a transcription-mediated amplification, detection with protection assays and then the result with a luminometer.

[Slide.]

In terms of assay calibrators or controls, the assay would contain an internal control. We have different calibrators who have three replicas of negative calibrators and two each of the positive calibrators. Calibrators are used to set cutoff as well as to set the ramp-up liquid area.

[Slide.]

As a performance, I will be talking about focussing analytical sensitivity and subtype detection. The first thing I will talk about is the sensitivity for

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HIV detection. Here, what we did is to do serial dilutions of the WHO standard going for 300 to serial International Units per ml. Each of these dilutions were tested at 30 replicas by either the triplex assay or the duplex assay for comparison. And then what we do is we use PROBIT analysis to determine the 95 percent detection probability for each assay plus the confidence intervals.

As you can see, the detection probability at 95 percent for HIV is 64.9 for the triplex assay and 50.3 for the multiplex or duplex assay and the confidence intervals overlap indicating that there are no statistical differences.

[Slide.]

Next I am going to show some data from testing of subtypes, HIV subtypes. The way we do this work is that we purchase from different vendors infected specimens with different subtypes. They are serially diluted to 100 or 30 copies per ml and then we test, in this case, six replicates of each and we show here, which is the positivity rate in each case.

As you can see, in all the cases, we detected 100 percent at 100 copies per ml and we have a very high percentage detection as well at 30 copies per ml.

[Slide.]

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This next slide is a continuation for the rest of the HIV subtypes. As you can see here, we are able to detect with the test not only the Group M strains but also N and O. The assay has very good sensitivity for O for 100 percent detection at 100 copies per ml.

[Slide.]

Here I am going to show the data for analytical sensitivity for the HCV. Again, we do the same type of a study as was shown before for HIV. We have serial dilutions of the WHO standard from 100 to serial International Units per ml. 30 replicates of each were tested for the triplex or duplex assay and then we used PROBIT analysis to determine 95 percent detection probabilities and confidence intervals.

Sensitivity for HCV is very high. It is 2.9 International Units per ml for the triplex assay and 2.4 for the duplex, again with overlapping confidence intervals.

[Slide.]

The detection of HCV subtypes are shown here. Again, here, we tested genotypes from 1 to 6. We have serial dilutions to 130 copies per ml which is the detection for--we have detection for duplex or for triplex. We see, in some cases, the triplex assay formulation actually has higher sensitivity for detection of some of

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the HCV subtypes. We work, as part of the assay, to improve sensitivity for some subtypes.

[Slide.]

Finally is the analytical sensitivity for hepatitis B. Again, here, we use the WHO standard, serially diluted from 135 to serial International Units per ml. Here are the detection rates of 30 replicas of each dilution. This gives us a 95 percent detection probability of 7.2 International Units per ml.

[Slide.]

Detection of HBV genotypes from A to G is shown here. Again, what we did was to purchase different specimens which were value-assigned based on the Roche monitor values by the vendor and then were diluted to 130 copies per ml and, in this case, 20 replicas of each were tested with a triplex assay. Here we see the percentage of detection. Again, in all the cases, we detect 100 percent at 100 copies per ml, a very high percentage, going from 100 to 80 percent at the 30 copies per ml level.

[Slide.]

As conclusions, the analytical sensitivity of the triplex assay shows no statistical differences with the sensitivity of detection of HIV and HCV with the HIV/HCV assay that is currently under review by CBER. We have

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95 percent detection of HIV at 16.9 International Units per ml or approximately 10 copies per ml, and 95 percent detection for HCV at 2.9 International Units per ml or approximately 9 copies per ml.

So this analytical sensitivity predicts that the clinical sensitivity of the triplex assay for HIV and HCV detection could be similar or better than the sensitivity that we have currently with the multiplex assay.

[Slide.]

The triplex assay is able to detect all different subtypes and genotypes for HIV variants as well as HCV and HBV. As we mentioned, especially we are able to detect N and O at very high sensitivity. This test also has improved detection of HCV genotypes, especially 2b, 3a and 4 and will detect HBV genotypes A to C at least 100 copies per ml.

[Slide.]

Finally, we found for detection of HBV that the analytical sensitivity of the assay for 95 percent detection is 7.2 International Units per ml or about 25 copies per ml, and 50 percent detection is 3.1 International Units per ml or 10 copies per ml. So, this predicts 95 percent detection of HBV DNA for 100 copies per ml in pools of 16 or 200 if we go for pools of 8, and 50

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percent detection for comparison with antigen tests is 160 copies per ml in pools of 16 or 80 in pools of 8.

So, based on the sensitivity and based on the data presented by Mike Busch regarding doubling time of HBV and the sensitivity of antigen tests, we say that the triplex assay pool test will detect infection twenty days earlier than the currently approved antigen test and nine days earlier than the other PRISM antigen test.

Finally, this work was supported also by the National Heart and Lung Institute under this contract.

Thank you very much.

DR. LI: Any questions?

DR. ZERLAUTH: You told us what we get, but you refrained from telling us when we get it.

DR. GIACHETTI: When we will have it? We just finished optimization of the assay and we start now to transfer it into manufacturing. So probably next year we can start to do some evaluations.

DR. LI: Actually, I have a question. How many HIV Group O specimens did you test and how do you determine the copy number?

DR. GIACHETTI: Good question. For Type O's, along the development of the assay--okay; for this particular triplex assay, we only tested a few O's. For the previous assay, we used the same primers, we tested at

at least 40 different O's. The determination of the copy number was done by the version of our quantitative test. We have a quantitative test based on TMA. The accuracy of detection was based on transcripts of O. So we have a transcript of O that is quantitated by OD and we determine from there the calibration.

DR. LI: So basically you used the palm technology to quantify the copy number and you used that as the standard?

DR. GIACHETTI: We validate by using transcripts. We did that because DNA doesn't detect it and the Roche assay also doesn't detect O's. So it was the best we had at the time.

DR. LI: Thank you.

Our next speaker is Andy Heaton from Chiron.

South African Experience with Single Unit NAT, Chiron

DR. HEATON: Thank you.

[Slide.]

It is a pleasure to be able to present the results of a study that was developed by Chiron and by the South African Blood Transfusion Service. It is a special pleasure for me since Dr. Hanes, who managed it, trained me in my early days and I have worked with him in research over a number of years.

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The use of Chiron Procleix Assay in South Africa is a different issue in that today you have mainly been hearing about the use of the assay to detect extremely rare events in a low-incidence population. But, in South Africa, HIV is almost a way of life. 15 percent of the South African population tests HIV-positive and they have an extremely high incidence. So this assay reports the benefits of an HIV/HCV NAT assay in a very high incidence environment.

[Slide.]

The purpose of the study was to assess the benefit of testing high-incidence blood donations to validate the performance in a less developed environment, to evaluate the feasibility for routine screening, to compare HIV and HCV NAT testing with routine EIA screening to try and quantify the level of improvement and, more specifically, to compare the residual HIV and HCV risk between different donor groups.

[Slide.]

The South African Blood Services are organized very much along the lines of American community blood centers and have done a remarkable job of preserving blood-recipient safety. One of the ways they have achieved that is by categorizing their donor base into high-risk donors and low-risk donors.

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The low-risk donor groups are categorized by geographic area, socioeconomic and age status into a low-risk group and then a secondary group of donors who are known to be from the higher-risk category are also collected. All the low-risk or low-prevalence group is used for transfusion and the high-prevalence group is then used as needed on an emergency basis.

For the purpose of this trial, the results were unlinked after recording, although we did record the demographics of the donor and, obviously, the results of routine serology. The trial ran from September of 1999 through May of the Year 2000. Operators were rapidly trained. Proficiency was rapidly established and we established an instrument-verification program.

[Slide.]

The results of the study, which was approximately 20,000 donors, was designed to have approximately half high-prevalence donors and half low-prevalence donors. 84 percent of the donors were repeat and 16 percent were first time. Approximately two-thirds of the donors were male and one-third were female.

[Slide.]

This slide summarizes, then, the numbers that we have observed in the performance of the study. I draw your attention first to the low-risk donor group which is down

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here. The low prevalence group had three positives and a total of 0.03 percent in this group which is approximately five times the prevalence of the U.S. donor group. There was no NAT yield case in this study group.

However, look at the high-prevalence group. First of all, 1.5 percent of your donor population in total was HIV-positive and, in fact, if we look at the first-time donors, 6.5 percent of first-time females were HIV-positive and approximately 3 percent of first-time male donors were positive, quite remarkable incidence.

[Slide.]

There was one yield case which occurred in a female in the repeat-donor group. The yield case came from a 32-year-old black female repeat donor who had donated 106 days prior to the previous indexed donation. The p24 antigen, the HIV-1 and 2 antibody was negative but the Chiron Procleix assay was positive both at a 1-in-8 dilution and a 1-in-16 dilution.

The PCR which was performed at Blood Centers of the Pacific was negative and the viral load was less than 100 copies per ml. We were unable to quantify the viral load in the yield case.

[Slide.]

We also had one Chiron Procleix-assay-negative, antibody-positive case. This was a black female, 33-years

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old. It was a lapsed donor who had not donated for approximately two years. The HIV-1 and 2 antibody test was positive. The Western Blot, which was repeated twice, was positive in all viral bands present. This sample was submitted to both Roche and Chiron Procleix repeat assay and, indeed, NGI assay and we were unable to detect viral RNA in any of the nucleic-acid testing tests.

The individual, so far as we know, was not on antiretroviral therapy and this represents a phenomenon which has been reported elsewhere in South Africa. The potential causes are antibody positivity with a low viral load, HIV-2 which is extremely uncommon in the Republic of South Africa, or a false-positive EIA or a false-negative NAT.

[Slide.]

There were seven cases which were positive for p24 antigen or were positive by the Chiron Procleix assay. The one, the top sample, was positive on the p24 antigen and negative for p24 antibody and was repeat male donor who had donated previously 56 days before.

The viral loads were generally relatively low, running the lowest, approximately 50 copies per ml and as high as 97,000 copies per ml.

[Slide.]

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Interestingly, there was very little HCV detected. We had to delete the dot in one center because one of the centers did not routinely defer HCV-positive donors, but there were 15 EIA-positive samples which were retested by Ortho HCV EIA 3.0 and RIBA 3.0, two of which confirmed positive, one from the high-risk group, one from the low-risk group, and both the NAT-positive samples were also RIBA-positive.

So, in this population, which is very high risk for HIV, there was, in fact, very low risk for HCV.

[Slide.]

In summary, we confirmed the operation or feasibility of the assay. The prevalence in the HP donors was 1.5 percent, the high-prevalence donors, 0.03 percent in the low-prevalence donors. In a separate study with Mike Busch where we used the detuned assay, we ascertained that 18 percent of the infections were recent, using the detuned assay. That suggests that their method of donor screening is as effective as ours is in the U.S.

Assuming a constant ratio between the U.S. HIV EIA and NAT ratios, we predict that the South Africans would identify 1.4 donations per year in their low-risk population and 7.9 donations in the high-prevalence population, assuming that 10 percent of the high-prevalence donations are used for transfusion.

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We concluded that they could replace HIV p24 antigen with NAT testing and, of great interest to them, since they are very worried about the cost, we looked to see whether individual testing of high-risk donors would result in a similar risk ratio to 16 pooled testing of--sorry; the other way round--of high-risk donors. We concluded that that would not be the case. You would have to do individual testing of both categories of donors.

Finally, the HCV NAT testing could be used to confirm the HCV EIA test results since the RIBA test was extremely expensive for them. The South African Blood Transfusion Service has subsequently concluded that it should introduce NAT testing and is now pursuing discussions with the Ministry of Health on the strategy of how to implement this nationwide.

Thank you.

DR. LI: Any questions?

DR. ALLAIN: It is not a question. It is more a comment. About the beginning of this year, we have published very similar data on a group of blood donors from Ghana with a prevalence of 1.7 percent of anti-HIV. We found 1 per 1600 NAT-positive, antibody-negative, which is pretty much similar to what you have found here.

DR. HEATON: Yes. Certainly, the unscreened donor population has a very high incidence, indeed. Under

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these circumstances, NAT testing really contributes very greatly to improvement in public safety.

DR. LI: I just have a thought. Because you say you can use this test to confirm the HCV area test, can you do the same for HIV?

DR. HEATON: Yes, indeed, you could do the same with HIV although, in this case, we did have the one case which has been reported elsewhere in Africa of a long survivor. So there was one case here of an individual who was EIA-positive and Western-Blot-positive who was not NAT-positive even using the most sensitive NAT technology available to us. So I would be hesitant to recommend it for that use in this context.

DR. SCHOCHETMAN: I want to reiterate what I said this morning and that is we found one here in the United States who was NAT-negative, EIA-positive. The only way we are able to confirm it is by finding proviral DNA in the PBMCs. So, again, it is not just simply unique to Africa or South Africa. You can actually find it here in the United States. So, EIA-positive, NAT-negatives, can be infected.

DR. HEATON: Yes. It becomes much more of an issue in countries which don't have good foreign-exchange acts because for them the confirmatory testing is a big financial burden.

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DR. LI: Our next speaker is Jim Gallarda from Roche.

Plans to Transition from Minipool Testing to Single-Unit NAT Testing, Roche

DR. GALLARDA: Good morning.

[Slide.]

I want to thank the FDA for putting me on the agenda for this topic. I want to give you an overview of what Roche has been looking at as far as the plans to transition from minipool testing to single-unit NAT testing.

[Slide.]

One of the things that we have done in the past few years is we have really benefitted from our work in Japan where we have a very active multiplex program right now screening the Japanese Red Cross blood supply where we have learned a lot about instrumentation features that are critical to incorporate into a single-unit NAT system as well as the reagent chemistries for multiplex PCR.

So the things that have been a challenge is to accommodate the viruses that really behave completely differently during the course of infection from parvo B19 which can reach to the gazillion copies per ml in blood down to HBV or HAV--HBV which can have just a few hundred

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copies in the ramp-up period and this intermittent blip phenomenon in the pre-ramp-up period.

So, kinetics have played a role in our design features for a single-unit system. The other issue, of course, is the logistics in different laboratories of different sizes. Do you put all your eggs into one box that is pretty expensive which will do everything and, if it breaks down, you have a problem or do you build modularity into your system. So these are features as well that we have taken into account in looking at what would be important factors to consider.

[Slide.]

System characteristics that we are looking at are that this should be a stand-alone system for automated PCR. This is from the specimen preparation point through amplification and detection. We have gotten feedback that it would be advantageous from a logistics perspective to have a feature built into the system to allow for splitting the specimen or the extracted eluate.

We are looking at a generic nucleic-acid-isolation procedure which is robust and can isolate simultaneously both DNA and RNA viruses or other nucleic acids from nonviral counterparts. We are looking at a 5-prime nuclease detection system which we call TaqMan which is otherwise known as real-time kinetic PCR. So, with

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every cycle, you can actually see a progression of an amplification event rather than waiting for the end of the cycle to be completed until you get your result.

Of course, a significant feature is to make sure that the system, both hardware, software and reagent are regulatory compliant both for U.S. and non-U.S. regulatory agencies.

[Slide.]

Some other factors to consider in the general features are that we recognize the importance that, for the most part, batch is the mode of the day for PCR testing. However, there would probably be instances where you would want to have a random-access feature built in simultaneously. So this features prominently in our system and to have a continuous loading of specimens, disposables and reagents so you don't have to interrupt a run if you are running low on any of those items.

I think one of the biggest things that we have been working on is to build flexibility into a system so that it can accommodate both pooled testing and single-unit testing mixed. So where, in the case of parvo B19, it would make no sense to do single-unit testing, it would make sense to do pooled testing compared to low-titered viruses, perhaps that would be at a much lower copy number where single-unit testing would give you incremental yield.

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One of the most striking things we have recently discovered is that the system can offer multiplex amplification with inherent real-time differentiation. So, again, if you have a multiplex assay, you don't have to wait until the end of the run to determine do you have a positive result. You see a growth curve with each cycle.

Simultaneous with that, you can distinguish which analyte is being elevated with each cycle so you know, during that time, which analyte is responsible for the positive signal.

For a single-unit sample scenario, now you know the individual and the analyte detected. If you are running a minipool configuration, you only have to deconstruct the pool and not the analyte. So the system should also incorporate discriminatory algorithms as well.

[Slide.]

For workload, of course high-process flexibility which, in our minds, means multiplex and single-target resolution, pools versus single-sample testing and, importantly, I think we are looking at the future of nonvirus-type detection. So, like a quantitation of leukocytes post-filtration, bacterial contamination, there are a lot of other markers including genetic markers that we are looking at that might be suitable in this type of system.

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The specimens should, of course, accommodate multiple anticoagulants. Andy Conrad mentioned the importance of increasing your volume to accommodate relative sensitivity requirements. That is an important feature to drive the sensitivity of your assay, to make sure that you are closing the preseroconversion window as much as possible.

[Slide.]

The assay and, in fact, the system, itself, should track all elements of the process so we are requiring the complete process control. There should be full process internal controls, as Micha Nubling mentioned this morning, where, from the lysis step, you control and you, in fact, know that every step of the process has occurred from lysis through detection.

This also holds true for the positive and negative controls that go along with the kit as well. And then, if a customer requires an external run control, that should be built in as a flexible item on the menu.

[Slide.]

As far as analytes that we are looking at, we are going to go after an HIV-1 complete which includes Groups O and M. Actually, it would include Group N as well, and HIV-2, HCV and HBV, parvo B19, hepatitis A virus and then the flexibility to add other analytes as required. So

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these are all factors that we have been considering in producing a system that will meet the future's needs for single-unit NAT testing which would also include a flexible menu to allow minipool testing if it makes sense.

I think that is it. Thank you.

DR. LI: Questions or comments?

DR. ZERLAUTH: As you may guess, the same question; when do you think we will have it?

DR. GALLARDA: I was supposed to have a canned comment if you asked me that question. So the canned comment is that we remain to be silent on that. Sorry.

DR. ZERLAUTH: It is a nice program, though.

DR. LI: I have a question. For your single-unit testing NAT test, do you have use ultracentrifugation to pilot virus?

DR. GALLARDA: No. That is a good point Dr. Li brings out. This system is you load blood samples onto a rack, push a button and walk away. So there will not be a pretreatment for that step.

DR. NAKHASI: I have a question. This multiplex which you are thinking about, what orientation is it? Is it like a microarray type of thing, because I counted at least eight or nine agents there. What is the design? Is it individually or--

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DR. GALLARDA: We have done a couple of prototypes. One of them is to take a kitchen-sink approach and have everything in one reaction. And then we look at different combinations of analytes together that we split out as particular matching partners. So there is a lot of flexibility, both in the viruses that you want to put together in a multiplex as well as the floors that would go along with the detection of the specific analyte for that virus.

So it is not a microarray design. It is a TaqMan based--however, it does allow a lot of flexibility.

DR. LI: Our last speaker is Akira Yoshikawa from Japan.

Japanese Experience with Single Unit NAT

DR. YOSHIKAWA: Thank you.

[Slide.]

The Japanese data crossed JRC is the only one responsible organization to provide units to transfusion and plasma fraction from voluntary donors. The Japanese government required JRC to do NAT for HBV, HCV and HIV-1 from March 1 of this year for all blood products. JRC had already started 50 minipool NAT screening of all donated blood from February last year by using multiplex reagent.

[Slide.]

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There are three NAT centers and 77 blood centers in Japan, Hokkaido NAT Center, Tokyo NAT Center and Kyoto NAT Center. The number of Japanese voluntary donors was 6 million per year.

[Slide.]

During the transportation of NAT samples, blood centers had finished the serological screening. After receiving the serological results, the NAT centers began to pool serological negative samples. The results are electronically transmitted to each blood center.

[Slide.]

In NAT centers only serologically negative samples are pooled. This machine excludes serologically positive samples and negative samples are there.

[Slide.]

This is a pooling machine. Centrifugation, uncapping and pooling and decapping is done automatically. The pooled samples are transported to the next steps.

[Slide.]

NAT results are transmitted to each blood center within twenty-four hours after blood donation. In Japan, the shelf life of platelets is 72 hours, three days. Therefore, the NAT results are available in each blood center before the release of the blood products.

[Slide.]

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A portion of all donated blood is stored for ten years at minus 20 Centigrade. This test-sample storage system began in 1996. So over 30 million sample tubes have been stocked in the Hokkaido NAT Center.

[Slide.]

Over 11 million, about a third of a million blood screening, has been carried out since July 1999. 209 cases for HBV, 43 cases for HCV and four cases HIV-1 were detected.

[Slide.]

95 percent detection limit of HVB is 22 to 60 copies per ml. HCV is 61 to 112 International Units per ml. HIV-1 is 33 to 66 copies per ml reported by Meng et al.

[Slide.]

This slide shows the relation between HCV DNA copies per ml and HBs antigen with CLIA S to CO. 181 HBV NAT positive samples are detected by 50 pool from February last year to October of this year. 76 samples, 42 percent, are CLIA negative.

[Slide.]

Among 181 HBV NAT positive, by our 50 pool 0.2 milliliter system, 76, 42 percent, could not be detected by CLIA HBs antigen test.

[Slide.]

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In summary, we constructed the NAT system from the serological screening test negative from nonremunerated voluntary donations. We supplied blood products to medical organizations implementing the NAT for HBV, HCV and HIV-1 for transfusion and so for fractionation. NAT screening is being done within one day of blood donation by a 24-hours-per-day seven-days-a-week working system.

[Slide.]

Between July 1, 1999 and November 15, this year, over 11, about 12 million, serological test-negative units were tested by NAT. As for HBV, a total of 209 cases with HBV DNA were detected. The sensitivity of a 50-pool NAT screening with an input volume of 0.2 milliliter specimen in our system is significantly higher than that of highly sensitive HBs antigen testing.

Among 181 HBV DNA positives by our 50-pool 0.2 milliliter NAT system, 76 cases, 42 percent, could not be detected by CLIA HBs antigen test.

[Slide.]

As for HCV and HIV-1, 43 cases with HCV RNA and four cases with HIV-1 RNA were detected. These cases were escaped from HCV antibody and HIV-1 antibody screening. We could prevent transfusion of these serologically screening tested negative HBV, HCV and HIV-1 NAT positive blood units.

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Thank you.

DR. LI: Any questions?

DR. KLEINMAN: For your HBV testing, I know in Japan you do a combination of surface antigen and anticore testing. So the ones that you take to NAT, for HBV NAT, would these be the units that would otherwise be transfused because they had an anticore and antisurface that made them eligible for transfusion?

DR. YOSHIKAWA: In Japan, HBV anticore are discarded so this NAT system, HBV NAT was within the window period.

DR. LI: If there are no other questions, let's take a fifteen minute break. Let's come back at 4 o'clock sharp.

[Break.]

Examination with Minipool NAT Screening for HAV/Parvovirus B19 Industry Presentations

DR. YU: My comoderator is Dr. Gerardo Kaplan. Our schedule is very, very tight. There are a lot of industry presentations so, again, we are going to limit per presentation ten minutes. However, we would like to have some short questions and comments so each presenter should give an eight-minute presentation. Since Dr. Yuwen did such a wonderful job, he is going to be the time-clocker for us again.

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Let's start. This is the experience with minipool NAT screening for HAV and parvovirus B19. This is for plasma for further manufacturing. Our first presenter is Dr. Charles Watson from Aventis. Welcome.

Aventis

DR. WATSON: Good afternoon. And, for those of you who have made it back, thank you.

[Slide.]

You all saw that. If you remember it. Wow.

[Slide.]

That's our test system and we are going to follow that with HAV and B19. One of the questions that might be asked because a lot of people are not really hot on HAV testing is why, why would we do it. To us, we have a technology and we had a problem, or a perceived problem in our industry.

HAV is non-enveloped. It is more resistant to virus inactivation. We have no serology test. Even if we use ALT, it is indirect and it really doesn't match what happens with HAV. We have two articles in the literature, although they were after we thought about HAV. The first article, from Bower et al., showed that you can have HAV RNA present with a normal ALT. The second article showed that a likely suspect of an HAV transmission that occurred

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in Europe was a unit that had a concentration of 2 to 10^6 GEs per ml of HAV while the ALT was normal.

So we had this in the literature and we thought we would develop a system.

[Slide.]

It is the same system we have for the other viruses. When you test 2 million to 3 million samples a year, you don't want to use two, three, different test systems. For these manufacturers out there, if you are going to do two viruses, I suggest you do more than two.

Our detection limit is 250 GEs per ml and that is calibrated against the NIBSC working reagent. We consider this a donor-qualification test. We will not defer the donor. We will only destroy the unit. Our goal was to improve the safety margin of source plasma. We wanted to minimize what went into production.

[Slide.]

What have we done? We started this in March of 2000. Actually, this is worldwide statistics. All this information is worldwide, not just the United States. The HAV data has been accepted for publication and the B19 data is in press now.

We have tested a little over 5 million donations. We did actually determine a donor frequency for the

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publication that we did and that is 1 in 20,000 donors. We find more of these than we do HIV, 1 in 120,000.

[Slide.]

Titers, we have found up to 10^7 GEs per ml with an ALT that was noneliminating. A single unit of plasma is approximately 800 mls so what you have got in one unit positive, at this level, is approximately ten logs of HAV. We have a window period, we call it a window period, of approximately 14 days.

[Slide.]

What I am going to do is show a couple of donors. These donors, a couple of previous donations, HAV-positive donations. We obtained some units to quantitate them. This work was done in Dr. Weimer's lab in Germany. You can see the ALT, which is at least twice normal, which means that we do not take the donor anymore and this unit is destroyed at the center. There is no way that we can test it.

This is the time frame from the first positive to the ALT elevation. You can see the concentration in this donor.

[Slide.]

We have basically the same situation in the next donor. We had three positives. We were able to get one unit here due to look-back with a very low concentration.

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These units were tested for quantification by themselves without a pool. Here we had eleven days. Again, this is probably an upper limit because we can't control when a donor returns.

The third donor, which is basically the same, shows that we have 10^7. You can follow the ALT which is relatively normal and it jumps up, although this donor had a twelve-day time frame before he came back again. So we wanted to demonstrate what we have there with ALT.

[Slide.]

If we move on to parvovirus B19, why? This virus is not just more resistant than enveloped viruses, it is resistant. It is a self-limiting infection. Again, it is a donation test. The concentration here can be up to 10^{14} GEs per ml or basically 14 logs. This one, if you don't control it in the collection center, you are going to find lots of positives, even if you have a detuned test.

High prevalence in the literature and why do we want to do it? Well, a lot of our product goes to immunocompromised patients. It goes to pregnant women and we thought that it was something that, since we had the technology, we needed to at least investigate.

[Slide.]

On our initial analysis, we confirmed, using a sensitive test, the positive rate of 1 in 800 donations.

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But we noted that about 1 in 13,000 donations had a concentration greater than 10^7. This is what we concentrated on. If you are going to put it in the laboratory and you have 1 in 800 donations positive, you might as well close the lab.

We did a pilot study, approximately 900,000 donations where we found about 1 in 8,000 donations positive. And then we did a secondary evaluation of it with about a little over 2 million samples, again about 1 in 8,000. This is a high estimate because we do not confirm at the positive level. If a positive is positive, we destroy the unit. We don't get the unit in to confirm the positive.

So if you have contamination that occurred at the center, and you will see it, we still count it as a positive. So this is a high estimation.

[Slide.]

Our test system is, again, the same. However, here you can see that our detection limit is 4.4 times 10^6 International Units. We basically had the same goal. However, we started before the FDA gave us their guidance and our target was 10^5, which we think they should adopt.

[Slide.]

We have tested 6 million donations. Again, be careful at the collection. Very high concentrations. This

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is a high estimate of your positive frequency but, at the same time, if you are going to test the unit, you are going to lose a unit because it can't go in the manufacturing. So why test the unit, from our point of view.

[Slide.]

What is the effect of this, the effect of both HAV and B19? Does it do anything? Does it reduce the viral load of the plasma pool, prior to going to manufacturing? This is similar to the slide we showed before. Again, you have the theoretical load and then you have the load remaining after you introduce the PCR test.

The benefit on screening is a little over 4 logs for HAV and 7 logs for B19. That is a pretty good reduction. So you are removing every positive. B19 removes over 9 logs of virus going into a production pool. For HAV, it removes 5 logs.

[Slide.]

For those of you in the back, this is green and red. To the left of the line here is, as you look at it, plasma that we evaluated on B19 in a retrospective manner. These are the fractionation pools that had no prescreening for B19. This line, the horizontal line, is 10^5 GEs per ml for the fractionation pool.

The green here is this first 115 fractionation pools that we made from prescreened plasma. All of them

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are below 10^5. Four of them are above 10^4. Two of these, I believe are 2 times 10^4. Every time you start something, everything gets screwed up.

[Slide.]

Another way of looking at this. The red is the initial study, not prescreened. 20 percent were positive. I think it was 5 out of the 30 or 6 out of the 30. The green was the 115 where we had 4 out of 115 pools. Since then, we have 130 pools beyond that where we have had everything less than 10^4. But that's okay. You guys can go to 10^5.

[Slide.]

Just because we put that in there. What does that mean? It may not mean anything. So what we did was we looked at eleven lots of our of our products, antithrombin-3, from pretested plasma and compared that to twelve lots from no testing of B19, what happens in a product. So you can see here that, when we didn't do any prescreening for B19 in the product, you have anywhere from 200 to 300 GEs per ml of B19 material, nucleic acid that you can detect.

However, if you combine the prescreening with, at least in our company is it a validated removal of 10.4 logs of B19, and you then look at the product, this test was run on a sensitive test that detected 33 GEs per ml was the

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limit of detection. It was less than that for every one of the lots.

[Slide.]

So, to summarize, for HAV, we find titers up to 10^7 with a normal ALT. Every positive unit at that level can remove 10 logs of HAV from manufacturing. We have supported our goals, or we have found information that said we set the right goals anyway, to reduce the virus load and limit that load to a defined maximum.

[Slide.]

For B19, now you are looking at a minimum of 10-log removal because you are only finding samples that are positive with a concentration of greater than 10^7. So you can go up to 10^{14}. We have shown the effect on the fractionation pools and actually it converts into something that you can look at on a product. Again, we have limited the virus load and we have reduced the load going in the fractionation.

[Slide.]

I would like to thank some people in Marburg, our research facility, and you for listening. And, for those of you that came back, thank you for coming back.

DR. YU: Thank you, Dr. Watson. Short comments and questions?

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DR. LI: My question is for both HAV and B19, how many viral particles do you have to get infused before you develop symptoms, clinical symptoms, that is?

DR. WATSON: I will leave that to the clinical people. Maybe someone in the audience? What is the infectious dose for HAV and B19?

DR. YU: Let me just answer. I don't think anybody knows, in a certain sense. However, based on the 1999, this is the Vitex experience, for SD-treated, pooled plasma, they found if you have the 10^4 genome equivalent per ml, and this is a 200-ml dose, they found those infused lower than the 10^4 genome equivalent per ml no transmission. Those who received high-titer ones, which is around 10^7 genome equivalents per ml, that it transmitted into recipients. They have the B19 transmission.

DR. MIMMS: Larry Mimms from GenProbe. Do you test for antiHAV and antiparvo titer and what was the effect on the titer of your product after removal of these high viremics?

DR. WATSON: Would you repeat your question, please?

DR. MIMMS: Do you test your product for antiHAV and antiparvo titer and, if so, what is the effect of removal of these high viremics on those titers?

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DR. WATSON: We do not normally test them. This is the first time we have ever tested any product and it was strictly to see what happened at the end after we removed B19.

DR. YU: Very good question. Thank you.

We really do have to move on. Our next speaker is Dr. Richard Smith from NGI.

NGI

DR. SMITH: Good afternoon.

[Slide.]

Today, I am going to briefly summarize NGI's experience to date in screening for both parvo and hepatitis A virus in U.S. source plasma. I am going to present the combined data for all the clients that we are screening for and you will hear later from some of these broken out for the individual clients.

I will describe the algorithms being used to identify the positive units and how they differ slightly from the algorithms used for HBV, HCV and HIV. I will also talk about the sensitivity of our assays and how often we are detecting both HAV and parvo.

[Slide.]

Screening for both viruses is being performed using the same three-dimensional matrices described earlier by Dr. Conrad that are tested for HBV, HCV and HIV.

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However, as I mentioned, a few modifications for these in-process tests have been incorporated.

[Slide.]

The resolution algorithm involves testing master pools representing up to 512 individual donations. If they are negative, all samples are released as not-implicated. If the master pools are positive, the 24 primary pools are tested to determine which are the implicated individual samples.

In the case of parvo, both the master pools and the primary pools are diluted before testing in order to reduce the sensitivity of the assay and avoid detection of the low-titer samples that Chuck Watson just described.

Implicated samples have then been tested individually or many of the manufacturers have now decided to stop testing after primary pool testing and simply discard all the implicated units without any confirmatory testing.

[Slide.]

There are advantages to this method. The obvious advantage to discard the implicated units without further testing is that fewer NAT tests are required. While a few more units might be discarded than absolutely necessary, in most cases, the cost of testing those units is more than

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the value of the plasma, itself, so it makes sense to circumvent the testing.

The primary concern with this approach is the inability to allow for--to know for certain that all the positive pools are truly accounted for. Occasionally, a low-titer positive can require additional testing to resolve.

For parvo, this really isn't a problem, however, since all high-titer samples should be implicated by all three primary pools and discarded. We know that the low-titer samples might go detected already. That is the purpose of diluting the pools for the initial screening.

For hepatitis A, however, this approach is sometimes being applied because the test is being considered an in-process test, but no such tolerance for low amounts of virus has been recommended or justified that I am aware of, yet.

[Slide.]

This slide just shows the raw Southern Blot data from an NGI UltraQual assay. Without going into too much detail about the process, I just want to make the point that assay sensitivity can be controlled both by the amount of starting material from which the nucleic acids are extracted and by the total number of amplification reactions performed.

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Here we have two membranes, one on the left and one on the right, each showing the results of duplicate sets of 30 reactions. For example, we have, in position 21, you can see position 21 in four separate cases. The results of those would be combined in order to come up with a consolidated result. Any one reaction being positive would cause the assay to be reported as positive.

So duplicate reactions are therefore performed when more sensitivity is required.

[Slide.]

In the case of parvo, we don't need the increased sensitivity. That is why we actually dilute the samples to decrease the sensitivity. We use one reaction per primer set. There are two primer sets that we use for the parvo assay. The 95 percent detection cutoff of this assay is approximately 23 copies per ml and we further desensitize that assay, as I mentioned, by diluting the master pools.

Each fractionator determines for itself what sensitivity they want to achieve the goal of less than 10^4 copies per ml in the manufacturing pool. Those with very large starting pools have typically asked us to dilute the master pools 1 to 1000 which, in effect, dilutes the individual sample 1 to 500,000.

Those who have relatively small manufacturing pools have us dilute the master pool only 1 to 50 for an

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overall dilution of about 1 to 25,000. That includes the dilution factor of 512 samples in the master pool and you get 95 percent cutoffs of about 12 million in the first case and 600,000 in the second case.

[Slide.]

I am not going to present a lot of data about parvo because I know there are a couple of other presentations where there will be more numbers. To date, the majority of samples we have screened in master pools, the master pools have been diluted 1 to 1000. We have screened about 5 million samples so far and found 730 master pools positive.

In most cases, only one sample is implicated but there are matrices that contain more than one high-titer parvo-positive.

[Slide.]

The rate of positive master pools has ranged from about 1 in 9 to 1 in 13, and that correlates with the dilution factor that is used for the master pools.

[Slide.]

For HAV, the assay we are using to screen is almost identical to our recently approved HCV assay. We extract from 1 ml. We perform four amplification reactions. The 95 percent detection cutoff was determined using material from NIBSC to be about 3.9 genome

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equivalents per ml which translates to about 2000 copies per ml in the individual sample.

[Slide.]

We have tested almost 5,000 pools of 512 representing almost 2.5 million donations so far for HAV and identified eleven positive donations to date. To this point, all matrices have been resolved to the individual sample including the confirmatory tests. The overall rate, so far, is one positive per 225,000 donations. Those eleven positive donations came from only six donors.

So, in the majority of cases, we have detected more than one HAV-positive donation per donor.

[Slide.]

This slide shows the titers of viral loads of eight of the positive donations representing four of the six donors. We found that they ranged from 2.2 million copies per ml all the way down to 650 copies per ml. Ten of the eleven titers are well above the 95 percent detection cutoff which would still be significant in small manufacturing pools but one was close to our mean detection point. That is down near the 50 percent line. That line represents the mean detection cutoff.

Each donor is represented by a different color and symbol. You can see that donor ramped up to about 420,000 copies per ml seven days after making this

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donation. The other donors, with multiple positives, are represented by the green squares, the blue circles.

None of the positive donations were separated by more than seven days from the earliest HAV-positive detected. However, no donor follow up was carried out either.

[Slide.]

I will just leave with a couple of questions. First, is the HAV a reportable infection? I know that earlier we heard that it was not. However, this question keeps coming up and I keep hearing it from people. It appears that some states may have regulations about whether or not you really need to report this to the local health agencies and, if you report it to the local health agencies, do you need to report it to the donor. We are still waiting for definitive answers on that question.

Second, should look-backs or look-forwards be performed? Our one 650 copy per ml donation would suggest that yes, we should perform look-backs but studies still need to be performed to determine what length of time the look-backs should cover.

Finally, what sensitivity should be required? There is still no recommendation on that. We can increase or decrease the sensitivity of the assay by changing the input volume and the number of reactions. We have just

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picked the sensitivity that we get with the assay we use as performed for HCV.

So those are questions for people out here. I am going to end by asking you questions instead of waiting for questions from you.

That's it

DR. YU: Those are nice questions. Thank you, Dr. Smith. So we are open for short questions and comments.

DR. KAPLAN: What is the intended use of the assay? Is it donor screening or an in-process test?

DR. SMITH: It is an in-process test as we are currently using it. There is no donor notification. However, that is all up to the fractionators who would perform the test as they will request us.

DR. KAPLAN: But you are going to do it on real time, aren't you?

DR. SMITH: On what?

DR. KAPLAN: On real time. You are going to test samples as they come immediately?

DR. SMITH: Yes. We are testing for the plasma--this is all for U.S. source plasma, remember, so they have time to discard those units once they are found positive.

DR. BIANCO: Celso Bianco, ABC. I am a little bit concerned about the ideas of look-back. You are going

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to test these units months after they are collected. The infection is gone many months after that. There is nothing else they can do. Notification to state health departments; they want to be notified immediately because--

DR. YU: Please keep the questions short.

DR. SMITH: I agree. I know there is a lot of controversy about notification. I think that you could still perform look-backs without donor notification just to find those units that might have low titers. As far as notifying the local health agencies, that would be more of a way of them tracking what is going on with HAV rather than something to help the donor.

DR. MIMMS: How did you assign your concentration values for HAV copies per ml. Did you participate in the WHO study? Are you using their standard or do you have your own standard?

DR. SMITH: Yes. Yes. And yes. We participated in all the WHO studies. I think there were two rounds. And we also are using their standard. We used the NIBSC's standard for the determination of the sensitivity and we use their standard on every assay that we perform.

DR. MIMMS: What conversion factor do you use between International Units per ml and copies per ml? Is that published?

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DR. SMITH: Well, they don't have International Units. It is genome equivalents per ml and we use 1 to 1 for that because we determined our sensitivity using their standards. So we are talking about genomes. It is apples to apples in this case.

DR. YU: Thank you. There is no international standard for HAV yet. One question. This is my personal opinion about this. I think if you have got a NAT-positive, HAV-NAT-positive, you should ask the CDC whether it should be reported because some states it is a reported disease, according to CDC, for HAV.

DR. SMITH: That confuses me about the earlier presentations.

DR. YU: No; that is another thing. We won't talk about that. Thank you very much. For whole blood, it is a separate issue altogether.

Our next presenter is Dr. Lorraine Peddada from Alpha Therapeutics.

Alpha

DR. PEDDADA: Thank you.

[Slide.]

This afternoon, I am going to give an update on our parvovirus in-process screening program. We just started December of 2000. We are sending our samples to National Genetics Institute, as Rich stated, for the

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UltraQual assay on diluted samples. Our system is designed to capture units that have approximately 10^7 copies per ml.

[Slide.]

The objectives of my study initially were to optimize our in-process screening protocol, to estimate the viral load in the units that we capture, to determine the serological status of the donors in the captured units and to determine the viral load and antibody titer in our manufacturing pools.

[Slide.]

We are using the three-dimensional pooling scheme and the assay sensitivity as reported to me when we started the program was 40 B19 DNA copies per ml.

[Slide.]

We performed the screening in two phases. We have been screening for about ten-and-a-half months. So, in the first phase, we started with a qualitative assay using 1000-fold diluted samples. This will give us a sensitivity of about 40,000 copies per ml. Then the overall sensitivity in the individual donation is about 20 million copies per ml. All positive master pools were resolved by screening the primary pools with a quantitative assay and we had a set cutoff of approximately 1 times 10^5 copies per ml.

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So we screened the source plasma for about six months using the phase-1 protocol and then we moved on to a phase-2 system where we are screening the primary pools from positive masters with primary pools that are diluted 1000-fold and we are using a qualitative assay.

The overall sensitivity at the master-pool level and the individual level remains the same.

[Slide.]

So far, we have tested 3,504 pools. Approximately 33 were positive on the first round. Then, when we tried to resolve the pools, only 217 were confirmed positive; that means, above our cutoff. We were able to triangulate and identify suspect positive units that we discarded.

[Slide.]

Looking at the same results on a unit basis, from 3,500 pools, that represents about 1.8 million units. There were 217 high-titer positive master pools and, from those master pools, we captured 471 high-titer units. This represents a frequency of about 26 donations per 100,000. This is an elevated number because, in our resolution algorithm, we are identifying negative units and discarding negative units, negative meaning below our cutoff.

[Slide.]

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I looked at the seasonal variation in the number of high-titer positives by month. This was beginning in December of 200 to October of this year. As you can see, in the months of June and July, the number of positive donations increased to about 85 per 100,000. This was very difficult for us to deal with.

[Slide.]

I looked at the serological status of 21 of our high-titer donations. Here, I have listed the log copies per ml and the number of units that were represented. In the first row, we have the antibody-negative donations. They represented 71 percent of the high-titer donations. So the titers range from 10^8 to 10^{12}.

We had four donations that were reactive for IgM and negative for antiB19 IgG. This represented 19 percent. One donor was reactive for IgG and one was reactive with both IgG and IgM.

[Slide.]

Next, I looked at the viral load. Approximately, let's say, 25 percent of the high-titer donations had titers less than 10^4. More than 30 percent had titers greater than 10^{10}. The remaining titers just fall in between here.

[Slide.]

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Since we are just beginning our studies, right now, we have to improve our screening method. We plan to do this by lowering the dilution of the master pools so that we will capture master pools that have 10,000 copies per ml as opposed to 40,000 copies per ml. In this way, we will be able to remove all donations with titers about 10^6 DNA copies per ml.

We need to assure the efficacy of our B19 screening method by determining the B19 titer in the manufacturing pools and we want to assure the safety of our B19 screening method. I have said the wrong thing. We have to look at the B19 titer in the manufacturing pools and make sure that it is less than 10^4 and we want to be assured that our B19 antibody titer in our manufacturing pools is equivalent to what it was before we initiated high-titer screening.

Thank you.

DR. YU: Thank you, Dr. Peddada. Any short questions and comments?

DR. KLEINMAN: Lorraine, I was a little confused on one of those slides, right towards the end, when you talked about the viral load of the units and you had quite a few that had less than 10^4. How could you detect those if the assay system was sensitive only down to 10^7?

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DR. PEDDADA: As I said, in our triangulation system, we triangulate and we identify positives and negatives. We just take them all and we throw them out. So some of the units identified by triangulation are negative, are low titer.

DR. KLEINMAN: But shouldn't triangulation give you one specific unit?

DR. PEDDADA: I hate to admit this but, in some cases, we have 35 positives per pool in the peak months of June and July.

DR. KLEINMAN: I see.

DR. PEDDADA: So if you have twelve possible triangulations, you may only have four positives in that pool. That is probably part of it.

DR. KLEINMAN: Thanks.

DR. LI: Does the viral load fluctuate for a specific individual?

DR. PEDDADA: We are not studying individuals, but we expect the viremia to last only two weeks.

DR. LI: (comments off mike)

DR. PEDDADA: Because we want that person when they are producing antibody. What I was trying to show with the antibody data was that the viremic donations are antibody-negative. So we are throwing out the high titer. We are not losing antibody.

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DR. YU: I am almost sure subsequent presenters will give you some viremia data. Dr. Zerlauth is going to give you some.

Our next present is Dr. Donald Baker from Baxter.

Baxter

DR. BAKER: To forestall any questions, I am not going to give you any viremia data.

[Slide.]

At the beginning, I would like to return to a question that Chuck raised which was basically why do this. To put it in perspective, with our current generation of products, and I am speaking with respect to all of Baxter products which they have manufactured in the last six years, the products manufactured from plasma which was unscreened for HIV or B19, in my experience, I have seen no HAV transmissions over a whole range of products and potentially two B19 transmissions which could be reasonably related to the plasma derivatives.

Unfortunately, with B19, there is a high community-acquired infection rate so it is difficult. I think we have maybe got one case in which is it pretty clear there was a transmission. So this is a very low-level problem.

In both of those situations in which there was reasonable evidence for possible B19 transmission, there

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was no long-term clinical effect. So this is a problem which is in a very different category than some of the others that we have dealt with with plasma derivatives.

[Slide.]

Again, in this case, I am not going to go into the details of the assays. Our pool testing is done by NGI and it is at a 1-in-1000 diluation of the master pool. Our production pool testing is done at our labs, at our Baxter labs, in Vienna with the intention of keeping the master production pools below 10^4 genome equivalents per ml.

Considering that we had two different labs doing the donor screening and the parvovirus screening, I was amazed that, for parvovirus, where we were shooting for the 10^4, that they were actually replicatable and there has not been a disagreement.

[Slide.]

Again, the data flow, obviously in these situations, since we are not going back to the donors, is much simpler and it has obvious advantages in manufacturing. [Slide.]

With respect to parvovirus, over the period February 2000 when we began through June 2001, we have tested a little over 2 million donations with 319 donations positive.

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I think the pool testing was wrong. I think that is my error. That should be 234 pools were positive with a plasma loss, as you can see, of about 0.015 percent. We have not experienced, as of yet, the problems with seasonal variation. This was something that we anticipated was going to be a problem but, so far, again, touch wood, that has not been a problem in our laboratories.

[Slide.]

HAV, again in contradistinction to Chuck's experience, we have had far fewer positives. That is something that we want to talk with him about, why the difference between us. We have only found four donations positive and that represented four donors.

[Slide.]

So, in summary, the system works. It is reproducible. We have had no positive manufacturing pools, which has been a good thing. And we have had minimal plasma loss.

Thank you.

DR. YU: Thank you, Dr. Baker.

Any questions and comments for Dr. Baker? Do you monitor antibody, like what Dr. Mimms asked a while ago? Do you actually measure anti-B19 levels in the product to make sure that--

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DR. BAKER: No; we have no specification for B19. We don't monitor it.

DR. YU: Just to make sure the screening does not decrease the dose-neutralizing antibody, the antihepatitis A or antiB19.

Our next presenter is Dr. Gerold Zerlauth from BaxterHylandImmuno. He has some viremic--you have the data for it.

DR. ZERLAUTH: Hopefully.

BaxterHylandImmuno

DR. ZERLAUTH: Thank you very much.

[Slide.]

I think most of you are familiar with the type of screening assay that we have introduced some years ago for screening parvo B19 and, more recently, for HAV. The system characteristics are that we are using a 5-prime nuclease assay, a TaqMan assay system. We have developed an internal quality marker similar to all our other tests which is added at the beginning and it is a real global marker.

One of these tests is laid out as a quantitative test when we discriminate the viral load of a minipool and a similar test has been made in a qualitative fashion which is what we call the Parvo Resolution PCR. This is fully

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automated. We don't touch these samples anymore, which gives us a lot of capacity.

The 95 percent cutoff value is 35 IU per ml and the assay, such as all the others that we are using, is validated according to the guidelines already mentioned and following exactly the same procedures.

[Slide.]

Looking at data that we have compiled from March '99 to February, 2001, in minipools, you can see that--we can see a lot of different things. One is that minipools can be fairly highly loaded, 10^9 IU per ml you can find in some minipools.

Interestingly, if you look at these bands here, you can see that either you have a low-level load in the minipools or a fairly high level which is usually caused by one highly viremic donation which causes so much noise. We also do see some clustering of viremic periods which, of course, is a little bit blurred by the fact that we have not matched all the bleed dates to the test date.

Due to logistic activities, we do not, of course, test the donor a couple days after bleeding, obviously. So it cannot be really seen as a 1-to-1 translation. But, overall, we do see periods where we have a lot of higher minipools as compared to other periods where the number of low-level minipools is much higher.

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[Slide.]

What does this mean when we use such minipools without screening or when we use such minipools without B19 screening, we experienced production pools that had up to 10^7 IU per ml. Upon introduction of this screening system, we could nicely reduce this number to about 100 IU per ml on average. In this year, we had none exceeding 1000 which gave an average number nicely below 100 UI per ml.

So this is a fairly effective system which allows us to reduce the intake of parvovirus by about five lots prior to production.

What I wanted to show you is simply that we have tested approximately 2,500 pools per year in the years 1999 and 2000 and 3,900 pools in this year which comprises about 5 million, a bit more than 5 million, donations over all. In the year 2000, I have just taken this year as an example--in the year 2000, we tested approximately 1.5 million donations and we had to discard 300 of them based on a cutoff of 6.5 times 10^4 UI per ml which is the current cutoff. The rate to discard is 1 in 5,000 donations.

[Slide.]

So much for parvo--oh, no; sorry. You asked me about the antibody situation. We have assessed the antibody level in production pools prior to B19 screening. This is the distribution of IU per ml of antiparvo B19

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antibody. So this is what we found. The percentage of pools having 5 to 10 and so on and so forth.

Upon introduction of this parvo B19 screening that I have shown to you, we have tested an additional 450 pools. As you can easily estimate, this situation, or this activity, did not change at all the antiparvo B19 antibody level.

So this procedure does not selectively remove antibodies which, of course, are highly welcome.

[Slide.]

What is also interesting is that we have found donors, and this is an example, which show high viremic level of virus as expected. This person had up to 10^{12} parvo B19. This is an older slide. It is still in Baxter Units but it is more or less equal to International Units. We followed IgM and also IgG here.

But what is interesting, in my opinion, is that the viral load in this donor did not decrease as fast as one would expect. Also the viremic phase was like in the picture book. But then this donor remained for a prolonged period of time at the level of 10,000 per milliliter which, of course, contributes for quite some time a lot of virus. However, as you can see, there is, at least for some period of time, a lot of antibody which makes this donation a valuable contribution in antibody.

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But it is just interesting that not all donors go back to zero. Quite a few donors may remain at an elevated level over a prolonged period of time.

[Slide.]

With respect to HAV, which we have introduced about a year ago, we also use a TaqMan-based system. Again, we use internal quality markers. This assay is validated as a qualitative assay in the absence of any international standard. As of yet, we have titrated that to 150 Baxter Units according to the now many-times cited validation guidelines.

We also have made a comparison to the NIBSC 97/540 or the following one, 99/733. And we detect the 1-to-10 dilution every time and the 1-to-100 diluation of these preparations in 93 percent of cases. So this is so much to the sensitivity. We will, of course, calibrate it the minute John is going to publish the data on this assay--oh, sorry; a standard.

[Slide.]

Interestingly enough, we cannot corroborate Jack's findings. We have tested 2.5 million donations so far and we had two positive donations which were derived from one single donor. So that is the current status with respect to B19 and HAV screening within Baxter in Europe.

Thank you for your attention.

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DR. YU: Thank you, Dr. Zerlauth.

Any comments, questions?

DR. GALLARDA: Gerold, your internal quality marker; is that a full process control?

DR. ZERLAUTH: A full--

DR. GALLARDA: Your internal quality marker; is that a virus-like full process control that controls for lysis through detection?

DR. ZERLAUTH: Right. This is similar to all the other assays. It is added at the beginning and is taken through the whole process.

DR. GALLARDA: It is a protected genome?

DR. ZERLAUTH: Yes.

DR. YU: Any other questions?

Let's go to very last speaker from the source-plasma industry, Dr. Todd Gierman from Bayer.

Bayer

DR. GIERMAN: Thank you. I appreciate the opportunity to contribute to what I think is a topical and informative program.

[Slide.]

This you have already had a little preview to. It is late. I think I can get through this quickly. This is really just to remind me to tell you where we started, how did it begin. Basically, we initiated a program a

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couple of years ago to really do some low-key surveys of our sample minipools that were coming through our high-throughput testing facility. We basically collect these as unlinked samples and test them using an existing assay that we were using for investigation in the laboratory. On a monthly basis, we do about 50.

So, basically, through the course of the year, I think what is most relevant is we were able to ascertain that we could expect a hit rate on the minipool level of about 9.2 percent. This was important for us to understand in terms of assessing how might the introduction of testing impact our logistics in the high-throughput facility.

We also predicted that, with the assay that we were using to do the survey, that we would probably pick up, through the course of a year, 1 in 1000 donations.

[Slide.]

With that in mind, we--that was a little intimidating, actually. We also had noticed some seasonal variation as well, so we felt there were some things that had to happen to our assay to make it appropriate for the high-throughput testing environment.

Basically what we strove to do was to adapt an existing assay to insure suitable and reliable performance in a high-throughput environment by reducing assay sensitivity to strike a balance between the reduction of

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risk and operational feasibility, introduce an internal control to monitor assay performance relative to individual samples. The original assay that we had had no internal control and it has proved quite valuable to have that.

Improve assay specificity to limit potential for spurious results; we were using a targeting strategy from the literature but had concerns about homology between the region of the B19 genome and some human genomic sequences. We also realized it was important to integrate the assay into the current operational work flow and documentation systems. So one of the main objectives was to use the common sample-prep procedure for HIV and HCV that was already in place. Also, we didn't develop this on the research end but consulted in the process to develop a donation-management algorithm for reactive donations.

[Slide.]

Just a quick overview of what our assay looks like. As I said, we used an existing extraction method which was the Roche Multiplex Extraction which was in use for HCV and HIV. We modified primers from an existing targeting strategy, as I said, to improve--what we felt would improve the specificity. We added internal control.

You can kind of see, if you are looking at this on gel, the sizes. We target the NS-I gene of the genome and we utilized an existing microwell plate platform that

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was in the marketplace that is manufactured by Digene. That utilizes a proprietary antibody that will capture the DNA-RNA hybrid. An RNA probe is used for the detection to insure specificity.

[Slide.]

Basically, from the time that it was decided by senior management that this was an issue that we needed to take care of, we were basically given six months to put this in place from where we stood with what we felt was a very low-level stage of development. We agreed that we could do that with the caveat that we went into this with what we were calling a research-phase implementation.

The way that we set this up was that this would be implemented as part of the current operational testing at the Raleigh test lab. They would utilize sample receipt, handling and pooling that was already in place, and they would actually perform the test. They would record results and keep records in the current system.

What we decided we would do through research would we would provide oversight relative to the technical performance of the assay and we wanted to make improvements to the process. So we have been heavily involved in tracking and trending data. We are involved in troubleshooting when it is needed, and it has been needed on a few occasions.

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We do extensive analytical characterization of reactive and nonreactive samples that come through the facility. This is the way that we monitor our progress to see how we are doing. We are continuing the development of the assay parameters. We are hopefully wrapping that up here in the next month or so, and we intend to, then, validate this final configuration.

We also implemented what I would guess I would characterize as a modified process of change control. We felt that, in order to have this structure work, we would need a fairly high degree of flexibility. We would need to be able to respond rapidly to any detrimental changes that we may have introduced as part of the development process. That really hasn't been the case to any great degree, but, basically, we have two keyholders. Barbara Masecar is one and I am the other, and we consult heavily before making any changes and document changes, of course.

[Slide.]

So, to get to the numbers. Since July of 2000, when we implemented, we basically have the numbers through October at this point, we have tested roughly 3.5 million donations in a total of about 36,000 minipools. We have come up with a little over 1400 reactive donations.

These have been resolved from 980 reactive minipools. This is kind of interesting because this gap

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has closed in the time that we have been doing this. I would attribute this in part to the change in the collection system that we had in place. I think, as Barbara mentioned earlier, we were using an open-drip system and we went to a closed tube in December. I think that is when this gap really began to close.

So we don't get as much carryover, or sample-to-sample contamination I think is what the case is. We have got a hit rate of about 1 in 2400 donations, and that is one for every 37 minipools. Our analytical sensitivity is somewhere around 1000 International Units per ml.

Just to provide you with a trend, in terms of the incidence during this period, we have seen some seasonal fluctuations. We really had a spike in April. I think, as Dr. Zerlauth mentioned, this does not correlate with bleed date but test date. We could estimate for the majority of these samples, they were bled two to four weeks before. But it could be further out than that, even.

So, anyway, we had this spike in April and then what has really been a decline, a slow, steady decline in the incidence. This, what appears to be a spike here, is probably more due to accounting. During this period of time, we did have some difficulty with interference in our samples and a lot of the testing was carried over from June

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into July. So this may be a bit of a smoother downward trend in reality.

[Slide.]

Just to give you a breakdown of some of the analytical studies that we have done in terms of quantitating viral loads in the individual samples that were found in the minipools, our major concern is with units that have viral loads greater than 10^6 copies per ml. We have not yet converted the quantitative assay over to International Units.

But, basically, what we are seeing is 30 percent of what we interdict falls in this range, 10^6 to 10^{13} copies per ml. If we break this down further on a log basis, 13 percent fall between 10^5 and 10^6 copies per ml, 17 percent between 10^4 and 10^5, 12 percent between 10^3 and 10^4. This 28 percent represents very low titer units and probably some cross-contamination.

[Slide.]

So, in summary, we have developed a PCR-based colormetric detection system for parvovirus B19 on an existing microplate platform. We have pursued a strategy of differential screening to facilitate operation feasiblity and maintained effective antiB19 antibody titers in the products.

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We have established assay sensitivity to insure compliance with industry and regulatory standards for plasma manufacturing pools. During our research phase of implementation since July, 2000, we have tested more than 3.4 million donations and interdicted greater than 1400 individual units.

[Slide.]

Just to flash the acknowledgements. This basically indicates that we have put a tremendous effort and resources into this project.

DR. YU: Thank you, Dr. Gierman. Do you monitor the manufacturing pool?

DR. GIERMAN: What we do currently--we will be monitoring the manufacturing pool in compliance with the PPTA standards. What we do currently is, as I indicated, we collect the negative minipools and we construct what we call mock manufacturing pools from the negatives and we monitor that way. We found out we have been very successful in reducing viral loads.

DR. YU: Thank you.

Any questions or comments? If not, let's move to another set of presentations. Our moderator is Dr. Gerardo Kaplan.

Blood Bank Experience

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DR. KAPLAN: Thank you, Mei-ying. We have three talks on the blood bank experience in NAT for HAV and parvo B19. The first speaker is Leslie Stringfellow from GenProbe.

GenProbe

DR. STRINGFELLOW: Good afternoon. I would like to thank the organizers for inviting me to speak today.

[Slide.]

The GenProbe-Chiron product strategy for HAV and parvo B19 has been to develop in-process TMA assays for recovered detection of B19 and HAV and recovered plasma. The analytical sensitivity targets for both of these assays are as follows: parvo, about 500 to 1000 copies per ml and HAV about 100 copies per ml with analytical specificity for them at about 99 percent.

[Slide.]

These also have an internal control for monitoring inhibition and they will be the Procleix assay formulation like you have heard from other GenProbe folks today. We also use a semiautomated system and the assays are also compatible with the automated platform. We are considering pool sizes of 16, 128 and 256 making this more complicated.

[Slide.]

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The assay protocol that we use is the same as you have seen from other GenProbe people. This is just to remind you of that fact.

[Slide.]

I am going to speak mostly about parvo today. I have one slide of data on HAV and will come to that at the end. We received several specimens from our friends at Chiron who qualify them for us. The first set they purchased from SeraCare. They are IgM-positive. Five out of five of those were positive by TMA.

Also, another set identified by Chiron that were IgM-positive and also PCR-positive by their nested PCR assay. All six of these were, again, positive by TMA. Most recently, we tested the WHO International Standard and our 100 percent positivity rate is at about 500 IUs per ml. This is in an n of 30. This translates into 300 or 400 copies per ml using the conversion factor that Dr. Saldanha talked about this morning.

[Slide.]

We tested reproducibility on two days of the same operator of a sample diluted to 1000 copies per ml originally quantified by NGI and a normal negative. 50 replicates for both of these. You see on both days, the 1000 copies per ml specimen was 98 percent and the normal negative was negative both days.

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[Slide.]

We have also tested analytical specificity. These are normal negatives received from the Kansas City Blood Bank. We had a total of 462 that we tested. You can see that most of them, all but three, are below our cutoff of 1.0. We had three positives here.

[Slide.]

For an initial reactive rate of 0.6 percent, we retested these by TMA. They were positive again for a repeat reactive rate of 0.6 percent. These were also confirmed again by our friends at Chiron to be PCR-positive. So this gives us an analytical specificity of 100 percent and, potentially, a prevalence of 0.6 percent, admittedly on a small population.

[Slide.]

How, for HAV, without a WHO standard, we have to turn to something in-house and so what we have done for most of the time we have been working on HAV is to use the transcript from a cloned target. That is what you see here in the first portion of the slide. This was quantified by OD, two separate operators, three separate dilutions and two separate spectraphotometers.

You can see that we are pretty good at 50 copies per ml. When we use the WHO working standard, which Dr. Saldanha has told us today is about 2,000 genome

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equivalents or copies per ml. At a 1 to 10, we are about 70 percent positive. Less than that, we fall off quite substantially. So if we take all of these together and consider that the WHO is probably a better measure, we are close to maybe 200 copies per ml.

[Slide.]

So, in conclusion, or TMA assay systems seem appropriate for in-process testing of recovered plasma. Our analysis sensitivity and specificity goals for parvo have been met. For HAV, we are probably within about two-fold of the sensitivity goal. We haven't tested specificity yet. And we are going to begin an in-house research study using ARC specimens initially testing in pools of 16 and positives of these will be diluted eight-fold equivalent to pools of 128.

That is all I have today. Thank you.

DR. KAPLAN: Any questions?

DR. ZERLAUTH: It is not a question about the time, but will you have all these assays in one thing, in one tube, in the long run?

DR. STRINGFELLOW: I think that a lot of this has to do with the regulatory strategy and what our customers want. Currently, we are in two separate assays.

DR. KAPLAN: Your three positives in the 400-and-something, were they antibody-positive, too?

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DR. STRINGFELLOW: These are normal negatives and we didn't have them tested for antibody.

DR. KAPLAN: So you don't know.

DR. STRINGFELLOW: We don't know.

DR. KAPLAN: I have a question. What is the intended use of the test? Basically, will it be done in real time?

DR. STRINGFELLOW: Again, this has to do with our customers, but the intended use is an in-process test for both of them.

Thank you.

DR. KAPLAN: Thank you.

The next speaker is Christoph Majewski from Roche.

Roche

DR. MAJEWSKI: Good afternoon.

[Slide.]

First of all, thanks a lot to the organizers for giving us the opportunity to present our parvo and HAV assay system that we have developed at Roche but also thanks to the audience for those who actually stayed for such a long while to listen to the second-to-last presentation of today.

You may ask, why is Roche developing an assay for parvo and HAV given the hot debate we have about the

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significance of these assays in the diagnostic area. The very simple reason is that last year, in summer, we were approached by manufacturers of the plasma industry in Germany and all over Europe. They requested us to provide them some help in establishing a suitable quality in-process control for their blood products.

So, with this in mind, we actually had started testing by the end of that year, by the middle of last year, and by the middle of this year, we already launched the first product which is the parvo assay. Just a month ago, we launched HAV as the second product in this role.

[Slide.]

I am going to describe to you what this assay is actually about. In contrast to the normal platform that you are used to from Roche Molecular Systems, we are using here a platform from the Biochemical Group in Roche which, on one side, is a sample preparation system called the MagnaPure.

[Slide.]

On the other side is basically the amplification system, the light-cycler system, which is running the PCR reaction and is providing homogeneous detection in the reaction vessels. So this is real-time kinetic PCR and the sample prep actually is based on generic capture on glass beads of any nucleic acid, so it could capture DNA

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and RNA. But the protocol that we have come up with is actually optimized for parvo and HAV virus.

[Slide.]

So I am providing you with a brief summary about the assays that we have so far, the kit concept and the specifications of these assays, which are mostly valid for both of these analytes. So both assays come in a pack size of three times 16 results which is larger than 48 reactions considering the controls that you have to run.

The extraction protocol is optimized for the MagnaPure. However, it also works with other sample preparation procedures. I will at least mention some data that have been generated with the NucleoSens machine.

So the detection platform, as I mentioned already, is the light cycler and we are using not the TaqMan principle in this case. We are using something which is called the hybridization probe. It is very similar to TaqMan. It just uses two probes labeled with fluorophors that hybridize during the PCR reaction. And it is actually a dual-color assay because we also run an internal control, a homologous coextractate with a target nucleic-acid standard.

Furthermore, you can actually choose. The product comes with standards that we provide already prediluted. But you also can use, and Boston has actually

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calibrated the WHO standards. But you also can use the WHO standards by yourself if you prefer those, actually. We are using an external target-specific curve which is predispensed and you will see how that looks in a second.

[Slide.]

The data so far; the analytical sensitivity, this is true for parvo. We have 58 International Units per ml at 95 percent. If you are using 1 milliliter of serum for the sample prep. And I should mention, the MagnaPure is a very flexible platform. It allows you actually to use as little as 200 microliters for the preparation.

But, in this specific case, we have used 1 milliliter and that is the sensitivity that you actually can obtain using this amount of starting material. The dynamic range is 10^9 copies so, reliably, you can actually quantify in the area of 100 to 10^6. The variance that we observe is 2.3 percent on the threshold value at 10,000 copies for HAV. Of course, this is concentration-dependent or virus-number dependent, the variance that you will observe.

But it will actually go down as you can see here on the example of the parvo virus to 0.67 percent intra-assay the 100 copies and 0.54 percent at 10,000 copies. So I should mention here, with regard to the sensitivity, we have similar values established already for HAV. For HAV,

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we actually have a decreased sensitivity due to the RT PCR procedure.

Just to give you an idea. So this would be translating to ten copies of parvovirus in the reaction vessel. The sensitivity for HAV is like 50 copies of HAV virus genomes in the reaction vessel.

So the whole time-to-result for the PCR and detection process is just one hour. I mentioned already the extraction volume is variable. If you consider the extraction procedure that we recommend, the overall assay procedure time is two hours for the complete assay.

[Slide.]

So, basically, this is how the setup looks like. You can see here, this is the standard curve that we are running. It is just six dilutions of your various concentrations of parvo or HAV virus. Then you have placed for 24 samples. The green and red bar means that you have the target-specific signal as well as the internal control in each of these reaction vessels and you detect them simultaneously. Of course, we are always running a positive and negative control.

[Slide.]

The results that you would be seeing are looking like that. So the dotted lines are real samples. Let me just check it briefly. Yes; the solid lines are real

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samples and you see a very high concentrated sample here of medium concentrated and a lower concentrated. You see the dotted lines are basically the DNA standards that are coming with the assay.

You don't have to worry about the screen overall because the machine or the software of this machine will actually provide you with a printout of the concentration of the viruses. So this is nothing that you have to do manually. But basically what you see here is the fluorescence profile of a typical PCR reaction and it provides quite a nice reality check.

In fact, if you look here, like at Cycle 15 already you can make a decision whether one sample, whether a high-titer sample, would become positive and Cycle 15 will be something ten to fifteen minutes, if at all. So, already, after ten to fifteen minutes, you have quite a nice impression whether your sample is a high-titer or a low-titer one.

[Slide.]

This is actually providing and verifying the sensitivity data. Again, as you see here, the curve. We have a 95 percent detection limit for 58 International Units per ml. I should mention that with the NucleoSens procedure in the Netherlands the CBL has generated a slightly lower detection level of something lie 50

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International Units per ml which is not too different, though.

[Slide.]

This is the way we actually have checked the reproducibility for the overall procedure, just to give you an impression. So, basically, we have set up 30 different samples. Half of the samples have been pooled and have undergone sample preparation with the MagnaPure and then have been run in separate PCR reactions. Half of the reactions have been purified directly and they have also been analyzed.

As you can see, the CV between direct PCR from the samples, individually purified samples or pooled samples purified, is not differing by a large amount. The cycle threshold value that you are obtaining from these different procedures actually is in quite good agreement overall.

So this is an overall description of the CV of the assay system. This provides an overview of the cross-contamination check that we have done on the system so far. I don't know whether you can actually read that, but we actually looked at very high-titer power samples going up to 6 times 10^{11} equivalents and you can see quite nicely that there is almost no cross-contamination with the exception of one sample down here.

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We actually looked at quite a substantial sample number of 1,000 overall. The cross-contamination that we observed here is with the highest titer sample and we really don't know whether this did not happen before sample preparation due to contamination of the sample, itself.

So, overall, we now have looked at something like 25,000 samples. We have looked at pools of 48 and 480. So 25,000 samples means it is still less than the 100,000 that Mrs. Yu recommended. But we have a lot of collaborations ongoing and several numbers of labs are already testing these assays in Europe and will provide us with some results in the near future.

So, as I said before, these assays are available in Europe already and will be ready to go also at the beginning of next year here in the U.S.

So thanks for your attention and I am open to questions.

DR. KAPLAN: Any questions?

DR. CHUDY: Michael Chudy from the Paul Erlich Institute in Germany. Have you an explanation why, in high-titer samples with this increasing cycle number, the signal will decrease?

DR. MAJEWSKI: We call that the late-dose effect. You are referring to the plateau, the signal?

DR. CHUDY: Yes.

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DR. MAJEWSKI: So this is reannealing of the product that you are observing there. Because you are coming up with product quite rapidly and then what happens then is that you call it futile PCR, basically, so you have a competition between primers annealing to your product and the product reannealing, basically. It is a very common effect observed in real-time instruments.

DR. KAPLAN: I have a question. This system seems to be self-contained. Your ambition is that this will be sitting in blood banks and be able to do on-line testing?

DR. MAJEWSKI: Yes.

DR. KAPLAN: Is Roche planning to apply for a contained kit for the processing and testing?

DR. MAJEWSKI: Since this is meant as an in-process control assay, we are not--will actually go through any diagnostic approval procedures with this assay as it is here. Just the purpose of this is different. We just look at pooled samples. We are not interested, really, in tracing it down to the single-donor level right now.

DR. KAPLAN: Thank you very much.

The last speaker is Sue Stramer from ARC.

ARC

DR. STRAMER: As usual, I would like to thank my collaborators.

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[Slide.]

This was presented at AABB so, for those of you who have heard the session with the exception of the two slides, you can go back to sleep.

[Slide.]

As far as background, manufacturers of plasma derivatives are implementing NAT for non-enveloped viruses and such testing will likely be implemented for recovered plasma. Most parvovirus B19 NAT programs target the elimination of units greater than 1 million copies per ml. Studies of hepatitis A virus and parvo B19 frequencies in recovered plasma are limited.

One such study by Dodd and coworkers reported at the AABB in 1997 reported zero positives in 20,000 screened in pools of 512 for hepatitis A, RNA and seven in 10,000 for B19 DNA.

[Slide.]

Also, the three-year experience at Vitex for NAT screening final product that is 2,500 donations which is done at NGI for HAV or minipools of 100 for B19, this is for SC plasma which is pools of 2,500 donations, they report frequencies of one in 4,776 for hepatitis A and approximately one in 800 for B19.

We have obtained units from some of the positive subpools; that is, if a pool of 100 is positive at NGI, it

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is resolved into five pools of 20 and no further resolution is performed because there are no samples available. So we have obtained the subpools of 20 from the positive minipools of 100 for identification and characterization of B19-positive units.

Of 500 units tested from 32 positive subpools, only eight have tested B19-positive at NGI and the eight came from six pools. So, actually, 81 percent, 26 of 32, were false-positive minipool results.

[Slide.]

If we look at the positive results from the eight parvovirus units that I just mentioned, and you look at them in order of decreasing viral load, this is the high-titer unit that you would expect. Looking at the antibody results, IgM and IgG, as from the BioTrend kit, the high-titer unit, as one would expect, does not contain IgM or IgG. Then, as titers drop, virus is cleared by the production of antibody, first IgM followed by IgG.

So the low-titer samples here would be IgM-negative and IgG-reactive.

[Slide.]

Therefore, we know, from all the background studies I have shown you, that HAV is infrequent. B19 NAT false positivity may be common. Low-level DNA from B19-

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positive IgG-positive samples does occur and individuals with early acute B19 infection have high viral titers.

[Slide.]

So what we did was a follow-up study. We took unlinked samples to determine the HAV and B19 frequencies in recovered plasma. We took our NAT-negative HIV/HCV samples contained in PPTs and send them to NGI where NGI did their UltraQual test. The NGI UltraQual test, as described, uses two primers for HAV and B19 each tested in duplicate at NGI. The algorithm we followed for our pilot study was we sent blinded 512,000 donations. They were pooled by the Tecan into 100 pools of 512. They were tested undilute for HAV. If there was an HAV-positive pool of 512, they were resolved to individual donation and quantified.

We tested according to the 1-to-1000 dilution you have heard about for B19. If they were positive, we resolve individual donor, quantified and did antibody testing for IgG and IgM. However, if they were negative at a 1-to-1000 dilution, we wanted to see what the frequency was if we used a more sensitive B19 assay.

So we also tested undilute. The same thing if they were positive. We resolved, quantified and did antibody testing.

[Slide.]

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The results for HAV were zero of 100 pools were positive for hepatitis A. So our frequency was less than one per 51,200 donations. For parvovirus, we had three of 100 pools reactive at the 1-to-1000 dilution but four donations were positive in those three pools. So our frequency was one in 12,800.

If you look at the four positives here, again, we had two high-titer samples, both negative for IgG but one of them was positive for IgM. We had two lower-titer samples that actually should have been a surprise to be reactive. They were both in the same pool because they were below the level of detection of NGI's test at 1.2 times 10^7. One of them did contained IgM.

[Slide.]

Continuing to look at the results for the remaining 97 pools that were NAT-positive at a 1-to-1000 dilution, 34 were positive if we tested them without dilution. Without dilution, these contained--actually, this should be 95 positive donations. That is a frequency of one in 528 so, clearly, an unacceptable frequency.

If you look at the titers here, we did have some moderate titer samples that contained IgM. Two out of three contained IgG but the IgG concentrations certainly were lower than strong positive samples. The lower-titer samples, which were, undoubtedly, the result of

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contamination or low-level positives had various variable concentrations of IgM and higher frequencies of IgG.

[Slide.]

In addition, what we are doing before we implement any type of screening program is minipool testing for HAV or B19 is we have implemented now--on our manufacturing pools of 3,250 liters, we have started HAV testing. Because of the infrequency of positives occurring in HAV, we have moved forward with doing manufacturing pool screening. So I wanted to report to you the results of that testing.

So, again, our manufacturing pools contain 11,500 donations or 3,250 liters. The time period I am reporting is from May of this year through October with results of 157 pools. We did get one positive pool and, actually, that is the pool that Rich Smith of NGI showed that I will show again.

The frequency, then, of this one positive in the 157 manufacturing pools, if you calculate it out for the volumes I told you, is a frequency of one positive sample in 1.8 million donations. If you calculate what the titer would be in the positive unit, it would be greater than or equal to 1 times 10^5 copies per ml to be detected in this system.

[Slide.]

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This is the autoradiogram that Rich showed earlier with the two primer pairs each run in duplicate. Here are positive controls. This Well 1 also contains positive controls. Negatives are on the end of the first row in all systems and you can see that they are all negative. The positives, with, I think, the exception of this one, were all positive and the test sample, run in quadruplicate--here is one positive result, a second weaker positive result, a negative result and, certainly, a very strong positive result. So this manufacturing pool was destroyed.

[Slide.]

In conclusion, or in summary, blood collectors considering implementation of B19 screening will have to evaluate NAT methods that are relatively insensitive to prevent issues from contamination and detection of low-level NAT positives. So what we reported was a frequency of greater than 1 in 10,000 using the insensitive method with 1 in 25,600 being high titer acute viremic IgG-negative donations.

If we tested by the sensitive method, we had an unacceptable frequency of 1 in 528 with various concentrations of IgG- and IgM-positive samples.

[Slide.]

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High-titer screening methods may not capture all infectious B19-positive units. However, infectivity of antibody-reactive low-titer positives is unknown. This study defines expected yields of parvovirus B19 if sensitive and insensitive NAT methods are used. This study also demonstrates the infrequent occurrence of HAV in recovered plasma ranging from one in 476,000 to one in 1.8 million donations.

[Slide.]

I want to also discuss with you what we are planning to do or what we are having discussions within Red Cross for how we will continue. Since we are doing HAV currently in manufacturing pools, it only makes sense, especially we had one positive manufacturing pool, to do prescreening before we do that.

So, what we will do is we will send samples--that is, pools--of 16 after the completion of testing for HIV and HCV to NGI. NGI will pool to pools of 128, do testing using an UltraQual 2 ml RT PCR test that we have qualified for this purpose. If they are negative, plasma will go for further manufacturing. If they are positive, we will resolve to pools of 16 and discard all in-date frozen products consistent with an in-process manufacturing model.

The qualified sensitivity of this method is 32 copies per ml per donation at 95 percent confidence. So

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we plan on implementing this sometime next year, probably in June.

[Slide.]

But long term, our goals would be to bring a test in-house under our control that we would be able to do real-time testing on in which a pool size would be determined. Reactives would be resolved to the individual donation. No product would be released unless HAV and B19 tests NAT-negative.

B19 sensitivity, we would set initially a sensitivity level per donation at about greater than or equal to 10^5 copies per ml and it would be for plasma only; that is, we would make no claims for labile products. However, labile products would never be distributed because we wouldn't release any products until we had NAT-negative results.

Over time, we need to determine the needs for recipients of labile products. This was one of the conclusions from the NIH workshop that was held on parvovirus so that work still needs to be done. In a program such as this, donor notification, management of products from NAT-reactive donors, previous donations and recipient tracing would need to be determined and the time line for such a test would be dependent on the regulatory policy and availability of a test kit.

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Thank you.

DR. KAPLAN: Any questions? I have one. It looks like ARC is planning to do donor screening for HAV and parvo B19. We heard from several manufacturers of kits that the intended use of the kits will be in-process. Can you comment on this?

DR. STRAMER: Obviously, the manufacturers said they want to meet the needs of their customers. So, if customers--now, when I described screening, I mean, it may be FDA interpretation that what I described is screening. But, as far as the intended use, the intended use is for plasma only because we also have platelets and red cells. We have to deal with those as well.

We don't believe it would be a good practice to distribute red cells and platelets when we know the corresponding plasma would have been screened out as positive. So we would still define this as a plasma test only. But we have the consequences of how to manage red cells and platelets. So I still think it needs to be determined what the regulatory decision is about this type of test.

DR. KAPLAN: Any other questions? Thank you.

[Whereupon, at 5:30 p.m., the meeting was recessed to be resumed at 8:30 a.m., Wednesday, December 5, 2001.]