at

ATDEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

APPLICATION OF NUCLEIC ACID TESTING

TO BLOOD BORNE PATHOGENS AND EMERGING TECHNOLOGIES

OBRR/CBER/FDA WORKSHOP

Wednesday, December 5, 2001

8:30 a.m.

Lister Hill Auditorium
National Institutes of Health

MILLER REPORTING COMPANY, INC.
735 8th Street, S.E.
Washington, D.C. 20003-2802
(202) 546-6666

at

8600 Rockville Pike
Bethesda, Maryland

MILLER REPORTING COMPANY, INC.
735 8th Street, S.E.
Washington, D.C. 20003-2802
(202) 546-6666

at

C O N T E N T S

at

at

C O N T E N T S (Continued)

PAGE

at

P R O C E E D I N G S

DR. MIED: Good morning. Thank you for your attendance this morning and especially your prompt attendance in being ready to go promptly and on time. I have been asked to make a couple of announcements; first of all, that the GGP document, guidance for industry, from the FDA has been posted to the FDA website. This was yesterday. The title of the guidance is The Use of Nucleic Acid Tests on Pooled Samples from Source Plasma Donors to Adequately and Appropriately Reduce the Risk of Transmission of HIV-1 and HCV.

I have a copy of this here that I will let you look at but it has been posted on the FDA website which is www.fda.gov/cber/whatsnew.htm.

We have a full agenda today of scintillating talks. I hope they are at least as interesting as those yesterday which was a wonderful session.

VI. Potential Replacement of Tests by NAT

(Biswas/Mied)

DR. MIED: Without any further delay, let's get into this morning's session. We are going to talk about potential replacement of serologic tests by NAT. In particular, we are going to talk about p24 antigen. Sue Stramer from the Red Cross will discuss the data that they have collected on p24 antigen.

at

As you know, FDA previously outlined criteria that a manufacturer would need to meet and data they would need to supply to justify the replacement of p24 antigen with a NAT test.

After that, we will talk about the possibility of discontinuing anticore screening. We will hear about HCV core antigen. We heard a little bit about that yesterday. We will hear a good bit more today in Dr. Lee's talk. Then Mike Busch will conclude the session with some information on supplemental testing algorithms, although there is another Sue Stramer talk in there about where surface antigen stands relative to HBV NAT in terms of sensitivity.

So, to tell us about the replacement of p24 antigen screening with HIV-1 NAT, here is the Executive Scientific Officer of the American Red Cross.

VI. Potential Replacement of Tests by NAT (Biswas/Mied)

Replacement of p24 antigen screening with HIV-1 NAT-S

DR. STRAMER: Thanks, Paul. Good morning.

[Slide.]

As Paul just described, I will be talking about studies with Red Cross and others to eliminate p24 antigen and replace it with NAT performed on pools of samples. This study has been done in collaboration with GenProbe,

at

Chiron and National Genetics Institute. And, as always, I want to thank my collaborators.

[Slide.]

Since the implementation of p24 antigen screening in March of 1996, there have been six antigen-only window-case donations that have been identified at the American Red Cross. As reported by ABC yesterday, there have been additional cases by the ABC of which three are real window-case donations and one was a false positive. So we put that together and we are talking about the grand total of 15 since the implementation of the test for a frequency, at least at the Red Cross, of 1 in 6 million donations.

[Slide.]

Since the implementation of HIV NAT screening in spring to summer of 1999, there have been eight NAT-positive window-case donations that have been identified, two of which were also p24 antigen positive. So that already should give you a flavor of the relative sensitivities of the two cases. Of the eight we had, only two were detected by p24 antigen. So that gives us a yield of 1 in 3.3 million for NAT positive and p24 antigen yield cases, but that reduces, if you back up those two cases, to 1 in 4.4 million NAT-only yield, as I presented yesterday.

at

Due to a low antigen yield and the improved sensitivity of NAT screening of HIV, replacement of antigen with HIV NAT should be possible.

[Slide.]

On September 18, FDA licensed NGI's HIV and HCV UltraQual PCR tests as donor screens for use in pools of up to 512 donations. Of interest is that the FDA also approved the use of this test, the HIV RT PCR, with approved pooling algorithms as an alternate to HIV p24 antigen tests for screening of source plasma.

On October 4 of this year, there was a CBER submission of a BLA amendment to Chiron's Procleix, as I said, the GenProbe assay, to eliminate p24 antigen upon licensure of NAT.

[Slide.]

As Dr. Mied said, there have been criteria outlined by the FDA to eliminate p24 antigen so the steps are there. All we have to do is plug in data for the steps and hope that the data support the test's elimination. So the criteria include the fact that NAT must show greater than or equal sensitivity to p24 antigen in the window period as shown by the following studies; repository p24 antigen positive, antibody negative, window period donations since the implementation of p24 antigen must be NAT reactive.

at

In seroconversion panels, NAT must detect p24 antigen with sufficient sensitivity to offset any dilutional effects. Prospective clinical-trial data must show HIV RNA is detected at comparable or greater frequency than p24 antigen in antibody-positive and antibody-negative individuals. NAT must be capable of detecting all HIV subtypes. And NAT must be capable of reproducibly detecting samples that are weakly p24 antigen reactive.

I am going to show you data that fulfills each of these criteria.

[Slide.]

But, prior to that, I just want to show you some of the NGI data since they already have a license for HIV-1 NAT with the elimination of p24 antigen. Some of the NGI data include the collection of 347 potential HIV window-period plasma samples that they collected from their repository from various HIV screening methods.

If you look at the breakdown of these 347 samples, they break down into the following groups here as outlined by the presence of various serological markers and nucleic acid. So, if you look at the undiluted sample by PCR, a 1 to 512 diluation by PCR, p24 antigen and then antibody, you can basically see which categories are positive relative to numbers.

at

So, for the UltraQual, we have the most samples reactive at the undiluted state, 73 percent. If you run the dilution, we lose some samples, going down to 60.5 detection. p24 antigen is least best, then, of these three at 40 percent detection. So you can basically how the window of what is detected reduces and then p24 antigen HIV-1-2 antibody picks up some of the p24 antigen and then moves out forward into full seroconversion as antigen and virus is replace, or eliminated, neutralized.

[Slide.]

Looking at a subset of these samples, of the 347 that had at least one reactive marker, and looking at a 1 to 512 dilution versus the p24 antigen, either Coulter or Abbott--whatever was reactive is included in this table; both assays were run--we have 140 samples that were reactive by antigen but 210 samples that were reactive at PCR at the dilution.

140 samples were concordant. There were no samples that were PCR negative and antigen positive, but there were 70 samples that were detected by PCR that were not detected by p24 antigen.

[Slide.]

To go into some Red Cross data to give you background on the performance of the p24 antigen test since implementation, this shows you our repeat-reactive samples

at

divided by the results of confirmatory--that is, neutralization--testing.

If you look at the red bars here, the assay of the Coulter test that we are using has a cutoff of 40 percent, equal to or greater than 40 percent being the criteria for positive on the neutralization test. If you look at these 180 samples, you can see that there are few that are antibody positive that confirmed at low levels neutralization. But the vast majority run between 90 and 100 percent or over 100 percent neutralization.

So these are strongly positive samples. But then we had a series here of 197, or greater than 50 percent of our samples, that were not antibody positive. These are not yield samples but were false-positive samples. You can see that the vast majority have low levels of neutralization, around 40 percent. They have low signal-to-cutoff ratios in the assay. They do not repeat if you perform the neutralization test again. They are RNA negative for HIV. They are also RT negative by a total assay for reverse transcriptase in a subset that we have looked at.

So, by any assay that we have looked at, and in follow up of most of these individuals who have come back and have not been reactive for HIV, we know these are false positives.

at

[Slide.]

If you look at the repeat-reactive rate of the assay or the consistency of performance of assay based on how many samples are submitted for confirmatory testing, with an FDA-licensed test, you would expect consistency lot-to-lot over time.

The green line here gives you the total of samples submitted for neutralization. The yellow line, or the number that are nonneutralized or indeterminate according to the criteria, you can see this line is anything but straight. There were some production problems here which began to shortly resolve, didn't resolve completely.

Then we had the aftermath of September 11 where donations really increased so this number went up as far as total in indeterminate. And then we switched vendors in the middle of October and our repeat-reactive rates now have been really, really low. But the point of the matter is that this test has not performed consistently.

[Slide.]

Looking at our seroconversion cases that we detected with p24 antigen, there are five here. They are not very clear. But the point here is in gold. You can see all of their quantitative viral RNA loads. The second column is the p24 antigen signal-to-cutoff ratios. In each

at

case, RNA was positive at the index and in all follow-up samples where p24 antigen was not.

The peak p24 antigen sample corresponded with the peak viral RNA that was detected showing basically that there is a 1-to-1 relationship of p24 antigen and viral RNA early in the ramp-up phase of viral replication, so one would expect that the highest viral loads would occur during peak antigenemia.

[Slide.]

This shows the same type of profiles for our first two NAT-reactive donations identified in pools. This sixth sample here was the sixth p24 antigen identified. It was also the second NAT positive that we had. But the point of the matter is still the same; RNA is positive at the index and all follow-up samples whereas p24 antigen is positive for only a short time and the peaks of the two assays correspond.

This is the third case. Same point.

[Slide.]

We also took some of our antigen samples, as required, and diluted them. At that point, we are using pool sizes of 128 in our NAT program. So we diluted our antigen yield samples to a diluation of 128 and assayed them to see if they were reactive. And they were. Here are their viral loads.

at

We also took the first HIV NAT case identified in the United States in a pool of 24, also diluted it to a pool of 128. Again, that was reactive. We took our first two cases that were identified, both in pools of 16, but just to be consistent and to see how far we could take this, we also diluted them 1 to 128 and, again, they were reactive.

Interestingly enough, those that were antigen positive, consistent with what I said, at the highest signal-to-cutoff ratio, the TMA test, and those that were NAT only, which represent earlier phases in time, have a little bit lower S-to-COs but certainly had no problem being detected.

[Slide.]

Looking at profiles specifically, I will just show you one example. This is the development in a plasmapheresis donor of HIV-1-2 antibody, p24 antigen at a window period reduction of five days, quantitative PCR performed by NGI with an improvement in detection of two days. If we add the TMA assay in dilutions, which are the open triangles, it gives us the same first day of detection as p24 antigen. Running a neat sample, we get an additional five days in this particular panel.

at

But the point here, whether it is diluted, neat, GenProbe, NGI's Quant method, all are more sensitive than p24 antigen. And this is a reproducible finding.

[Slide.]

Such that when you put all of these panels together which we, in our study, included 25 such individuals, or 92 samples that were antibody negative, if you look at the agreement between p24 antigen and the GenProbe assay, you can basically see that there are 29 samples that were detected by NAT that were not detected by p24 antigen. There were no samples that were p24 antigen reactive that were not detected by NAT.

[Slide.]

If you take this to the diluation of 128, instead of 29, we now have 21. But the point is still the same. It doesn't matter how many samples they are, but all of the samples are in this category, that are NAT positive even in pools of 128, p24 antigen negative.

[Slide.]

If you put all the data together, even combining the antibody positives in this study, we looked at two different lots of the GenProbe test. This was submitted in our IND just to make sure we saw reproducibility. This is the number of samples that were detected by the TMA assay

at

undilute; 89 to 90 percent detected still at a diluation of 128, but only 50 to 60 percent detected by p24 antigen.

Interestingly enough, we can see similar relationships even though this talk isn't about HCV relative to another test that should be eliminated which is ALT.

[Slide.]

In looking at a compilation of seroconversion panels by Mike Busch, just to look at how much RNA is at the p24 antigen assay cutoff, this is a regression analysis of 146 samples and the cutoff here at the p24 antigen corresponds to about 10,000 viral copies per ml with this range, 596 to about 200,000.

[Slide.]

Now switching to antibody-positive populations, over this period of time, when we integrated NAT with our serology testing, we looked at how many p24 antigen samples neutralized and then how many were NAT positive. So we had 31 out of 34 repeat reactives that neutralized by the antigen-neutralization assay of 580 donations submitted for p24 confirmation. 321 were from allogeneic donors.

But of the 31, eleven, because we had some autologous and non-Red-Cross donors in here, we didn't have NAT results for. But the ones that we did, which were 20,

at

all 20 were NAT-reactive by the MultiPlex test and by the discriminatory HIV test.

What I find very interesting about the study, of these 34, there were three that did not neutralize or that were indeterminate by the neutralization protocol and three of those were NAT reactive. So we could confirm the antigen reactivity by NAT by not by the corresponding neutralization assay.

[Slide.]

If you merge, over the same period of time, all the NAT reactives with all the p24 antigen reactives, we have 20 samples that were positive by both assays. There were no samples that were p24 antigen positive, NAT negative. There were three samples that I already discussed that were antigen repeat reactive and NAT positive but couldn't be confirmed by the antigen neutralization test. But there were an additional 281 samples that were p24 antigen negative and NAT positive.

So there were 284 in total, or 88.5 percent that were p24 antigen negative, NAT positive.

[Slide.]

Looking at the GenProbe clinical-trial data to get some prospective data in here, it included about 200,000 donations screened. There were 54 antigen repeat-reactive samples. Looking at the results of the

at

confirmatory neutralization testing, there was one QNS sample, 48 samples that could not be neutralized, were NAT negative and antibody negative.

There were two, as I showed you in our study of all of our donation testing since March of '96, lots of false positives. Here, this study, as one would expect, did find some false-positive neutralizations, too. They were NAT negative by the GenProbe test, antibody negative.

One donor we didn't agree to follow up but when the index sample was tested by a supplemental NAT, it was negative. One donor did come in for follow up and was NAT negative. They were also negative for p24 antigen and for HIV antibody. So, again, these are false-positive neutralization results.

If we look at the study for the thirteen NAT reactive donors that were identified, there were an additional three samples here that were both antigen repeat-reactive and NAT reactive. But then there were ten samples that were antigen negative and antibody positive. So we have a total of thirteen HIV-infected individuals who were identified, all identified by NAT but only three of thirteen were p24 antigen confirmed positive.

[Slide.]

Looking at subtype detection, the studies that we put into the BLA amendment included dilutions of various

at

subtypes of HIV. I only show you the lowest copy level that was tested. All replicates of all samples tested at 1,000, 300 and 100 were detected as reactive.

I just show you 30, which is below their label claim for sensitivity. The vast majority of samples are detected. One or two wrapped and most are not detected in one to three of the subtypes, but the vast majority are reactive at the lowest dilution.

[Slide.]

Also looking at samples of various subtypes, these are the viral loads of those samples testing them neat and then performing a 1-to-16 dilution, as we do for pooling, you can again see 100 percent detection of all subtypes assayed.

[Slide.]

Looking at assay reproducibility of weekly antigen repeat-reactive samples, what we do in the Red Cross is for every NAT run, there are four external run controls and the external run controls have to meet the required specifications or the run is not considered valid. These are the four samples, but one of them, we do include as a weak p24 antigen control.

We selected a sample, or series of samples, by the Coulter assay that ran a cutoff on the antigen assay that is an S-to-Co of 1-to-2, diluted that-1 to-16 and we

at

run that sample on every single assay. As far as viral copies, because now we have gone through three lots of such a reagent, it ranges from 2400 to 6800 copies per ml.

Looking at the performance of these run controls when we did our pools of 128, here you have the negative, here you have the HCV-reactive control. Here you have the HIV-1-reactive control. And here you have the p24 antigen control that you can see runs very comparably to the HIV control but actually runs a little bit higher.

[Slide.]

This is only about 400 datapoints, so we have now extended it and we have compiled all the data that we have to the end of October which now includes over 17,000 runs. You can see the same patterns. We have never had a failure in all of these runs with a p24 antigen control not being reactive. So this has performed very, very well and we are confident in every run that the assay is more sensitive than p24 antigen by virtue of including this control.

[Slide.]

So, in summary, in all categories of samples tested, HIV NAT was more sensitive than p24 antigen and meets the FDA criteria for elimination. In antibody-negative populations from the NGI study, p24 antigen missed 27 percent of the samples, 70 of 258, relative to pooled NAT and that was in pools of 512.

at

In the Red Cross study, p24 antigen missed 23 percent of samples, 21 of 92, relative to pooled NAT. So these numbers are pretty comparable. In antibody-positive populations in the Red Cross study, p24 antigen missed 88 percent of the samples, 284 of 321, relative to pooled NAT in pools of 16. In the GenProbe study, p24 antigen missed 77 percent of samples, 10 of 13, relative to pooled NAT, again very comparable numbers in antibody-positive and antibody-negative populations.

So we believe, with the use of an approved pooling algorithm, p24 antigen can be replaced by NAT. One question that I have is will the NAT assay require FDA licensure prior to elimination of this redundant test.

Thank you very much.

DR. MIED: Thank you, Sue. The answer to your question is yes.

DR. STRAMER: Instead of you asking me a question, I asked you a question.

DR. MIED: Sue, in terms of specificity, I know you focused mostly on sensitivity, what would you say is the average repeatedly reactive that you see for antigen testing?

DR. STRAMER: It varies by manufacturer. With the Coulter test prior to that mountainous range that I showed you, we were running consistently, actually, at

at

about a repeat-reactive rate of 0.03 percent. With the Abbott test, we are running less than 0.01 percent. So we have improved specificity. So, with NAT, at 0.01, let's say, that is 1 in 10,000 repeat reactive.

With NAT, as I presented yesterday, we see 1 in 25,000 as being false positive.

DR. MIED: Questions for Sue? Mike?

DR. BUSCH: Sue, and maybe Paul, with approval of this recommendation, and we delete prospectively antigen, all of these donors who have been historically deferred, either the repeat-reactive neutralization-negative, so-called indeterminates, but also these false-positive neutralization cases that I think we have worked up and have shown are RNA negative, is it your expectation that we will just be able to blanket reverse their deferral and mail them letters that they can donate, or do you think these people will all have to go through a formal redraw, reinstatement, algorithm?

DR. MIED: I think that is something we will have to address, Mike. We really haven't looked at that direction yet. But it is certainly a concern that donors who should be reentered can be in the future. So it is something we will look at.

DR. ALLAIN: I was interested in your nonneutralizable p24 antigen. What was the viral load, the

at

subtype and did you have any idea about the sequence of this p24 antigen that looks like potential variance?

DR. STRAMER: No. Actually, I still have the repository samples. We haven't done anything with them. I can't even tell you the percent neutralization offhand. But, I mean, all of those data are available. I just don't have them here.

DR. ALLAIN: The viral load; do you have that?

DR. STRAMER: I don't have the viral load, no, because routinely we only do qualitative testing. So I have a qualitative result. I would have to submit those samples for viral-load testing but it would be an interesting--

DR. BIANCO: Sue, in the early presentations that Paul and FDA made of the potential for elimination of p24 antigen, they restricted it to source plasma. Do you think that, with this beautiful data, we will have an opportunity to do it also for whole-blood donors?

DR. STRAMER: You are asking if we will be able to eliminate the test for whole-blood donors?

DR. BIANCO: Yes. That is your intent.

DR. STRAMER: Right.

DR. BIANCO: I am directly, or indirectly, asking Paul.

at

DR. STRAMER: Right; okay. That is what I presented; right?

DR. MIED: Yes. You were presenting whole blood.

DR. STRAMER: The intention is that for the whole-blood industry, we have collected enough suitable data so the test should be able to be eliminated. We have submitted the data for FDA consideration so it is up to FDA review to see if the data are acceptable.

DR. EPSTEIN: I think I can clarify what you are getting at Celso. The way the FDA looks at it, we look at an approved NAT test and if that test qualifies to eliminate p24 antigen, then we approve that, if you are using that test, you can eliminate p24. So the conundrum here is that the test that we approved happens to be labeled only for source plasma because that is what it was developed for.

That is why the approval to eliminate p24 is only for the use of that test in that setting. But our expectation is that, as we license other tests in a broader use for whole blood, we will be able to do the same. That is, I think, the point of Sue's presentation is that it looks like we will be able to.

DR. MIED: Mike, to address your point on reentry of donors, the discussion yesterday, the reentry algorithm that we are considering for reentry of NAT-deferred donors

at

also includes a group that were antigen repeatedly reactive and were indeterminate on the antigen test, whether invalid or nonneutralized.

So I think we will have a mechanism in the future for getting those donors back in.

DR. STRAMER: The way you presented it would be that they would have a follow-up sample.

DR. MIED: That's correct.

DR. STRAMER: And they would test negative by all FDA-licensed tests and then be eligible. So Mike's question was could we just have a blanket reversal of the deferral so that if we follow the algorithms that were presented yesterday, then the answer would be no, assuming that is the way it falls out.

DR. MIED: Right. And that was my initial response to Mike. I think that is something we have to look at.

So, Sue, the bottom line of what you are telling us is that you see no concern for the NAT systems you have looked at, at least, replacement for p24 antigen with NAT?

DR. STRAMER: Yes; I think the studies have been, if I say so myself, pretty thorough both from the standpoint of looking at all of NGI's data, looking at the data from prospective screening using p24 antigen and from prospective screening using NAT under IND. So I,

at

personally, see no concerns and think the test should be eliminated.

DR. MIED: Thank you, Sue.

DR. STRAMER: Thank you.

DR. BISWAS: The next three talks will be on hepatitis. The next talk will be given by Dr. Steven Kleinman from the University of British Columbia. He will be talking about the prospects for discontinuing anti-HBC screening in the post-NAT era.

Prospects for Discontinuing anti-HBC Screening in the Post-NAT Era

DR. KLEINMAN: Thanks, Robin. And thanks to the organizers for inviting me.

[Slide.]

What I would like to do today is address a somewhat confusing area, I think, and that is HBV with its serological testing and potentially with NAT testing. The question that I am posing is whether HBV NAT screening of donated blood with implementation, if it occurs, and we don't know if that will occur yet, will that allow for dropping anticore testing or speculating at the end HbSAg testing.

[Slide.]

The recent developments in blood-donor screening with regard to HBV, and we heard yesterday that minipool

at

NAT is being done in the source-plasma sector, with regard to whole blood, HBV NAT in minipools or on individual donors is being considered for routine blood-donor screening, actually minipools, probably, to begin with.

At the same time, you will hear, in the next talk, that HBsAg assays with improved sensitivity are also under review by FDA so we should have more sensitive surface-antigen tests. The question is, based on these developments, we get to the possibility of discontinuing anticore testing if we can show that very few, or no, potentially infectious units would be missed by dropping this test.

[Slide.]

So I would like to start with discussing the contribution of anticore testing to blood safety vis-a-vis hepatitis B. I want to show you some background information and then report on two recent studies.

[Slide.]

What we have known for a long that surface-antigen-negative core-positive units have been implicated or, more recently, proven to be the source of transfusion-transmitted hepatitis B. A number of case reports from the late 1970s showed this. The transfusion-transmitted virus study which was conducted during that time published a paper in 1995 with six probable cases. These cases are all

at

probable because we have a patient with post-transfusion hepatitis B and at least one of the units has anticore, but the link between the two has not actually been proven that that unit was causing the HBV.

Then J.P. Allain, who is in the audience, published a paper recently about two probable transmissions from anticore-positive units in the U.K. Recently a paper came out from Japan in Transfusion in which they had investigated two cases of post-transfusion hepatitis B and showed that there were two donors, one in each case, who were surface-antigen-negative, core-positive, and could actually be shown to have hepatitis-B DNA, one at low copy number, 400 copies per ml, the other, they were not able to quantify.

I think these two cases actually better establish infectivity but the background cases, I think, show us that the phenomenon has been occurring for last twenty years.

[Slide.]

We can look to liver transplantation. I am sure everybody is familiar with this--and know that just because you are anticore-positive doesn't mean that you have cleared the hepatitis-B infection. There are multiple studies in liver transplantation that have documented high rates of hepatitis B transmission from anticore-positive liver donors to anticore-negative recipients.

at

[Slide.]

So it is hard to look at infectivity in blood-donor populations unless you have a post-transfusion ongoing study. But one can look at HBV DNA positivity rates in various populations. There are numerous papers in the literature on this. The problem is that they don't show consistent results.

You have to break the papers down into two categories. One is to look at the data in low HBV endemicity areas such as the U.S. Here you get rates of somewhere between 0 to a high of 1 percent of persons who are core-positive surface-antigen-negative having HBV DNA. A study done in Kansas City actually tried to calculate the frequency of this and came out with 1 in 46,000 positive transfusible components; that is, these units were negative for all other markers, positive for anticore and had HBV DNA.

But if you look at donors in other countries or in patient groups, you can find that HBV DNA is present in high percentages of anticore-positive donors, from 2 to 6 percent. In donors and in certain patient groups, they can have an HBsAg silent infection in 10 to 30 percent.

[Slide.]

So, with that background, I want to report on a study that the REDS group, in conjunction with Abbott,

at

recently concluded and presented at this year's AABB annual meeting. In this study, we used a repository that we had in REDS. REDS collects samples from five U.S. blood centers. We froze about 15 percent of the donations from the years 1991 to 1995.

From that repository, we selected samples that were originally positive by the Abbott Corzyme test, negative for surface antigen on routine EIA screening and negative for all other viral markers.

[Slide.]

Within this larger population, we imposed a few other criteria as they take the samples on to PCR testing. One is that, because of the known nonspecificity of the particular anticore test that we were using, we needed a second anti-HB-core test to make sure the patient really was core-positive. We used the PRISM HB core test or the Chemiluminescent assay which is not yet licensed in the U.S. but under FDA review.

[Slide.]

We also looked for the presence of antibody to HBs or HBs antibody and quantitated that, again, by using a PRISM assay. We arbitrarily took at cutoff value of 100 International Units per liter and we basically said that, in order to go on to PCR, the units needed to be anticore-positive by both assays, the screening assay and the PRISM

at

assay, and to either have absent anti-HBs or HBs below 100 International Units. The reason for that is there are very few, if any, reports of PCR-positives in persons who have anti-HBs at titers of greater than 100 International Units.

[Slide.]

So, for our PCR testing, we first carefully aliquoted the repository samples. May Kuhns' lab at Abbott did the research PCR assay using a primer and probe sequence in the conserved core region. Sensitivity of 95 percent at 50 copies per ml. We did replicate sample preparations and detections and any sample that showed an initial positive PCR result, we aliquoted two additional independent aliquots, tested one at Abbott and the second one at NGI.

[Slide.]

We started with about 5 million donations in the repository. Over 40,000 were Corzyme reactive and met our criteria. Of these, we have 50,121 that were available for further evaluation. When we evaluated these 50,121, if you can concentrate on this line here, you will see there were 387 that were core-positive by the PRISM assay and lack anti-HBs and there were another 2,963 that were core-positive that had anti-HBs. I will break that down in the next slide.

at

You can see that our criteria for low anti-HBs, there were 844 samples, 387 with absent samples. So we started with 1,031 samples that were eligible for PCR testing.

[Slide.]

Unfortunately, we could not test all of these. We had some issues about whether the donors had been deferred or were still eligible to donate based on different algorithms at the Blood Center. Since we didn't want to get into a problem with recall of units, if we did find positives, we confined the testing to the indefinitely deferred donors.

We had 498 of these. They were more at one particular site so we adjusted to get comparability. Eventually, we tested 395 samples in the DNA PCR assay.

[Slide.]

The results are here, broken down by the groups. You can see that we had four positives. We tested 107 that lacked anti-HBs. All four positives came from this group. All the donors who had anti-HBs at low levels were HBV-DNA-negative. So, of the 395 selected samples, four, or 1 percent, were positive.

[Slide.]

Here are the four samples. We tried to estimate their DNA copy number. This was done by the number of

at

replicate reactions that were positive at NGI and the relative intensity of the band. So, at best, it is semiquantitative. But you can see two of these we thought were very low titers, 10 to 30 copies per ml, two of them a little bit higher, 50 to 100 copies per ml. Only one of these had anti-HBe. They all lacked anti-HBs.

When tested on a more sensitive surface-antigen assay, the PRISM assay, only one of these four samples was positive.

[Slide.]

This is a little complicated, but we could back-calculate based on our selection algorithm if we went back to all units that were donated, how many would actually be positive. So we had a rate of 1 percent, but, again, we had to project that on all 50,121 tested samples. So that 1 percent rate, we felt, would hold for the 1,031 and then we assumed that the rest of the samples with the high anti-HBs or the nonreproducible core would be HBV-negative.

Then we had our overall close to 1 percent core--positive rate. The bottom line is we came up with a number that, if you took a unit off the shelf that was anticore-positive and otherwise transfusable that there would be a 1 in almost 50,000 risk that that unit had HBV DNA.

Then the inference is that those units would be infectious. We don't know that for sure but I think any

at

unit with HBV DNA we would want to regard an infectious. So that is sort of the benefit of anticore testing in the current--at least in the U.S. environment from 1991 through 1995 where these samples came from.

[Slide.]

So, again, some caveats here. I think I have gone through this. Certain assumptions. Obviously, our yield could be limited by the sensitivity of our assay. Although it was sensitive down to 50 copies, we might need even a more sensitive assay. We couldn't drive this assay because we had very limited sample volume based on repository aliquots.

Again, these were from repository samples. We were not successful in bringing the donors in so we always have the problem of not having confirmed these.

[Slide.]

However, our conclusions from this study were all four units lacked anti-HBs thus increasing the probability that it might be infectious. The use of the enhanced sensitivity surface-antigen assay only detected one out of four of the units and, actually, our rate of 1 in 50,000 indicated that, had we not been doing test, transmission of HBV from this source would be higher, probably, than what has been estimated for window-period units.

[Slide.]

at

So that is the first study. I am going to present a second study with data supplied by who else, Dr. Susan Stramer, who seems to drive a lot of the data in this field, but an American Red Cross study along with NGI. Let me go through that now.

[Slide.]

This study had, as its primary objectives, to determine the rate of HBV DNA in, again, core-positive units. But these are units for which the plasma would be used for further manufacture. In addition to determining the rate, the issue was to quantify the HBV DNA levels so that the Red Cross could operationally select an appropriate pool size for performing minipool NAT on all anticore-positive donations as a way of improving the viral safety margin in their submitted plasma pools.

Obviously, their pool size ultimately would be dependent on the assay analytic sensitivity, the input volume and the pool size that they would take.

I am not going to get into the Red Cross conclusions operationally because I don't know what they were, actually, but what I do want to show you is how the data is relevant to the issue that we are discussing today.

[Slide.]

What they did was they took samples from all five of their NAT labs, approximately 3,000 samples. These were

at

anonymized so that donors could not be traced. These were done from contemporary samples this year. They were negative on all other screening assays.

The sample source was a frozen residual EDA plasma from the PPT tubes that had been used for HIV and HCV, so these were optimally collected samples. Then they were assayed at NGI for HBV DNA using their assays that I think they use for source plasma, or similar assays.

They used four different primer pairs, assay in duplicate. And they call a sample positive if DNA is detected on any one of the eight reactions, as you saw yesterday. Each unit had a 0.5 ml input volume. At this input volume, their sensitivity for their assay was 36 copies per ml.

Any positive samples were then quantified by their quantitative assay which has a sensitivity of 100 copies per ml.

[Slide.]

What you can see here is, in the 3,000 samples, 19, or 0.63 percent, of these units were demonstrated to have HBV DNA. Eight of them were quantifiable. All the quantifiable units had relatively low levels. Two had 100 copies per ml. Three had 200 copies per ml. Three had 500 copies per ml. And eleven were not quantifiable. We assume that means that they were below 100 copies per ml

at

but were, in fact, positive because they were picked up on the qualitative assay.

[Slide.]

So, putting this data together with the REDS study, I will summarize the studies on this same slide. You can see here the REDS study samples from 1991 through 1995, the Red Cross in the Year 2000. REDS ultimately tested 395 samples by PCR through the selection algorithm. The Red Cross tested all comers and that was 3,000 samples. We had four DNA-positives. They had 19 DAN positives. You can calculate the rate. We had to calculate the rate in REDS by the algorithm I showed you. Obviously, the rate in the Red Cross study is direct.

So our rate for core-positive surface antigen-negative unit was 0.24 percent. The Red Cross rate was 0.63 percent. Calculating back to how frequently this would be in a unit that was otherwise acceptable for transfusion, I showed you the REDS rate was 1 in 49,000. The Red Cross rate, when you calculate back, was 1 in 37,000, very comparable numbers. Copies per ml of HBV DNA were low. All of the REDS samples were equal to or below 100 and, in the Red Cross study, two-thirds of the samples were equal to or below 100, very consistent data across two studies with independent study designs and different laboratories performing the tests.

at

[Slide.]

So I think we can draw some pretty strong conclusions from this. Number one, anticore-positive, NAT-positive, units have low DNA copy numbers. This is supported by data I think we can hear people in the audience from other countries have found similar things. When they are positive, they generally have very low copy numbers such that minipool testing, under current formats, would not be expected to detect them in the majority of cases, or almost all cases.

Therefore, if we were contemplating dropping anticore testing, this would be very unlikely and I would say not acceptable under the context of minipool HBV NAT testing. Otherwise, we would impose a risk of about 1 in 50,000 potentially infectious components for HBV reentering the blood supply.

[Slide.]

Maybe if we move to individual donation HBV NAT, we would be able to detect these units. But it would need to be a highly sensitive individual donation NAT. Given the performance of these tests at low copy number and Poisson distribution considerations, it is still possible that some units could be detected some of the time, maybe at a 50 percent detection level, but that means they could be missed some of the time as well.

at

We wouldn't know that until we do more studies. So I think the conclusion here is discontinuation of anticore testing might be possible but only if a highly sensitive individual donation NAT is developed. This may require, to get the kind of sensitivity we want, fairly large sample volume inputs. Of course, this can be a limitation in blood-donor screening. So I don't think that the conclusions are in on this yet.

[Slide.]

How do we explain these units? Why do we get anticore-positive units that are DNA-positive? Nobody knows for sure but the most likely explanation is that these represent people who have chronic HBV infection in which surface-antigen levels may have been higher previously but they have declined to subdetectable levels.

Alternatively, these could be people who are acutely infected and never really develop enough surface antigen to be detected. So, at least in these units, and this is only a subset of units--but, in these units, HBV DNA is present where there is no HBsAg and so at least we should look at the question. Maybe HBV DNA will be more sensitive than HBsAg and maybe if we can't drop anticore testing, could we drop HBsAg testing.

I will come back to that in the next few slides.

at

Finally, a second potential explanation for these kinds of phenomena are that we actually have mutations in antigens detected by surface antigen and we are not able to detect it in the assay systems that we use. Clearly, mutants exist and they have been reported in other parts of the world. But, to my knowledge, none have been reported in the U.S. So I don't think that is the explanation for the samples in our studies.

[Slide.]

So, if then turn to surface-antigen testing and ask the question could we ever drop surface-antigen testing, where do we go with that? Number one, clearly, this has been a very robust and important test in blood-donor screening. It has been around and it has worked for 30 years so I think that anybody who wants to say we can drop this test has a large burden of proof to bear.

But, in a sort of theoretical concept, they are, in a sense, measuring part of the same phenomenon and that is direct viral detection although we know surface antigen really doesn't have to be an intact virion.

So, as background, we get to the situation in that these two assays, surface antigen and NAT, would need to be compared in at least two different situations. One would be the window-period situation. Of course, this would all be anticore-negative so you would have to rely on

at

one of these two tests to pick up these window-period donations. We will hear about this in the next presentation by Sue.

The second situation would be what about in chronic carriers. How do the two tests perform in chronic carriers? We know that most chronic carriers are also anticore-positive so you could say it doesn't really matter how the two perform because our anticore test will pick up most of our chronic carriers anyway. But I don't think that would be very reassuring. We would want to pick the chronic carriers up by whatever direct assay we used as well.

[Slide.]

So, do we have any data to bear on this? The bottom line is we really don't have a lot of data that we can use at this point. We know that biology indicates that the majority of carriers make lots of surface antigen compared to HBV DNA and that is they make lots of defective particles and that is why we can find surface antigen so readily.

If you look at older studies, and I was reminded yesterday by Dr. Allain that a lot of the older studies are not even this good. But one of the studies that we did in REDS, at least, indicated that approximately 5 percent of surface-antigen core-positive donors are HBV-DNA-negative;

at

that is, we can find DNA in 95 percent of the cases but we miss it in 5 percent. Other studies suggest we miss it in more than 5 percent. But much of this is dependent on how sensitive the PCR assay is. Clearly, these assays have gotten better over the years and the studies haven't been done with contemporary assays.

So I think they need to be repeated to see what the sensitivity of HBV NAT is in the context of current testing. Just a point here that illustrates that in a recent paper by Seto in Transfusion, tucked away in one of his tables, you have two cases that they reported from surface-antigen-positive donors who are anticore-negative who are NAT-negative and were inferred to be chronically infected, and these people were originally negative on PCR testing with small input volumes.

But, when they increased the input volumes, they were able to demonstrate DNA. So it may be that DNA is present in these surface-antigen-positive samples but you may really need to drive the sensitivity of the assay to find them.

[Slide.]

One other group that is the chronic carriers who are surface-antigen-positive but lack anticore. These seem to constitute about 2 to 5 percent of surface-antigen-positives that are detected in U.S. blood banks. We looked

at

at this subgroup in REDS in a study we reported several years ago. We were able to show actually that some of these donors were window-period cases. They weren't chronic carriers. They had very high HBV DNA levels, hugely high titers. And they were in acute infection.

However, some of these donors were HBV-DNA-negative. Unfortunately, we could not conclude in this study, because we didn't have donor follow up, whether theses surface-antigen-positive core-negative donors were infected with HBV, whether their sample had been contaminated for surface antigen because of lab procedures or whether they were false positive for surface antigen.

So I just show you this to say that that is a subset of donors that needs to be studied and there are no studies that are definitive in that subset at this point.

[Slide.]

So I think that if we want to entertain the question about surface antigen and its future, we really need to do a large contemporary study using highly sensitive HBV NAT and we need to follow up--we have to have follow-up sampling of donors to determine truth because we are trying to evaluate one assay against another and we don't know which is really the gold standard.

But we could depend on donors having reproducible results or seroconverting to anticore. We would need to do

at

this to generate data concerning the possible replacement of surface antigen by HBV NAT in the context of continued anticore testing.

Finally, this type of study, obviously, should do quantitative viral loads so you could get the information as to if we were going to drop this test, whether it would be feasible in minipool formats or it would require individual donation testing and then how sensitive that individual donation testing would have to be.

Thank you.

DR. BISWAS: Steve, thank you very much for that very interesting talk. I think that will be sort of a wonderful template for further discussions on this very important topic.

One or two questions I have. One is simply a definition. When you talk about window period, I take it you mean the period sort of pre-acute before the HBsAg comes.

DR. KLEINMAN: Right.

DR. BISWAS: The reason I ask that is that, traditionally, for us old folks in hepatitis, the window period has been when the HBsAg comes down in the acute phase before HBsAg comes. That traditionally has been the window period. So I think we need to be clear when we have

at

these discussions what we mean by window period in hepatitis B.

DR. KLEINMAN: Right. This is nouveau fashion window period, new contemporary windows. So it would mean the preseroconversion window, not that anticore tail.

DR. BISWAS: The other thing is that in the two studies that you presented, the REDS and the Red Cross study, the HBsAg testing that was negative, this was all done in licensed tests; correct?

DR. KLEINMAN: Correct.

DR. BISWAS: So they were not done in more sensitive tests under development.

DR. KLEINMAN: That's correct, except in the REDS study, we did test those four samples that were positive. Only those four samples were tested by PRISM using, I guess, whatever--I am not sure what cutoff, whether it is the one that is under evaluation by FDA or a less sensitive cutoff, but three of the four were negative. So it does imply that they might be missed.

Certainly, one consideration is whether to take those Red Cross samples and to test them by PRISM surface antigen. I guess that is a possibility. There are a number of other purposes of that study that I described from the Red Cross so I don't know whether that will be done.

at

DR. BISWAS: Lastly, when you were preparing for this talk, did you see anywhere in the literature that there have been, say, chimpanzee studies done on HBsAg-negatives anticore-positives irrespective of the NAT?

DR. KLEINMAN: There is one study that Fred Prince recently reported on where he injected--I can't remember if it was two or three chimps with units that were NAT-positive, surface-antigen-negative, anticore-positive. But they were also anti-HBs positive. So they were not necessarily the most likely to transmit units.

One of the theories is that if you have anti-HBs, you might complex any virus that could transmit. He was only able to--the bottom line is his results were he couldn't transmit--those units did not transmit to the chimps. But he used relatively small inoculation volumes into the chimps.

So I think it was interesting results but hard to know whether you could generalize from the animal model. But that would imply that the units were not infectious. But my personal feeling is, in the absence of conclusive evidence, you would have to think that a unit that has HBV DNA, given the fact that there are such high rates of transmission from needle-stick injuries--admittedly, that could be from people with very high titers.

at

When you are transfusing a blood unit or a unit of platelets with 50 ccs, you don't need very many copies per ml to get, I think, a reasonably high inoculation volume. So it may be possible that not all these units would transmit, but I think we would need good proof that they were nontransmitting units.

In the absence of proof, we would have to take the conservative attitude. Here I am preaching to the FDA to take the conservative attitude. It is kind of funny. We need to take the conservative attitude that these were potentially infectious units.

DR. BISWAS: Thanks a lot. Conservative with a small "c." Any more questions?

DR. BIANCO: Celso Bianco. Steve, at a rate of 1 in 50,000--that is 260 potential transmissions a year. Why don't we see that?

DR. KLEINMAN: We don't see those 260 because we are doing anticore testing. So the question is why didn't we see them before we did anticore testing? I think it is an important point and that is probably--if HBV is going to be transmitted in the transfusion setting, for the most part, it is going to be handled by the recipient. We know that at least most adults that contract HBV resolve the acute infection and don't go on to become chronic carriers.

at

So I don't have the explanation other than to say that post-transfusion hepatitis B could often be a subclinical infection with no chronicity. Therefore, you could ask the question how important is it to actually prevent an infection like that.

The disease burden brought on by transmitting HBV I think is likely to be very low. And that is an important consideration, I think, in any policy discussions.

DR. ALLAIN: Steven you mentioned the studies done in the higher prevalence area, and we have done one of those. We have found eight samples out of 576 individuals antiHBsAg-negative to be DNA-positive. As you showed, all of them are below 200 International Units per ml.

The second important information, and we did that in collaboration with GenProbe, is that 98 percent of HBsAg-positive were DNA-positive. If I can offer an opinion, if I had to drop one test out of HBV DNA, HBsAg and anti-HBC, I would drop HBsAg, the reasons being that HBV DNA has an advantage in the preseroconversion period and also a little bit of an advantage, much less than in the seroconversion period, at the late stage of chronic carriage.

The advantage of anti-HBC is that possibly you can avoid some infectious unit that would be missed by HBV DNA. So this pair of tests seem to me to most effective

at

from a safety point of view, probably not from an operational point of view.

DR. KLEINMAN: Thanks, J.P. I think one of the reasons blood bankers, for the last few years, have been thinking about dropping anticore is, of course, that has been a relatively nonspecific test and a test with a high repeat-reactive rate. So the benefits of dropping it in terms of lack of appropriate donor deferral and availability outweigh surface antigen. In other words, the benefits to the system, if you could drop anticore for safety concerns, would be a lot more availability of blood than dropping surface antigen.

But I think, from the safety viewpoint, I agree with you. Now that we have the data, dropping anticore in the context of putting in HBV NAT, anticore would seem to be more important than surface antigen although we may have a cocktail of all three.

DR. BIANCO: I think we should do one more question. Larry?

DR. MIMMS: This is Mimms, GenProbe. I think that surface-antigen mutants have been discovered in just about every population that has been examined thoroughly in the right way. There are examples in the United States. One was from McAffee a few years ago. It was really an interesting sample. It showed an insertional deletion of

at

two amino acids in the A antigenic region. So there are examples, I think, in virtually every population of S mutants.

DR. KLEINMAN: Thanks, Larry. I misspoke on that. I guess I should have said they were not as common in the U.S. and, because they are relatively uncommon, they probably don't explain the phenomena here. But thanks for the correction.

DR. BISWAS: Thanks, Steve. Our next talk is by Dr. Susan Stramer of the American Red Cross. She will be summarizing the recent HBV studies including our own. Her talk is entitled Comparative Sensitivities of HBsAg and HBV NAT Assays.

Comparative Sensitivities of HBsAg and HBV NAT Assays

DR. STRAMER: Thanks, Robin.

[Slide.]

As Robin referenced, I will be summarizing two recent studies that looked at comparisons of HBsAg and HBV NAT assays. Both were presented at the AABB this October, so what we did is we just compiled these two studies into one presentation that I will review.

The authors on the FDA study, which was a collaborative study, are listed here and the authors on the

at

Red Cross study are listed here. I would like to acknowledge all of them.

[Slide.]

Also, we would like to acknowledge the NAT Study Group which was very important in the structure of all of these studies providing support and a really good working group to outline and to implement a lot of the studies that we have talked about in the last two days.

[Slide.]

As background, HBV NAT has not yet been implemented for blood-donation screening. This reflects the likelihood the newer HBsAg tests may have equal sensitivity to prototype HBV NAT assays using pool sizes of 16 to 24 donations. In order to evaluate this, the two studies were performed comparing HBV NAT and HBsAg. I will refer to them as the FDA study and the ARC study.

[Slide.]

By way of background, I want to show two profiles of HBV during seroconversion. This is time on the X axis, viral load, and signal-to-cutoff ratio. Here you can see HBsAg and HBV DNA early in seroconversion during viral ramp-up and then clearance by the appearance of antibody later in time. These are cutoffs theoretically imposed by an NGI assay just to see where detection would be. In this case, the first HBsAg-positive sample corresponds with the

at

first DNA-positive sample if one were to use a pool of 512, for example.

Here you would see one positive DNA sample but below the cutoff of the assay. So, again, the low viral load seen is seen in this seroconversion.

[Slide.]

Here, again, you see the same thing where HBsAg antigen and DNA rise very early and rapidly in the case of this panel and then decrease with the development of antibody. Actually, with core antibody, this person didn't develop a strong anti-surface response but did have a strong anticore and was reactive in surface.

But the point of the slide here is there was a small shoulder of three samples that were below the level of detection by a DNA-pooled test.

[Slide.]

Compiling 13 of such plasma panels and looking at the viral loads during different phases of seroconversion, and, again, two different cutoffs by an NGI pool test, here we have the pre-HBsAg-positive samples using currently licensed tests. The median of the samples in these thirteen plasma panels was about 600 copies per ml which was below the cutoff of the assay. So the majority of the samples would not be detected and using a conventional

at

cutoff, we would have only detected three, so, relatively poor yield of a pooled NAT test.

[Slide.]

Then, coupled with that, we know there are newer HBsAg tests in development. So, if we look at a theoretical time line on the X axis here, viral load, again, on the Y axis, if we look at 25 panels, the HBsAg EIA test that we use today would cover relative to this period of time during the HBV window.

Then PRISM, or looking at more sensitive surface-antigen test, we would go into the preseroconversion period at about 6.8 days, detecting those samples that had about this viral load, up to 3,500 copies per ml. But then there would be a period of time where DNA would still be positive HBsAg-negative even by the most sensitive tests and these would correspond to samples having very low viral loads.

As Dr. Kleinman just went through, as PRISM cuts into the period of the window that is traditionally the anticore window where core and surface antibody are coming up, here, again, we see very low viral copy numbers.

[Slide.]

One thing that we do know is that if we look at FDA-licensed tests and some of the unlicensed procedures that we have, we know from earlier studies that there is

at

about a one-log variation in detection of purified nanograms per ml, purified HBsAg, by the various assays.

So, knowing this, we wanted to expand the dataset and actually see what the variability was and then compare this to the use of NAT either in a single-donation platform or in pool testing. And that is background to the two studies.

[Slide.]

So the FDA Study goals were to estimate the increase in the yield of HBV infectious units detected comparing current HBsAg assays, newer more sensitive assays that are not yet licensed, pool testing methods for NAT and single-sample NAT techniques.

[Slide.]

Ten samples from each of ten selected seroconversion panels were chosen for both HBsAg and HBV DNA testing or 100 samples. Samples represented the viral pre-ramp-up period and viral ramp-up phases. There were also controls of 28 samples which included the CBER lot-release panel that contains various concentrations of HBsAg. The WHO HBV DNA standard that were run at three different dilutions, 40, 400 and 4000 International Units per ml, and then negative samples. Fourteen samples were prepared and they each were run in duplicate.

[Slide.]

at

All 128 samples mentioned were coded and tested. Seven HBsAg tests were performed under code by the FDA. HBV NAT assays were performed by four manufacturers and these included another series of seven tests; a 1-to-512 dilution, a 1-to-1200 dilution from source plasma, two whole-blood assay formats, one run in a dilution of 1-to-16, one run at 1-to-24 and then three manufacturers tests run in single donation.

[Slide.]

The analysis performed included an estimate of the HBsAg concentrations of the cutoff using the CBER lot-release panel, and that is nanograms per ml; an estimate of the viral load at the assay cutoff using the WHO HBV DNA standard, and that is reported in International Units per ml; a comparison of the viral detection in pre-ramp-up and ramp-up phase specimens; an estimate of the viral load at the cutoff based on the HBV doubling-time model that Mike Busch talked about yesterday; to compare the window-period differences between the HBsAg assays and those assays relative to NAT performed in pools or in single donations.cc

[Slide.]

And to take those window-period estimates and then project increases in yield of HBV-infectious units

at

detected, again, as I said, based on window-period differences and known HBV incidence.

The incidence for this study included the REDS HBV incidence of 5 per 100,000 person years. I just included the ARC HBV incidence because the REDS includes--well, it does include three Red Cross centers from three large urban areas but just to compare this to the systemwide incidence data over a similar period of time, which was a little bit lower but overall comparable.

[Slide.]

Now, to look at the results or the analysis that I presented in the order that I presented them. First, we had the CBER lot-release panel. Here you can the assays, the seven HBsAg assays (A) through G. The unlicensed assays are indicated by parentheses so here we have the three unlicensed assays which actually, overall, performed better than the current licensed assays.

The range reported here was 0.09 to 0.63 nanograms per ml so it is comparable to the earlier range I showed you of 0.08 to 0.7. So we are basically seeing, in a larger dataset, that these results repeat.

[Slide.]

Looking at the WHO standard, here you have the same overall pattern but now assays (A) and (B) switched so we have (A) being the more sensitive followed by (B). The

at

range here in IU per ml 88 to 1,014 International Units per ml. I just did the conversion factors of four copies per IU because I only can think in copies per ml.

So, for those like me, it is about 350 to 4,500 copies per ml. But the point here is using two different ways to analyze differences in HBsAg sensitivities, you basically see the same overall trends that most of the currently licensed assays have lesser sensitivity than the new assays under FDA review.

[Slide.]

Looking at the pre-ramp-up and the ramp-up HBV, the ten seroconversion series, the 100 samples, ten of them came from the pre-ramp-up period. 90 came from the ramp-up period. If we list the assays in order of sensitivity, the seven HBsAg assays, there was only one pre-ramp-up sample that was detected and it had a very low S-to-CO ratio. So we don't know how reproducible that would be.

But looking, then, at the range of detection of the ramp-up samples, it is 61 percent to 31 percent, or I should say 31 percent to a high of 61 percent. This translates, at the assay cutoff, to copies per ml of 568 for the most sensitive assay to 10,000 copies per ml. So that is quite a range. And 95 percent confidence intervals are provided.

[Slide.]

at

If we take this last column of data and plot that just so we can see relative differences, it results in this plot where here we have the unlicensed assays against copy detection of the assay cutoff versus the currently licensed assays.

[Slide.]

If you take the doubling-time graph that Mike presented yesterday, although I don't think he presented it for HBV, this comes from a compilation of 23 seroconversion panels taking the quantitative viral loads and applying a longitudinal regression analysis. So we get a viral doubling time line here.

If you apply that line to looking at viral loads here, log of viral loads on the Y axis and window-period reduction on the X axis, what was done is the least-sensitive and the most-sensitive assays were plotted here to look for window improvement. So we have the best assay detecting 568 copies per ml and the least sensitive detecting over 10,000 copies per ml, how does that translate to window-period closure?

Looking at it in terms of the longitudinal regression analysis, it closes the window by 11.5 days.

[Slide.]

Looking at this with the actual data using a modified Markov model which didn't look at any quantitative

at

data but just evaluated each assay for window-period closure directly, if we look at the two unlicensed assays against two commonly used--if we look at the unlicensed assays which had the best sensitivity against two commonly used licensed assays, instead of generating an 11.5-day window-period reduction, we actually generate a little bit longer window-period difference, 12 days to 15 days, where these positive values represent the window-period reduction achieved by the use of the unlicensed test.

Both of these were significant. Looking at the other unlicensed tests, they were nonsignificant because the window-period reductions were less.

[Slide.]

Switching to looking at the seven NAT assays examined, here we have the ten pre-ramp-up samples and the 90 ramp-up samples. So here, unlike the HBsAg assays, we did detect more positive samples in the pre-ramp-up period. As one assumes, single-unit testing performed better than doing pooled testing. The same held true for ramp-up period. Here we had over 80 to 99 percent detection of the 90 samples in the ramp-up period by single-unit NAT testing versus lower numbers for pooled testing, 56 to 71 percent.

If we compare that to what I showed you for the differences between the seven HBsAg assays, that range for

at

detection of ramp-up samples was 31 to 61 percent, so we had more detection here with the pooled NAT tests.

[Slide.]

However, if you translate this all looking at the different HBsAg assays, the two that I showed you that were unlicensed, the most sensitive, two commonly used licensed assays against the single-unit formats for NAT and then the pooled formats for NAT, the plasma and the two whole-blood manufacturers, we see that, with single-unit testing, we do get significant, in all cases, improvements in window-period closure by NAT as compared to any HBsAg assay.

These are all the window periods given here. In comparison to licensed tests, we see a range from 25 days as a low to 36, just over 36, days as a high. So if we have single-unit assays, they do outperform all the HBsAg assays.

Looking at pooled NAT window-period reduction relative to these tests, we don't see as high window-period closure. In fact, with the case of assay A, we actually see that the HBsAg relative to these two pooled NAT tests actually had better window-period closure than did pooled NAT.

[Slide.]

So if you put all of the data together to conclude with the benefit of the new NAT detection methods

at

are as compared to licensed methods, we saw window-period reductions for the new HBsAg tests ranging from 11 to 15 days, for pooled NAT from 9 to 11 days, and for single-unit NAT, from 25 to 36 days, an applying incidence of 5.1 per 100,000, and translate this to 10 million donations annually, this gives you the number of units that we would detect.

So, looking at the newer HBsAg tests, about 15 to 21. Looking at pooled NAT, 13 to 15. So anywhere from 13 to 20 using any of these two technologies. But, with single-unit NAT, again based on low viral load early in seroconversion, this would give us the greatest improvement with a yield of 35 to 50 projected per year.

[Slide.]

Looking at a second study, the Red Cross study, we did something similar. Ours was not done under code. We actually obtained 17 additional seroconversion panels. So if you look at these two studies together, because they were unique panels, we looked at a total of 27 commercial seroconversion panels.

These panels were newer and not yet characterized by bioclinical partners so we helped them characterize it and use the data for the purpose of this study.

What we compared was the Abbott PRISM, the current Procedure C by Abbott and the current Procedure B

at

by Ortho and the Genetic Systems 2.0 static assay with the data provided by the vendor. We didn't actually run this assay but BCP already had.

This included 225 samples. The PCR testing was done by NGI using their UltraQual test and 156 of the 225 samples, or 69 percent, were positive by the qualitative test. All Qual-positives were refluxed down to the Quant test which is 100 copies per ml relative to the Qual test at 4 copies per ml. If we had a discrepancy that is Qaul-pos, Quant-negative, for the purposes of analysis, assigned 50 copies per ml.

HBsAg concentration were determined using purified standards. In regard to neutralization testing for PRISM, rather than running all the PRISM-positive samples by neutralization, all we did is we would run the first in a PRISM-reactive series or any sample having and S-to-CO of 1-to-2.

[Slide.]

The cutoffs used to extrapolate--unlike the FDA study, we didn't actually run pooled samples, but we extrapolated cutoffs used for pooled testing and the cutoffs that we used were 1600, 1000 and 320, depending on what type of endpoints you were looking at for detection of NAT, a 50 percent or a 95 percent endpoint.

at

But the important part is if you look at three different cutoffs relative to HBsAg, I think that is the valid comparator.

[Slide.]

I will show you for three assays the detection of the HBV DNA samples, the 156 samples, against three HBsAg assays. Here you have the viral load on the Y axis. So here you have the cutoff for HBsAg and the cutoff that I mentioned of 1600 copies for NAT.

The box that is important here, or the quadrant to look at, is the quadrant here because it shows those that would be HBsAg-negative but pooled NAT or NAT-positive if we were to use a cutoff of 1600. So, using this particular test, we have 36 samples that were HBsAg-negative and NAT-positive at a cutoff of 1600.

[Slide.]

Going now to another FDA-licensed test, 36 reduces to 21. So this assay did have better sensitivity, slightly.

[Slide.]

Going to an unlicensed test PRISM, instead of seeing 36 or 21 samples, we actually only saw five samples here in the quadrant that is HBsAg-negative, NAT-positive. I didn't discuss the quadrants in the other slides, but they had 71 in one sample in these quadrants. So,

at

interestingly enough, an additional five samples were picked up here that were HBsAg-reactive but would be negative by NAT at a 1600 copy cutoff.

Actually, one of these samples had a relatively low viral load similar to one of the samples that Dr. Kleinman showed in his previous presentation. So there would be some low copy-number samples detected.

[Slide.]

That was with a theoretical cutoff of 1600 copies per ml. But what if the cutoffs were reduced to 1000 or 320? How many additional samples would be picked up by NAT that would not be picked up by HBsAg. So, going from 1600 to 1000, we basically get no improvement, one sample that was already picked up by PRISM, one sample here that would have been negative by a current test and another negative by another current test.

But if we drop the cutoff to 320, we get more substantial improvements, especially in the HBsAg-negative samples. So we would be looking at a minimum, I think, of a cutoff closer to 320 rather than that of 1600 or 1000.

[Slide.]

Looking at the observed data, plotting viral loads over time, looking at each assay, this is when the first PCR-positive sample was detected and their viral loads, a median of just over 100, again, low copy number.

at

The last PCR before any HBV test was detected, HBsAg test was detected, as positive. Here we had 21 days, again, of relatively low viral load.

But PRISM samples, or I should say the PRISM population is here with its median at about 10,000. Then the other assays.

[Slide.]

That was observed data. But what if we do a linear regression to try to estimate what is the actual viral copy number that corresponds to a signal-to-cutoff ratio of 1 by these assays? How much virus does that correspond to?

So we did the regression with either the first positive sample and the last negative sample, the first HBsAg-positive to the last HBsAg-negative or we used four samples in the analysis or we used six samples in the analysis to make it more robust.

But, in either case, for PRISM, you see the S-to-CO of 1 corresponds to about 1400 copies per ml and for two licensed assays, they correspond to much higher viral loads of about 4,000, 5,000 to over 10,000 copies per ml, again, which is comparable to what I showed in the FDA study.

[Slide.]

So, summary and conclusions in this study and reflecting back on what we saw in the FDA study,

at

significant differences in sensitivity do exist between HBsAg assays. The detection of purified HBsAg ranges from greater than 0.7 nanograms for licensed assays to 0.1 to 0.08 nanograms per ml for Abbott's PRISM.

This difference translates to a mean of 17.5 days or 20 cases detected per 10 million donations. That is using our incidence of 4.5 per 100,000. Interestingly enough, from the FDA study looking at best to worst, they showed an 11 to 15 days improvement with 15 to 21 additional cases identified per 10 million, so a very comparable outcome.

HBV DNA can be detected for a mean of 21 days in these samples prior to the appearance of HBsAg even using the most sensitive HBsAg test. The median HBV DNA titers in HBsAg-negative samples are 100 to 500 copies per ml with 75 percent less than 2,000 copies per ml.

[Slide.]

This is just more detail. PRISM detects HBV DNA, at least in this study, at 1400 copies per ml plus higher viral loads for currently licensed assays and we saw that a cutoff of 1600 was about equivalent to pooled NAT so that we would want to drop the cutoff either for pooled NAT or an individual HBV DNA cutoff of something not to exceed 320.

at

The use of a more sensitive HBsAg assay appears to be equivalent, as I just said, to the performance of pooled NAT using a cutoff of 1,000 to 1,600 copies per ml.

Thank you.

DR. BISWAS: Thank you very much, indeed, Sue. I really hate to do this but we are so far behind--it is more than half an hour--unless there are any sort of burning questions, I think we really ought to move on to the next speaker.

Our next speaker is Dr. Stephen Lee from Ortho Diagnostics. His talk is entitled Use for Serological Tests for HCV Core Antigen for the Detection, Diagnosis and Monitoring of HCV Infection.

Use for Serological Tests for HCV Core Antigen for the Detection, Diagnosis and Monitoring of HCV Infection

DR. LEE: Thanks, Robin.

[Slide.]

Most of my presentation is going to be about the use of HCV core antigen detection technology in blood-screening application and also diagnosis and monitoring of HCV infection.

[Slide.]

But before I get into that, I wanted to briefly touch on the importance of HCV antibody testing in the

at

current NAT environment since it seems to be a topic that is germane to this forum. These are summary data I obtained from a recent TTVS and REDS study that was published recently where they looked at 105 donor-recipient pairs where the recipient received one NE-HCV-reactive unit from a donor.

They looked at rates of transmission as judged by seroconversion of the recipient compared to the RNA status of the donated unit. As you can see, there is a very high rate of transmission in the RNA-positive unit but there are also two anti-HCV-reactive units that are RNA-negative that resulted in transmission.

[Slide.]

This is data from the same study. Again, these are the two cases of anti-HCV-reactive seropositive units that resulted in transmission in the recipient. But there were also a significant number of units that were discordant between the two RNA tests. Of these, seven of the eight resulted in transmission indicating that a low viral load in the seropositive unit can also result in transmission at a rate that is comparable to the rate of transmission with higher viral load.

[Slide.]

There is a lot of data on this slide. I apologize. What this is is actually data from a

at

seroconversion series. Actually these next two slides are profiles from the plasma donors that were studied as part of the presentation that was made yesterday in the collaboration between Leslie Tobler, Bayer and Chiron looking at seropositivity comparing second- and third-generation immunoassays in early seroconversion.

This is a plasma donor undergoing seroconversion who maintains a very low level of antibody over a period of six months such that it is nonreactive in second-generation assay, reactive in 3.0. The individual shows no development of antibody in RIBA over a six-month period yet, throughout this period, the individual is strongly reactive in a licensed diagnostic anti-HCV assay and is RNA-positive throughout.

[Slide.]

Similarly, in a second case, again, the individual is discordant between 2.0 and 3.0 over a five-month period. In this case, the individual develops a positive response in RIBA but there is no evolution of the pattern in RIBA 3.0. The This individual maintains reactivity to C-100 and C-33C throughout, again strongly reactive in the licensed diagnostic assay. This time, the individual is sporadically positive by PCR.

[Slide.]

at

Moving on to HCV core antigen detection, this is, obviously, serological detection of circulating HCV core protein, represents an alternative marker for viremia. It has applications for early detection of infection preseroconversion before development of anti-HCV, also, diagnosis and confirmation of viremia, a quantification of viral load and monitor on patients undergoing treatment. I will show some data on those applications later.

[Slide.]

We distinguish between two types of HCV core antigen detection, at least based on the stage of seroconversion. Detection of so-called free HCV antigen--that is, before the development of anti-HCV--and this is obviously important in terms of early detection of infection. This is the basis for the current HCV antigen 1.0 screening assay which is in use in some countries in Europe.

Then detection of what we call total HCV antigen--that is antigen that has become complexed with antibody following seroconversion. This is the basis of the second generation assay or total HCV core antigen which has application, obviously, for diagnostic testing and also potentially for early detection also since it can detect by free and total antigen.

at

It has also been developed as a more sensitive in terms of analytical sensitivity. I will show some data on that also.

[Slide.]

This is simply the assay format. It is a standard microwell format using anticore monoclonals coated on microwell which trap HCV core antigen. The bound antigen is then detected by additional anticore monoclonals which are conjugated to an amplified peroxidase system.

[Slide.]

In terms of blood-screening application, obviously there are some very attractive features of the technology with regard to identifying preseroconversion units. Clearly, it is compatible with currently automated immunoassay systems. It is suitable for high-throughput screening.

There is a comparatively short time to result for release units and it is cost-effective and easy to implement. Obviously, these factors have weighed significantly in the implementation of this assay for donor screening in some countries in Europe.

[Slide.]

This is a typical seroconversion profile. This is data actually generated with the 1.0 test showing that HCV core antigen and RNA develop at approximately the same

at

time, at about 46 days in this case relative to the evolution of antibody. Again, in the plateau phase following ramp-up, there is a relative high viral load as judged by antigen and RNA.

[Slide.]

In terms of the current status of the HCV antigen 1.0 assay, in terms of blood screening, the assay is now widely used in some countries for screening blood donations. Most of the donations in Italy are currently been screened with the assay. It is a very widely used test in Spain and, I believe, all the donations in Poland are now being screened with this assay.

There have been various published reports looking at the time-to-detection of HCV infection of the HCV antigen assay compared to NAT. Generally, the consensus values from these studies have been that antigen is detectable within two to five days of detection of RNA.

The sensitivity in the plateau phase of preseroconversion where viral loads are very high has also been shown to be very good, 94 percent in one study. There have also been studies showing that specificity has proven acceptable for donor screening. So, at least in some countries, the HCV 1.0 assay has provided a significant improvement in transfusion safety over the use of anti-HCV screening alone.

at

[Slide.]

These are, again, data generated from the HCV 1.0 test looking at viral load in preseroconversion specimens when those specimens were categorized by their signal-to-cutoff in the HCV antigen assay.

This is a study of 128 preseroconversion specimens of which 94 percent had detectable HCV antigen. The majority of these 78 percent had strong signal-to-cutoff in the ELISA and an average viral load of approximately 900,000. There was a group that had signal-to-cutoff between 1 and 5 and had an average viral load of 150,000.

Then, of the 6 percent that were nonreactive in the ELISA, the average viral load was 43,000.

[Slide.]

This is more recent data that is in press from a study that was conducted in Spain, again just showing the identification of preseroconversion specimens by routine application of the HCV antigen 1.0 assay. This is a group in Barcelona who identified a preseroconversion specimen that the initial donation was strongly reactive in the ELISA confirmed by the neutralization procedure, had a high viral titer and then the individual was brought back a month later and was still reactive in the antigen assay, confirmed, again, high viral titer.

at

And now there was the beginning of the evolution of antibody and elevation of ALT.

[Slide.]

So, typically, we see evolution of RNA and antigen at at least comparable times in the preseroconversion window. Studies from post-transfusion cases have indicated it is approximately two weeks following infection and there is a rapid viral ramp-up phase during which HCV antigen and RNA develop very rapidly followed by a plateau phase of relatively constant, although with some variation, in the level of RNA, then, with antibody developing, at least by third-generation tests, being detectable, on average, at 70 days following infection.

So we knew from studies that the HCV antigen assay was relatively sensitive in this plateau phase but we also wanted to look at the sensitivity relative to NAT in this early ramp-up phase.

[Slide.]

The following slides are part of a study that was done in conjunction with the REDS study group from Westat who looked the sequential samples from 37 donors who were in the ramp-up phase preseroconversion. Then these studies were tested for viral load and used to construct the linear-regression model showing a doubling time of

at

approximately 15 hours in terms of viral load relative to the time from index donation.

These specimens were, then, also tested on both the antigen 1.0 and the antigen 2.0 assay in order to determine the sensitivity at cutoff in terms of equivalence to detection of RNA in terms of copies per ml.

[Slide.]

These are the results of that analysis. As you can see, the antigen 1.0 had sensitivity equivalent to 32,000 copies per ml whereas the second-generation test, which is more sensitive, had a sensitivity of 8,000 copies per ml. Then, when these were extrapolated from the linear-regression model, it was possible to compare the differential times-to-detection of HCV infection relative to an NAT assay assumed to have a sensitivity of 100 copies per ml.

This resulted in a differential between the first-generation test, an NAT of 5.2 days, and, for the second-generation test, the differential of 3.8 days. I didn't put the confidence intervals in this slide. They were relatively narrow, plus-or-minus two days in both cases.

Clearly, this differential, in terms of time-to-detection, is relative to an overall preseroconversion window of approximately 60 days most of which has a

at

relatively high-titer viremia which is not, obviously, part of this study where we were looking at the ramp-up phase.

But these differentials, in terms of days, can then be used to calculate differentials in terms of yields, in terms of the differential detection of NAT and the antigen assays when applied to the blood-donor population based on calculated incidence rates.

The differential was approximately 6 units per 10 million units screened for antigen 1.0 and the differential for antigen 2.0 versus NAT was approximately 4 units. You can, therefore, approximate that the calculated yield per 10 million units screened would be 56 for NAT, 52 for antigen 2.0 and 50 for antigen 1.0.

[Slide.]

Going on to the diagnostic application which, I think, is also very important, I mention that the diagnostic application requires the pretreatment of specimen to dissociate bound antibody in order to detect the core antigen. This involves a pretreatment which is heated for 56 for 30 minutes.

The pretreated specimen is then run through the immunoassay which is a very similar format to the blood screening assay. I should say, in the diagnostic assay, at the same time, a standard curve comprised of four calibrators with known amounts of core antigen is run on

at

the same plate and then the results from those specimens are used to calculate a standard curve.

Then the signal from the specimen can then be extrapolated from the standard curve to generate a quantitative result in picograms per ml. The overall turnaround time for the assay is around three hours.

[Slide.]

This is data from Dr. Fabiani in Angiers, France, who has studied the correlation between HCV antigen levels and viral load by RNA testing in chronic, untreated, patients. She found a very strong positive correlation with a correlation coefficient of 0.8, a slope of 0.9 in this study. Again, this is the viral load as plotted in the logs of International Units per ml, as compared to antigen, which is the log of the picogram per ml multiplied by 10,000.

[Slide.]

Again, this is similar data comparing the distribution of viral load as measured by PCR, bDNA or HCV at testing. Again, the plot is on a log scale and the only transformation is that all of the picograms per ml were multiplied by 10,000 to get them on the same scale.

You can see the distribution of viremia is judged to be very similar by all three methods. In fact, the mean, in terms of logs, is indicated in the bottom.

at

[Slide.]

The next series, actually, of slides show a series of patients who were studied under therapy looking at the pattern of evolution of both HCV RNA as well as HCV antigen. These are what Dr. Fabiani terms ultrafast responders. You can see a very rapid elimination of RNA under treatments. The X-axis shows months following treatment and also follow up after cessation of therapy.

Then the same patients were studied looking at HCV antigen levels. You see a very similar profile in terms of the elimination of HCV antigen in these patients.

[Slide.]

This next set represents relapsed response where you saw initial elimination of RNA followed by rapid rebound in RNA levels after cessation of therapy. The results in the HCV antigen testing, in terms of the quantitative load of picograms per ml, is a very, very similar pattern in these patients.

[Slide.]

These are slow responders to combination therapy. But they did maintain response after cessation of therapy and, if you looked at HCV-antigen profiles, again, it is a very similar pattern of evolution of HCV antigen.

[Slide.]

at

These are another group of partial responders where there was a slow decline in RNA levels but then a rebound following cessation of therapy. The results, in terms of HCV antigen level were also very, very comparable.

[Slide.]

Then, finally, a group of nonresponders which maintained relatively high levels of RNA throughout. Again, the profile in this group was very similar when antigen was quantified. So, in her patients, she was observing very similar clinical information when she studied HCV antigen levels compared to RNA. In fact, she has calculated virtually identical predictive values for both assays in terms of predicting response to therapy.

[Slide.]

This slide just addresses specificity in low-risk donors that the second generation assay is also very specific. There are actually no false positives as judged by repeat-reactivity and the specificity, based on initial reactivity, was 99.8 percent. I am indicating that this assay can also be applied for low-risk screening.

[Slide.]

So, in terms of the application of HCV core antigen for monitoring of patients, it does appear that it can provide valuable data on clinical and therapeutic progress. It represents a separate measure of viremia

at

compared to RNA analysis. I think it can be considered complementary to NAT in as much as, while NAT, with greater analytical sensitivity is obviously a preferable technology for determining endpoint of therapy, the HCV antigen measurement may have great value in terms of developing algorithms, particularly in the early stage of therapy.

The quantitative amount of antigen appears to be directly related to viral load as judged by RNA and the assay is very reproducible. It obviously allows very frequent and repetitive testing schedules because of the convenience of the technology and it is compatible with an established lab ELISA environment.

So it may provide a very convenient and economical route to individualized patient treatment.

[Slide.]

Finally, in summary, just the HCV antigen technology does appear to provide a cost-effective alternative to NAT for identification of blood donations in the seronegative window phase and we expect to continue to have some significant global application in that regard. It is clearly suitable for large-scale screening to identify recent HCV infection which may be of value in epidemiological studies.

It provides an alternative marker for diagnosis of viremia and appears to provide an effective methodology

at

for patient monitoring in as much as it is quantitative, reproducible, provides a fast time for the result, is cost-effective and convenient and, obviously, allows more frequent patient monitoring which could be a significant advantage.

Thanks.

DR. MIED: Thank you, Dr. Lee, for that excellent summary of what is going on at HCV antigen testing.

I think we need to move right to our last speaker which is Mike Busch from Blood Centers of the Pacific. Mike is going to talk to us about how we can integrate NAT with supplemental serology testing.

Integration of NAT Results into Supplemental Testing Algorithms for Serologic Assays

DR. BUSCH: Thank you, Paul. This has been an exhausting morning, tons of really great data.

[Slide.]

This is relatively painless, I hope. Of course, we have implemented NAT for its benefit in detecting window-period infections. But, along, if you will, for the ride, we have gotten an enormous amount of NAT data on seroreactive donors.

I think many of us, for several years, have been trying to figure out how best to integrate these NAT data on seroreactive donors both in the context of resolving the

at

true infectious status of our donors for counseling purposes, particularly in the setting of HCV RIBA where we can define our seroconfirmed RIBA-positive donors into those who seem to have persistent or cleared infections and also reassuring the donors who have indeterminate negative results.

In addition, a number of us have looked at the potential that the NAT results, routine NAT results, that we obtained could obviate the need to do particularly RIBA testing when the NAT result is positive. I will present data to, I think, support that recommendation.

Then I think we heard yesterday that the NAT data actually may be useful to facilitate reentry either to give us more confidence in reentering donors, for example, with indeterminate results on serologic supplemental findings but also in the context of the actual reinstatement, itself, either on a separate bleed or, obviously, on the repeat donation.

The fact that NAT can or will be done, again, I think has given FDA a little bit more confidence in moving forward with reinstatement of donors.

[Slide.]

I thinking about this, there are kind of three levels at which the NAT results can be used in the various donor counseling and reinstatement activities. One is

at

simply to incorporate the NAT results into the donor notification message. I think most of the programs have actually already done this.

This is easily done for the cases where the NAT results really corroborate our serologic supplemental findings. This is broken into sort of two categories here. In the setting where you have a positive RIBA and a positive HCV NAT or a positive blot and a prospective NAT for HIV or a positive p24 antigen neutralization in a prospective NAT, the NAT results give us greater confidence in notifying these donors that they are infected.

I think, again, most of the notification materials that are going out now to donors do bring the NAT results into that counseling message in the setting where we have really concordant positive results.

On the other side of coin, if the supplemental data is negative and the NAT results are negative, those findings can, again, be incorporated into the donor counseling message further reassuring the donor that they are not infected. So that is the first order of application.

[Slide.]

The next is if the NAT results are discrepant from the supplemental serology, that can help us flag cases that we think may be erroneous or where the serologic

at

interpretations may be inaccurate or help us to identify possible problematic or interesting cases. Examples here; if we have RIBA-positive donors who are minipool NAT-negative, those donors can be kind of counseled or we can flag those as presumptive resolved infections.

Some data I will show you, we have looked at the value of doing individual donation NAT or follow-up testing of donors who have this presumptive resolved infection to identify whether some of these may be low-level carriers potentially and we found occasional cases where there RIBA, in fact, was a false positive so the two-band concordant nonspecifics, so flagging those kinds.

This has been important where we have technically a block that meets the current positive criteria but the minipool NAT results are negative. As I will show you on the next slide, what we have identified and reported over the last several years is the observation of false-positive Western Blot patterns as the criteria for a positive blot have been relaxed to detect early infection.

We know that that has resulted in misclassification of a small rate of donors as false positive. The negative NAT results really is extremely useful to help identify these sources of the discrepant results. Those are much more frequent than donors with

at

low-level viremia who might have been missed by minipool but be detected by individual.

Then we have also, over the last few years, seen examples of autologous donors on HAART therapy who have had negative minipool NAT due to the effectiveness of the antiviral therapy but a positive blot and also some examples of vaccine recipients, healthy low-risk blood donors who have participated in vaccine trials, who have presented with positive blot patterns due to the vaccine response but were flagged as probable false positives due to the negative results coming out of routine NAT.

Another example that Sue alluded to is with antigen we do have a serious problem with false neutralization results. These flag out as minipool NAT-negative which can, then, allow further investigation of those donors.

We also had, and I will show you examples, where the RIBA or Western Blot results may be negative or indeterminate but the NAT result is positive. Most of these do, in fact, represent two infections where the supplemental serology--again, all of these are EIA-reactive donations--supplemental serology wasn't able to confirm infection due to either a window period or a incomplete serologic response.

[Slide.]

at

Just one example aside from Sue of her follow-up work on cases of Western-Blot-positive donors that were TMA negative through the routine screening, so these were identified either through pooled or neat screening early on. But what you really want to focus on is the blot pattern.

These ones in white, here, are all false-positive Western Blots lacking the p31 band which is the main way that we serologically flag potential false-positive samples. All of these cases had incomplete blot patterns and were negative by individual donation PCR.

But, as we heard, they have identified, in further studies, two or three additional cases like these that had high OD on the EIA, had full-band Western-Blot patterns and were detected by individual donation testing. So these are the low-level carriers.

[Slide.]

A third level of integration of NAT into serological algorithms would be the consideration of actually discontinuing doing some supplemental serology in this cases. The best example, and I will show a lot of data on this, is EIA repeat-reactive donors who have positive HCV NAT. There is a lot of data that really supports that those donors without doing a RIBA can comfortably, I think, be notified that they are infected.

at

This is actually consistent with the CDC guidelines in terms of general practice where a person who you can either use RIBA or a nucleic-acid test to corroborate infection status in the setting of a diagnostic HCV screening.

In general practice, I think, there are a lot of both public-health and diagnostic settings where either you go straight to RNA or, even, just based on the S-to-CO results on the EIA may notify a donor as infected without incurring the cost of doing RIBA.

In contrast, if the EIA is repeat-reactive and the HCV NAT is negative, there is a general consensus, based on data that I will share, that one should probably perform RIBA in those settings, certainly in the donor-screening context, in order to better counsel and determine look-back policy.

If the donor is found to be RIBA-positive, they should be notified as a presumptive resolved infection although I will show you that one can identify some of these people as low-level carriers if you do individual donation NAT.

In contrast, if the RIBA is negative or indeterminate, you now have negative or indeterminate RIBA plus a negative RNA so these donors can be very confidently notified that they are not infected.

at

[Slide.]

Finally, just a fourth kind of level which requires full FDA guidance is the integration of the NAT into reinstatement algorithms. I think, in general, the feeling is that we should only attempt to reinstate donors who have a negative index donor NAT as well as serologic data that would support reinstatement.

In other words, if you are EIA repeat-reactive almost irrespective of the supplemental data, if you are also NAT false-positive or NAT-reactive at index there is a general consensus that--they are very rare, and given the rarity and the complexity and concern that probably reinstating concordant false-positive donors doesn't make a lot of sense.

We heard yesterday about FDA proposals to have sort of relatively more standardized reinstatement algorithms to reinstate either HIV or HCV false-positive donors at two or six months. These false-positive classifications really, for the first time, are including indeterminate donors. So this is really excellent that now a lot of these donors with indeterminate results, which amount to a high proportion of false positives, are going to be reinstatable because we have NAT in place.

FDA is asking for NAT on a follow-up separate sample which seems reasonable but, of course, these donors

at

will be repeatedly screened by NAT. Finally, in my opinion, there should be consideration of trying to reinstate these donors who historically have been misclassified false positives by serology, so not only the indeterminates but these false-positive blots, false-positive neutralization cases, given that we can perform NAT both on a follow-up reinstatement bleed and routinely.

[Slide.]

So the data that sort of supports these conclusions come from the large correlation analyses where we have taken the donations that have been screened in parallel by minipool NAT and serology and really have teased away the relationships.

The first slide here is based on 5,400 HCV EIA repeat-reactive donations to Blood Systems Lab. You can see that around 3,000 of these 5400, so the majority, about 60 percent or so, are actually RIBA-confirmed. Of those RIBA-confirmed, by the minipool NAT, 80 percent of these are found to be viremic.

So we do have, though, about 20 percent that, by minipool NAT, are virus negative. We will come back to that group. Among the indeterminates by RIBA we found 27, or about 3 percent, to be positive. This is a mistake. This should be 776, so 3 percent of the total, 803 indeterminates. When you look at the band pattern on these

at

indeterminates, about a third of them, nine of them, actually had multiple HCV antigens, typically four reactive HCV antigens, but were called indeterminates due to the SOD band also being reactive.

Actually, Leslie Tobler has a letter in Transfusion in press that will really focus on the rate of these false indeterminates due to SOD override of a positive. The majority of the rest were either C22 or C33 only.

In our study, we didn't find any RIBA-negative donors to be viremic. So the major message here is the ability to reassure a large proportion of these indeterminate negative donors with the negative NAT and the ability to take the minipool NAT results and notify these donors as infected or not and a consideration of actually not even requiring RIBA if you have a positive NAT.

[Slide.]

This is parallel data from the Red Cross, even a larger number, over 20,000 donations over about a two-year period, virtually identical results. 80 percent of their RIBA-positives were found to be viremic. A similar proportion, about 3 percent, of the indeterminates were found viremic with similar band-pattern distributions.

They did identify, in this larger denominator, nine cases of RIBA-negatives that were found to be viremic.

at

I don't know whether there is any follow up on these cases but we have seen, and I think Steve just showed, examples where, in a seroconversion, the EIA can come up positive shortly before the RIBA.

So one would not be surprised to find a low rate of people who are evolving EIA-reactive but RIBA-negative and, as we also saw, a modest proportion who are still in the indeterminate phase while being viremic. So, very consistent with our sort of understanding of natural history.

[Slide.]

We asked the question of what would happen if we took these samples of the RIBA positives that were negative by minipool NAT as well as a representative number of the indeterminates and negatives. We did full-input individual donation RNA testing on these samples.

At Blood Systems, we did this in both the period when we were screening in pools of 24 as well as pools of 16. When we were screening with pools of 24, we found 23 percent of the minipool-negative RIBA-positives to be viremic. But, as we have moved to smaller pool sizes, that rate has dropped. So, in a recent study, we found only 6 percent to be viremic, which is quite comparable to what Sue found in a study with the Red Cross where they ran NGI UltraQual PCR on 356 RIBA-confirmed positives minipool

at

negatives. Seven, or 2 percent, of these were found viremic.

So there is a small percentage of donors who are RIBA-confirmed, minipool-negative, who would be detected as viremic with individual testing. In one of these studies, actually Leslie Tobler with Chiron ran replicates. Some of these are only detected as viremic on one of two reps. So these are really very low-level viremics only detected with these high sensitivity qualitative assays.

We also ran a batch of indeterminates and negatives and we found one of the samples of 136 that was, again, an EIA-reactive RIBA-indeterminate minipool-negative. When tested individually, we found a low viral-load case there and none out of 100 negative.

[Slide.]

Actually, Steve presented this but I just--these low viral-load carriers, one could ask, who cares, what does it mean. There are people who are viremic who probably haven't been detected in clinical studies because the viremia is so low level that most of the commercially available assays don't detect this low-level viremia. So they probably haven't been studied.

Is there any evidence that these kinds of low carriers are worth detecting. Steve showed this. The important observation here--this was the quantitative Roche

at

PCR assay so not anything close to as sensitive as the AmpliScreen. But we did find these low-level carriers that were detected only by high-sensitivity TMA less than 100 or 200 copies.

They do transmit. So the important message here is that these low-level carriers that we would only detect with single donation, and they are shown here, actually, are infectious from a transfusion context. We are initiating a study now of recall of these donors to better understand their ALT evolution and other downstream characteristics.

[Slide.]

So, based on the HCV data, the AABB Transfusion Disease Committee has reviewed this and gotten input and has actually formally submitted to FDA, I believe, this algorithm revision. The key points here--I don't want to go through in detail--is basically if a donor is EIA reactive and NAT positive for HCV that RIBA becomes optional.

The donors are permanently deferred. They are notified that they are infected based on the EIA reactivity and the RNA status and referred for medical treatment with particular interest in recent evidence, especially of they are NAT only, that early treatment may be particularly effective.

at

On the other side of the algorithm, if the donor is EIA repeat-reactive but NAT negative, then we believe that RIBA should be performed. If RIBA is found positive, those donors should be permanently deferred, look-back triggered if a repeat donor, a consideration for a qualitative high-input NAT either by the blood center or certainly referral to determine whether this donor may be viremic even though our minipool NAT was negative.

Obviously, if the RIBA is indeterminate or RIBA negative, with the negative NAT, now, we can temporarily defer and anticipate reentry of these donors.

[Slide.]

With HIV, just a few slides. The correlations are quite similar, again 2800 samples from Blood Systems. 113 were found blot positive so a much smaller fraction of all donations that are EIA repeat-reactive are from infected donors than with HCV which is partly why, I think, there would be very little value to recommending not doing Western Blot because the vast majority of samples are RNA-negative and, therefore, you would have to do blot anyway.

In addition, with HIV, it is such an important infection for these people that getting a blot in addition to a positive RNA we think is probably indicated. What we found was that, of 113 blot-positive donors, 112 were detected by minipool NAT. One was negative. That one

at

sample, when retested by single donation, was found to be viremic.

Among our indeterminates, we found 0.4 percent to be infected. This may partly relate to the blot that is used at Blood Systems is relatively less sensitive. You will see some Red Cross data that is different. So a modest rate of donors are found in the indeterminate evolution stage. These were all p24-reactives.

We found two interesting negatives, blot-negatives, that were EIA-reactive that were viremic. Again, this blot is not as sensitive as the screening EIA so we have got a period where the blot is negative after the EIA has converted.

[Slide.]

Red Cross is using the Cambridge Blot, more sensitive. So they didn't have any of that. This is almost 10,000 repeat-reactive donations from Red Cross. They didn't have any blot-negative donations that were found viremic and a much lower rate of indeterminates that were found viremic, only five.

They did have a similar, about 5 percent, proportion of EIA reactives that were blot positive. About 95 percent of those were viremic. These 31 negatives, I showed some of the data on those in an earlier slide, again, about two-thirds of these were false-positive

at

Western Blots. The other third were people who had low-level viremia undetected by minipool NAT.

[Slide.]

We also have done some retesting of the samples that were minipool NAT-negative by individual donation NAT. I showed you the one example at Blood Systems that was a low-level infected donor found by individual donation NAT. In Sue's follow-up work on the 26 cases of minipool-negative blot-positives at Red Cross, only two of these were found to be viremic. The majority were these false-positive blots or were not detected as they had such low level viremia, they were not detected by the NGI UltraQual.

We did take on a modest number of blot indeterminates and blot negatives that had been negative by minipool to individual and didn't detect any viremics.

[Slide.]

Just in conclusion, through all this data, we think that the routine NAT results are really very important in our counseling of the EIA-reactive donors and that, as I have discussed, they should already be being incorporated in the donor notification and counseling programs and we think there is opportunity to integrate them into the actual testing algorithms and reinstatement programs.

Thank you.

at

DR. MIED: Thank you very much, Mike.

Unless there is as burning question for Mike, let's take a ten-minute break and be back at 10:56 sharp.

[Break.]

VII. Emerging Pathogens (Asher/Nakhasi)

DR. ASHER: Let's resume the remainder of the morning session. I am David Asher from the Office of Blood. Until now, we have been learning about nucleic-acid testing for five blood-borne viral infections. But, as James Gallarda reminded us yesterday, the technology of nucleic-acid testing is useful for improving the detection of a variety of other viral agents and for nonviral agents that can be present in blood.

Now we are going to get a review of some selected pathogens for which NAT might be a useful technology. To begin the late-morning session, I would like to introduce my Co-Chairman, Dr. Hira Nakhasi also of the Office of Blood.

DR. NAKHASI: Thank you, David. As Dr. Asher pointed out, we are shifting gears here. So far, you heard all the known pathogens, detection, technologies and all the data for HIV, HCV, HBV. Now we will talk about them, new emerging pathogens. As we know, there are a lot of them coming up. Also we will talk about the technologies, the new technologies and the process of diagnosis.

at

They are very fascinating topics, at least to me, because we are trying to learn about new pathogens. Based on our experience in the past, which we have discussed for a year and a half, we should build on those experiences and see how we can detect these new pathogens and use the new technology in that manner.

[Slide.]

I would just put a transparency here which is in the Italian language to emphasize the point that the latest issue of the CDC's Emerging Infectious Diseases basically has this malaria poster on the front page which basically tells you that those pathogens which we thought we had eradicated are now coming back.

Just to give you an example here. This used to be a travelling guide when you went to Italy I think in 1924 or some time. They would warn the visitors not to go to those areas, malarious areas. Since it was very popular in those days, they have kept it still to sort of emphasize the point that even though we have eradicated some of these things, we have to be very, very careful.

I think that will be the theme for the next session. At this point, I will invite Dr. Tabor who will give us an overview of the emerging pathogens and then we will go to individual presentations.

at

DR. TABOR: Thank you very much. I am listed to speak for forty minutes. I am not going to speak for forty minutes. I am going to retroactively cede some of my time to one or more of the speakers in this morning's session.

In recent years, the world's supplies of blood for transfusion and plasma for fractionation have been beset by the emergence of new infectious agents. Twenty-five years ago, the primary known infectious threats to blood were the hepatitis B virus, the agent of non-A/non-B hepatitis now known to have been hepatitis C virus in about 90 percent of cases, and cytomegalo virus.

Today, more than half of the blood community's efforts in infectious-disease prevention are focused on one virus whose existence was not recognized twenty-five years ago, human immunodeficiency virus Type 1 and on a prion whose existence was not known twenty-five years ago and that, even today, has never been documented to have been transmitted by transfusion, variant Creutzfeld Jacob disease.

Furthermore, twenty-five years ago, we would have been astonished to think that we would ever consider the small-pox virus a risk to the blood supply since that virus had been declared eradicated from this planet. Today, small pox is a concern for blood safety because bioterrorism is now a reality and because individuals

at

infected with small-pox virus can have a twelve-day asymptomatic viremic period during which they might donate blood.

The 1970s seemed to us today to have been a time of blissful ignorance about infectious disease threats to come, a kind of never-never land in which we believed that infectious agents in the text books were the ones that we would spend our careers fighting. I don't recall any discussions about emerging infectious diseases at that time.

However, by the 1980s, it was recognized that new agents could enter the blood supply. Although the first of these to be recognized was the human T-cell lymphotrophic virus, Type 1. The first to raise intense concern was human immunodeficiency virus Type 1.

At first, there was skepticism that such terrible new agents could enter the blood supply. Many investigators believed that acquired immunodeficiency syndrome was not an infectious disease. As the realization became undeniable that these were truly infectious diseases, at first it was felt that these viruses must have recently entered the human population and probably had spread to many continents at the pace of modern jet travel.

Some of the early cases of AIDS certainly arrived in some countries as a result of modern travel patterns.

at

Later it was recognized, however, that HIV-1 had probably been infecting humans in isolated areas for several decades or, perhaps, even longer and might have entered the human population from nonhuman primates.

In the case of HTLV-1, it is possible that this virus existed in humans as long ago as the Sixteenth Century based on a comparison of geographic distribution of high-prevalence countries with a history of travels of discovery and trade in past centuries.

In fact, infectious diseases can emerge from various sources. New variants can emerge from known agents that acquire increased pathogenicity. Agents whose usual hosts are nonhuman animals can acquire the ability to infect humans. Previously unrecognized infectious agents can become recognized due to increased virulence, increased disease surveillance or due to amplification resulting from increased exposure of susceptible populations, for instance, as result of modern travel patterns.

Wide-ranging travel from one continent to another, from rain forests to industry cities, has made the planet a global village in which an emerging infectious disease anywhere in the world can represent a potential threat to the blood supply in the United States.

Finally, the creative evil of which all men are capable can enable an otherwise quiescent infectious agent

at

to emerge as a threat to the blood supply. The transformation of bioterrorism from a threat to a reality in our world in 2001 has increased the number of agents that could emerge as infectious threats to the blood supply.

There are agents of bioterrorism that could be transmitted by blood transfusion if infected individuals donated during the asymptomatic periods during which some of these agents are already circulating in the blood. In addition, bioengineering could result in modification of additional agents so that they, too, could be transmitted by blood transfusion.

The world looks toward the United States for leadership in making blood safe from emerging infectious agents. This is due, in part, to worldwide recognition that the U.S. Food and Drug Administration is tougher and more vigilant than any similar organization in any country.

This is also due to recognition that advanced technology and an active and inquisitive core of scientists are generously supported in the United States through the coordinated resources of the U.S. Public Health Service agencies in order to identify and study infectious-disease agents.

For the past four-and-a-half years, the U.S. Public Health Service agencies have maintained a committee

at

on emerging infectious diseases. Co-chaired by a representative of FDA and a representative of CDC with membership from NIH, this committee meets regularly to evaluate new developments in infectious diseases that might signal the emergence of a new threat to the blood supply.

The committee maintains a database of known emerging infectious agents with the potential to enter the blood supply and it maintains a standard operating procedure for coordinating Public Health Service response to such agents.

When considering what infectious agents to pay attention to in order to protect the blood supply, it is important to remember that we must always be alert, we must always be flexible and we must always be willing to consider a newly recognized agent or even previously unthreatening infectious agent as a potential threat to the blood supply. The infectious diseases that have emerged in the past twenty-five years attest to this.

Thank you.

DR. ASHER: Questions for Dr. Tabor?

Thank you, Ed, for trying to get us back on schedule. The next speaker will be Michael Cannon of the CDC who will review for us human herpesvirus 8 and its relevance to blood safety.

at

DR. CANNON: Thank you.

[Slide.]

First some background. Herpesvirus 8 was discovered in 1994. It is an enveloped DNA virus and, because it has been shown to have a causal role in Kaposi's sarcoma, it is also known as Kaposi's-sarcoma-associated herpesvirus. Its closest human herpesvirus relative is Epstein-Barr virus.

[Slide.]

A number of serologic assays are used to detect antibody to HHV-8. They detect antibody to latent antigens or lytic antigens. A number of assay formats are used but currently there are no FDA-approved assays.

[Slide.]

Here you can see the worldwide seroprevalence of HHV-8 in healthy individuals. First of all, you can see that, in SubSaharan Africa, the seroprevalence is highest. It is intermediate in countries bordering the Mediterranean such as Italy and Greece and lower seroprevalence in countries such as the U.S. This correlates with the incidence of Kaposi's sarcoma in different countries.

[Slide.]

Here you can see in the United States, if you look at different groups having varying degrees of risk of Kaposi's sarcoma ranging from those with KS, HIV-positive

at

and negative men who have sex with men and then injection-drug users or STD-clinic attendees, what you see with the seroprevalence is there is a good correlation with risk of developing KS.

Here, if you look at the prevalence of HHV-8 DNA in peripheral-blood mononuclear cells, you see that you can detect HHV-8 DNA in blood but it is less frequent than antibody. Here, about half of the individuals with KS are PCR-positive in blood.

[Slide.]

In the United States, the primary mode of transmission or the primary risk factor is multiple male homosexual partners. However, in Africa, it has been shown that HHV-8 is transmitted primarily through close nonsexual contact, a mode of transmission that is probably rare in the United States.

[Slide.]

The possibility of HHV-8 transmission through blood transfusion was really first brought up in this paper by Blackbourne and colleagues where they found that one donor was repeatedly PCR-positive for HHV-8. The virus was shown to be infectious and brought up the question of is this an issue. Do we need to worry about this?

The basic questions we want to know are are blood donors infected with HHV-8? Is there transfusion

at

transmission occurring and if it does, in fact, occur, are people getting ill because of it?

Addressing this first issue, a number of studies looked at seroprevalence of HHV-8 in blood donors. The first thing to notice is there is quite a wide range of seroprevalence values. Also, you can notice from these PCR studies that there are relatively few studies that have been done looking at PCR positivity in blood.

[Slide.]

To sort of remedy these two issues, the CDC is involved in the collaborative study with a number of other investigators looking at blood-donor specimens from the REDS repository. Six different laboratories tested these specimens along with 40 positive controls, specimens that came from patients with KS.

[Slide.]

Here are the unpublished results. The first thing to notice is that, in all six of the laboratories, everyone identified the positive controls. This demonstrates that, collectively, the sensitivity of seroassays is getting quite good. The next thing to notice is, although there is a range of seroprevalence, there is some variation in blood donors as far as this range that was found by each laboratory.

at

We do see that each laboratory found a non-zero seroprevalence of HHV-8. Somewhat reassuringly, looking at 243 of these blood donors, 55 of whom were seropositive in at least one of the laboratory, none of them were PCR-positive.

[Slide.]

Moving on to the issue of whether transfusion transmission occurs, there have basically been two studies which addressed this. The way they have done this is look at linked donor-recipient pairs where the donor was seropositive and the recipient was seronegative. In both of these studies, they found no evidence of seroconversion among the recipients even though, in one of the studies, they did find that HIV was transmitted in these same pairs.

Is this proof that transfusion transmission doesn't occur or is it sufficient proof? The number of linked donor pairs were relatively small. Some of the transfused units were in a form that you wouldn't expect HHV-8 to survive. It may be possible that, at the time these studies were done, the sensitivity of the assays wasn't ideal.

[Slide.]

Moving on to the third question, is there disease caused by HHV-8. If you are healthy, you have a 1 in a million chance of getting KS on an annual basis. However,

at

if you receive an organ transplant, your risk becomes, actually, quite high, 1 in 80. If you have HIV, it jumps to 1 in 50. If you actually measure HIV seropositivity and HHV-8 seropositivity, if you are positive in both, you have a 1-in-20 chance, 5 percent chance, yearly of getting KS.

[Slide.]

In summary, the disease risk due to transfusion transmission of HHV-8 if it occurs is probably very low. The percentage of infected donors appears to be low. HHV-8 is highly cell-associated and is likely to be susceptible to lymphocyte depletion and so might not survive storage very well. Again, there is no evidence of transfusion transmission and you really need something else, in addition to HHV-8, to get disease.

[Slide.]

But that is not quite the end of the story. This is some evidence that this could be an issue. First of all, you can find HHV-8 in blood. Again, if you are immunosuppressed, you actually have quite a high risk of getting KS. Additionally, there is some evidence that HHV-8 may be transmitted through exposure to blood. So I am going to summarize a couple of studies that looked at this third point.

[Slide.]

at

Studies have found a link, for instance, in this case, a nonsignificant link, between needle sharing and HHV-8 seropositivity. In addition, a number of studies have found a link between injection-drug use and HHV-8.

Some of the limitations of these studies are an issue of power, having enough individuals to really address this hypothesis, and, in these studies, the issue of confounding, is it possible that sexual behavior is what is really driving transmission and injection-drug use is simply a marker for that sexual behavior.

[Slide.]

To address these two issues of power and confounding, at the CDC, we did a study looking at 1300 women who were followed up at six-month intervals for up to six years. They provided self-reported data on injection-drug use and sexual behavior. Then we looked at what are the risk factors for being seropositive.

[Slide.]

What you can see here is, in this column, we have injection-drug use going from the women who never injected to those who injected every single day of every single visit which could add up to six years. You can see that the HHV-8 seropositivity increases from just over 12 percent to over 35 percent, a significant trend.

at

It appeared to be specific to injection-drug use because if you look at smoking crack, there was no similar trend. In addition, when you look at HCV seropositivity, which can be used as a laboratory marker for injection-drug use, we also found a significant association with HHV-8 seropositivity.

[Slide.]

Regarding the issue of confounding by sexual behavior, one way we addressed this is we limited the analyses to women who had a relatively low sexual risk. What you can see here is as injection-drug use increased, you still see an increase of HHV-8 at seropositivity and a very strong association with hepatitis C seropositivity suggesting that sexual behavior can't explain the association you are seeing.

[Slide.]

Similarly, if you do multivariate models where you control for variables such as HIV and syphilis, markers of sexual behavior, you still see this increasing odds ratio, this significant association between HHV-8 and injection-drug use.

[Slide.]

There appeared to be a link. How strong was this link? If you look at how it compares to hepatitis C virus, here you have injection-drug use again. You see a moderate

at

increase in HHV-8 seropositivity whereas, for hepatitis C, a very strong association with any injection-drug use suggesting that transmission of HHV-8 through shared needles is likely to be quite more infrequent and less efficient than hepatitis C transmission.

[Slide.]

In summary, the evidence for this type of transmission is association with self-reported and laboratory variables. Specific to injection-drug use, there was a dose-response relationship where the more you inject, the more likely you are to be infected. This didn't appear to be caused by confounding due to sexual behavior. Finally, the link was weaker for HHV-8 than for hepatitis C.

[Slide.]

In conclusion, it is probably safe to conclude that the amount of disease due to transfusion transmission of HHV-8, if it, in fact, occurs, is likely to be really low. However, there isn't enough evidence to say that there is no risk. For example, we probably can't say, based on the current evidence, that then cases of KS or maybe 50 cases of KS a year aren't caused by HHV-8 transfusion transmission.

So we really need to do some further studies to definitively say whether this is an issue. So some of the

at

things we are doing, for instance, at CDC is looking at bigger studies of linked donor-recipient pairs, especially in Uganda where seropositivity is much higher in donors.

In addition, studies need to be done that quantify better how much KS is possibly occurring due to transfusion transmission. Finally, assays need to be developed and licensed if, in fact, it turns out that donor screening is indicated.

Thank you.

DR. ASHER: Thank you, Dr. Cannon.

Questions for Dr. Cannon?

DR. BIANCO: There are several assays. Do you want to talk a little bit about the specificity of those assays?

DR. CANNON: The specificity of the assays? Well, with regards to blood donors, it is a really difficult question. The reason is, first of all, if you have any population that the true seroprevalence is very low, you have to have very specific assays to deal with that. In addition, it turns out that antibody titers to HHV-8 are much higher in people with KS than they are in blood donors or people at lower risk.

So the big problem is determining who is really no infected. People have tried to get at that. Often blood donors are used at the uninfected group, sometimes

at

children, in the U.S. or virginal women. Some suggestions, as far as how to deal with that, have been looking at confirmatory assays and that sort of thing.

So it is not quite clear how good the specificity is. It is probably really good but the difference between 97 percent specificity and 99.9 percent specificity could tell the whole story. It is not clear where things like right now.

DR. BUSCH: A couple questions. Is there an animal model that one could do transfusion experiments in?

DR. CANNON: As far as I am aware, not yet. I think there have been some recent proposed animal models but, for a while, there was nothing that seemed to be a good model.

DR. BUSCH: Probably this would have to be speculative, but the relative potential role of serologic screening versus nucleic-acid and, if one did nucleic-acid, would one have to target the leukocytes or is the a plasma viremia?

DR. CANNON: It seems that the viremia is more leukocyte-associated. So, as far as whether serologic testing would be the best solution, it is not clear. It seems that the people really at risk are the people who are immunosuppressed. So it seems more of an issue of what you do for people with HIV who get transfusions or organ-

at

transplant recipients. Because the seroprevalence appears to be much higher than the DNA prevalence, then maybe NAT testing would be more appropriate.

DR. HARRISON: My concern with HHV-8 is not Kaposi's sarcoma. This is Chantal Harrison, San Antonio, Texas. Its association with body-cavity-based lymphoma and multiple myeloma where even there are small studies or case reports, it is almost 100 percent of patients with multiple myeloma are infected with HHV-8. The same with the body-cavity-based lymphoma.

As a physician that remembers, when I was in medical school a long time ago, multiple myeloma has changed completely on the severity and age of onset. When I was a medical student, multiple myeloma--most patients were after 70, 80--you know, died of something else but multiple myeloma. That is what we learned.

Currently, multiple myeloma affects people that are like 40 to 50. It is a very aggressive disease, very often, and they die from it pretty quickly. So I am concerned about whether that is related with HHV-8, the change in epidemiology.

DR. CANNON: Yes; that is a controversial issue as far as HHV-8 and multiple myeloma. Currently, the evidence seems, as far as the number of studies and things that have been looked at, that it appears unlikely that

at

HHV-8 is responsible for any significant proportion of multiple myeloma.

Again, it is still controversial and it hasn't been completely resolved. That is part of the reason why I didn't discuss it in the talk. Generally, it is believed in the field that it is not a cause of multiple myeloma. As far as body-cavity-based lymphomas, that is also a concern. Just because they are more rare than KS, I didn't mention it here.

DR. LI: Is there any data on organ-transplantation-transmitted HHV-8 infection?

DR. CANNON: Yes; there have been a few studies in Europe, Italy, that have shown that an organ-transplant-recipient became infected with HHV-8 through the transplanted organ. I am not aware of any studies in the U.S. and that is certainly an issue of concern.

DR. RIOS: Maria Rios. As you mentioned the HHV-8, it is totally associated with leukocytes as far as we know in all the organ transplant, it would be associated with transfusion transmission otherwise. So, probably, the leukocyte production situation that we are going into makes somewhat less relevant for blood transfusion. Is that correct?

DR. CANNON: That is what we think. That is why we are doing some other studies looking in Uganda where,

at

basically, the blood from the donor goes right into the recipient to looking at the TTVS study in the '70's where screening procedures were different, and then looking at current studies. But yes; that is what we expect.

DR. ASHER: The next speaker, also from the CDC, Dr. Gregory Dasch will review nucleic-acid testing for detecting blood-borne rickettsial pathogens.

Present and Future Nucleic Acid Based Methods for the Detection of Blood-Borne Rickettsial Pathogens

DR. DASCHE: First of all, I would like to thank the speakers for inviting me to talk.

[Slide.]

I am reminded a little of the last time I had a chance to talk to an audience. It is unusual for me. It was an avian producers organization and they gave me a lot of quizzical looks about why I was talking about Rickettsiae. It was the same background in terms of the emergence of these agents.

[Slide.]

At the risk of offending some of the people in the audience who are medical, I am going to review the emergence of new rickettsioses. Because we have a large number of them, this is going to represent a significant amount of the talk. I will briefly present some of the

at

present nucleic aid diagnostic tests in use, particularly within our branch in terms of clinical diagnosis.

I would like to review some of the recent literature that has come out in terms of the future of diagnostic testing with nucleic acid tests. Many of these procedures are being actually implemented in diagnostic procedures today. Then, finally, I would like to talk to you a little bit about blood-supply issues versus diagnosis.

[Slide.]

The general principles about the pathogenic Rickettsiae--I am going to talk about both Rickettsiae and Bartonella. Bartonella, historically, were associated with Rickettsiae because they are also Gram-negative associated with arthropod vectors, cause febrile illness and are susceptible to tetracycline. With the advent of 16S ribosomal sequencing, it became very clear that the Bartonellas reside in a different group of organisms and they also have the feature that, unlike the Rickettsiae, they can be grown on bacteriological media.

However, they are rather fastidious organisms and the cultivation time and conditions required, most of the standard laboratories for blood cultures do not do these.

[Slide.]

at

This is a 16S tree which gives you a summary of the association of the different rickettsial agents. I would just like to point out, as I just said, that the Bartonellas are found in a group which other members of this group are largely plant bacteria, Agrobacterium, Rhizobium. These are organisms that form symbiotic associations in legumes for nitrogen fixation and are, consequently, very important.

The Rickettsial agents is another group of the alphaproteobacterial organisms. Down here, we have two members that, by 16S sequencing, have been allied with Rickettsial agents, the Asian acute fever and a fish pathogen down here are gamma-group organisms more closely related to E. coli.

As you can see from the large number of organisms listed in this phylogram, we have a large number of rickettsial agents. In fact, the usual problem in our branch is that we have more diseases to study than we have people.

[Slide.]

This is a panel. Not all those organisms I showed in the previous one were pathogens of people. These are. I have excluded, for lack of space, the Bartonella agents on here. These are only the rickettsial pathogens.

at

To make this a little more clear to you, our concern about these rickettsial organisms, as a general class, are highly infectious by parenteral route. It only takes one organism to cause disease. These are arthropod transmitted. We have different vectors that are involved.

The human body allows for epidemic typhus. Although that is a scourge of the past in this country, as recently as 1997, there was an outbreak in Burundi of 50,000 individuals. In World War I, 3 million fatalities in Eastern Europe due to epidemic typhus and it was quite widespread in Eastern Europe in World War II. It is not gone from the world at all.

Murine typhus is worldwide in distribution. It is a flea-transmitted disease. Most of the spotted-fever group Rickettsia are tick-transmitted but, recently, there has been one addition which is flea transmitted and there is another one, rickettsial pox, which we have had some concern about at CDC in terms of diagnosis of small pox, and rule outs for rickettsial pox have been the order of the day. That is a mite-transmitted disease.

There is another one, Orientia tsutsugamushi, which is a mite-transmitted disease. The population at risk for that--it is endemic in the Asia-Pacific region where there are approximately a billion people, and, to put it in some perspective, seroprevalence rates of this

at

disease in many areas, or 70 percent of the population have antibodies to these organisms.

[Slide.]

Of course, Rocky Mountain spotted fever is the most familiar one to us in this country. Just to remind you that we still, despite having a great deal of advancements in diagnosis, have fatalities every year for this disease. It is treatable with tetracycline with proper diagnosis. This usually requires some feeling about the history of the patient in terms of exposure to ticks, recognition of seasonality factors.

Some of these things may not come into play in terms of a blood-transmitted case. You may have lost the linker to exposure and, therefore, you have no clue. This leaves the physician at a loss because of his confusion with a lot of other diseases which present with the general symptoms of rash, fever and various, headache and the like, rather nonspecific symptoms until later in the disease.

[Slide.]

This is just to show you these are obligate intracellular pathogens in Rickettsia coxiella.

[Slide.]

One of the interesting facets of this disease is that they have an actin polymerization-mediated motility.

[Slide.]

at

That is perhaps seen a little more closely in this slide where you can see they actually swim and this is a part of their process of dissemination and spread from their primary site of infection. This is true of pathogenic and nonpathogenic ones.

[Slide.]

This is a distribution map generated--it is on our CDC website--for rickettsial diseases, the distribution of cases across the country. Rocky Mountain spotted fever is not just a U.S. disease. It is found in Central America and South America.

It has three different vectors that are involved in its dissemination. In the Eastern United States, it is largely dermacentor ticks, the wood tick. We have the dog tick, Dermacentor andersonii in the West and, in Central and South America extending up through Texas, we have Amblyomma species of ticks that can transmit it.

[Slide.]

This is just to give you an idea of the distribution of ticks that are in the West. The green is andersonii, variabilis on the right. Then, in California, there is another tick, occidentalis, which hasn't been implicated as a vector of this but there are certainly Rickettsia-like agents whose propensity for causing of human disease has really not been evaluated yet.

at

[Slide.]

There are three ways in which we have information. This is a nationally electronic system of surveillance of reports from state laboratories. Rocky Mountain spotted fever is a reportable disease. There is a case-report form which is given directly to the CDC. Unfortunately, a lot of these cases are not adequately confirmed with serological tests so, down here, we have another bracket which gives the confirmed case. That makes it look like it is more of a stable disease.

So you say, why am I talking about this in terms of emergency of disease? We feel that there are other rickettsial agents in the United States beside Rocky Mountain spotted fever that are greatly underdiagnosed. We don't really know, despite the fact that this has been a long-time reportable disease, how accurate our information is on the distribution and the occurrence of Rocky Mountain spotted fever to the present time.

[Slide.]

In the United States, we have, classically, four different Rickettsial agents that are well known and characterized as cause of disease. I mentioned epidemic typhus. This is normally a human-body-louse-transmitted disease but, in the United States, we have a vector reservoir which is flying squirrels in the Eastern United

at

States and the Appalachian region. Both the ectoparasites lice and fleas there can transmit the disease. So people who keep them as pets are at some risk and hunters who shoot squirrels may come in contact with this.

Rickettsia akari is mite-transmitted. It is an urban disease associated with house mice. I have already talked about Rocky Mountain spotted fever. The other agent that we have at the top is murine typhus which is associated with rat fleas. This is an organism that is worldwide in distribution and very prevalent.

The new rickettsial agents in the United States to mention are one that is associated with Amblyomma americanum in the Southeastern United States. We have very high prevalence rates and blood-donor populations to spotted-fever group Rickettsiae. This is an agent that, probably like Rickettsia africae, is a relatively new agent and found in Amblyomma ticks in Africa, causes a relatively mild, self-limiting infection.

But, of course, the population that is at greatest risk to infection with these agents are exactly those who would be receiving blood units quite often in terms of organ transplantations, older individuals who have surgery and the like. These are the people that, more often, suffer fatal infections even with the availability of supportive therapy and antibiotics.

at

We are evaluating the risk of Rickettsia amblyommii. I will come back to this point in a little bit. Rickettsia felis is flea transmitted. It is involved in a possum reservoir. It presents a lot like a murine-typhus illness, but, phylogenetically, it is related to the spotted-fever Rickettsia. Finally, the Gulf Coast Amblyomma maculatum, we have evidence from case reports that haven't been published yet the Rickettsia parkeri may also be able to cause pathogenic illness.

[Slide.]

Of course, our group serves as the WHO reference center for rickettsial diseases and we have to deal with a large number of other agents that are found throughout the world. The association of different spotted-fever-group Rickettsia is very tightly associated with specific vectors that are found only in certain regions.

The classical diseases that we knew, and when I talk about new rickettsial agents, I am largely talking about ones that we have an understanding of in the last ten years. The Rickettsia conorii, the agent of Mediterranean spotted fever, is an urban disease associated with dog ticks. People in France who have a little garden with their dog, and the ticks will drop off the dog and if people are more accessible, they will transmit the disease

at

to the owners. It is also a great disease of German tourists going to Southern France.

Rickettsia sibirica has a very vast range across Siberia down into Asia and to China. It is transmitted by dermacentor ticks. Rickettsia australis is largely only found in the continent of Australia transmitted by other ticks including Ixodes ticks.

New rickettsial agents; Rickettsia africae. This is distributed by amblyomma ticks throughout SubSaharan Africa. Rickettsia japonica appears to be found in other areas of Asia outside of Japan. Rickettsia honei of Flinders Island is a small island off of Australia, but it looks like this is, perhaps, an agent widely disseminated in Southeast Asia because there have been recent reports out of Thailand and Malaysia of disease caused by this agent.

There is a fairly high seroprevelance in the spotted-fever group and we know that disease occurs, but it had not been implicated what agent was involved. But it looks like, right now, Rickettsia honei.

If would finally like to mention a cluster of other agents who are not--their disease potential is really just beginning to be appreciated. The Astrakhan agent and Israeli tick typhus have been sometimes lumped with Rickettsia conorii, but they are, indeed, different agents.

at

They have different vectors. They do present different clinical presentations.

There are three other agents which are not presenting with the standard spotted-fever-group symptoms which are, as I say, rash and high fever. One case, helvetica, has been associated with myocarditis and two of the other rickettsial agents with cerebral vasculitis and presentations of that type.

[Slide.]

Many of you are probably familiar with, when we talk about emerging diseases, the Ehrlichial agents are probably the dominant ones that people recognize. There are three classes there; Ehrlichia chaffeensis is a monocytic organism. E. phagocytophila is Ixodes-transmitted. The chaffeensis is Amblyomma-americanum-transmitted. E. ewingii is recently identified as another disease agent just within the last two years which presents like a monocytic ehrlichiosis. As far as disease potential, the most severe outcome, of course, is immunocompromised individuals.

[Slide.]

This is just a DiffQuick to give you an idea of the staining that is done for Ehrlichia chaffeensis. This is why it is quite often--we talked about it in an emerging-disease symposium, the great increase in the

at

number of cases of booth HME and HGE organisms. I would like to point out we really don't know how frequent these diseases are.

[Slide.]

If you look at the clustering of disease HME entrance, you see, with this, that we have a cluster of diseases in the Arkansas-Missouri area.

[Slide.]

We have got some in North Carolina, really heavy. It turns out it is one of these things where, if you look for the disease, you are going to find it. We have physicians and we have research groups interested in it. They are out there looking for it and they find it without any problem.

It is not diagnosed in communities where you don't have specialized people looking for it.

[Slide.]

This give you an idea of the area of distribution of Amblyomma americanum that would be affected by this. We now that there chaffeensis-related organisms found throughout the world and, unfortunately, we don't know a great deal about the epidemiology of a lot of these at present.

[Slide.]

at

The other emerging disease showing similar and later incidence is human granulocytic ehrlichiosis, Lyme disease. Most people who know about tick-borne diseases know about Lyme disease. This is the same vector that transmits Lyme disease. Of course, this has been our number-one poster child for tick-transmitted diseases in the United States and has served as a source of funding for a lot of projects on that.

The HGE incidence, just like my HME slide, is clustered around Minnesota and Connecticut. That is, again, where you have active groups who are going out there and are getting physicians aware of these diseases and making the diagnoses.

[Slide.]

That is not to say that it is not widely disseminated. If you look at scapularis and pacificus, the agent distribution is quite widespread so we expect to see a lot more cases as physicians are more familiar with these diseases.

[Slide.]

I would like to end up with just to point out why is all this happening. Ehrlichiosis is a new disease. Is it emergence of a disease recognition or is it really just that we have the tools now and we know it is out there. We didn't know before 1987 with the chaffeensis.

at

Retrospectively, it has been identified in ticks, in the case of phagocytophila, better known as HGE agent, human granulocytic ehrlichiosis agent, which was identified back as far as 1982 and 1984 in Wisconsin and New York, respectively. But the explosion of the deer population from 350,000 around the turn of the century to an estimated 26 million in the United States, this is the reservoir for the ticks and the disease and man incidently comes in contact with these and acquires the disease.

[Slide.]

I am just briefly going to give you two slides on Bartonella and then I will talk about nucleic-acid technology. Bartonella; classically, there are four diseases that are caused by bartonellosis. The hallmarks of these organisms is that they are well known for causing chronic infections. The classic one is Bartonella bacilliformis which is found in the Peruvian foothills. It is transmitted by sand flies, leads to chronic infection of erythrocytes. In the case of that organism, parasitemia of as much as 50 percent of the erythrocytes can be detected.

Trench fever is very well known from World War I and World War II. It causes recurrent fevers. Then, more recently, the emerging disease that has stimulated a lot of interest in the United States is that Bartonella henselae

at

is the causative agent of cat-scratch disease. There is estimated to be around 20,000 cases of cat-scratch disease. It can cause chronic infections including encephalitis cases have been described as a result of this organism as well as a lot of other presentations.

Endocarditis is another thing that is commonly associated with these.

[Slide.]

This dendogram shows you an idea of what we had--actually, this is a little bit out of date. It gives you an idea where Bartonellae fall in the phylogenetic tree and approximately ten years how many of these organisms we knew about, henselae being the most recent addition to them.

[Slide.]

The Bartonellae family now looks like this. There are symbiotic organisms. We don't really know what their disease potential is. They are in practically every vertebrate species, very high carriage rates of Bartonellae in the blood.

I just wanted to make sure you are aware of this. An example of this is cattle herds have been screened. They quite often will have 40 to 50 percent of the cattle herds will contain Bartonellae organisms in their blood. We know very little about what the disease potential of these is but there have been a few cases of human

at

infections with some of the agents that are related to these.

There are also a number of rodent-associated Bartonellae which have caused human disease including Bartonella elizabethae which is a rat-borne organism.

[Slide.]

So this is a quick summary of what the current diagnostic tests that are done at CDC in terms of clinical diagnosis in our laboratory. None of these tests are FDA approved. They are not CLIA approved. They are really research tools that we use. But just to give you an idea that there are a large number of targets that have been used for the different organisms.

One organism I have not described is Coxiella burnetii. I would just like to emphasize here there is one other thing with respect to bioterrorism agents. Three of these agents are on the select-agent list because of concerns about their potential for use in bioterrorism.

Acute-fever agent, Coxiella burnetii, has been weaponized. It is a very stable organism that is very easily transmitted by aerosol so we currently have a research group in our program on that aspect of Coxiella.

Rickettsia prowazekii and Rickettsia rickettsii are also select agents for which we get no money and support for bioterrorism efforts but there is a

at

considerable concern because epidemic typhus has also been weaponized.

[Slide.]

The future is now in advances in technology. I wanted to point out something about the existing tests. The problem we have is that most of them, because of the low carriage of organisms in terms of bacteremia, they are generally talking about less than 1,000 organism per ml of blood.

We have a large window period for most of these organisms. It can range anywhere from a low rate of onset or a very rapid onset of about seven days up to as much as 40 to 60 days are estimated, in some cases, in terms of how long the window between exposure to the organism and active disease occurs.

The low carriage of these organisms; most of the tests were developed for applications where there are large numbers of organisms. For example, in the arthropods, carriage rates may tend to be high per organism and they work quite well.

The current now technology, reverse-transcriptase PCR, multiplex, quantitated real-time PCR and chip technology.

[Slide.]

at

I think, given the time, I am going to skip through this. The main holdup on adapting the advances in nucleic tests has been that most of the Rickettsia laboratories are fairly impecunious. Government labs and grants are short in supply and people haven't been able to afford quantitative PCR machines.

In the last two years, that has changed radically and there has been an evolution of tests for practically every one of these groups and they are being rapidly uses. Reverse transcriptase, of course, has advantages because one of the problems we have found is that there is persistence of DNA after the ability to detect viable units has disappeared so that we think it is very important to be able to check active RNA transcripts for disease.

It also gives you the opportunity of getting multiple copies of those RNAs compared to the amount of genetic material and increases in sensitivity.

[Slide.]

So we have, as I say, a large list of agents. This is the quantitative PCR that has been developed at the CDC where we detect all the spotted-fever groups of agents quite efficiently. It doesn't pick up other ones. We have another group antigen target gene that we can use that will pick up all the Rickettsia.

[Slide.]

at

Of course, there are potentially target sites within--we have been using Cybergreen to be able to detect all these because there is sequence variation between them. You can make specific TaqMan probes and identify down to specific agents.

[Slide.]

One of the issues that has driven a lot of the work is the problem with low numbers of organisms. Immunomagnetic separation has been used to concentrate organisms, antibody-capture method and precipitation of DNA.

[Slide.]

Finally, what is the real risk? I am just going to say one study. One concern that drove a lot of our interest in blood study was the experience of a National Guard unit that was involved in a blood drive in Arkansas. Approximately 377 individuals donated 320 blood units that were subsequently transfused into individuals.

Then they went back to Iowa. A number of the individuals came down with diseases that were associated with Rickettsia. They were confirmed at the CDC. There were twelve individuals out of this unit that had Ehrlichia or spotted fever. There were eight cases of Rickettsia, three of Ehrlichia and one that was both.

at

Fortunately, there was no immediate transfusion as a result of giving these 320 units to recipients but we do have, in the literature, examples where transfusion-mediated Rickettsial disease have occurred.

Thank you.

DR. ASHER: Thank you, Dr. Dasche. I think we will have to dispense with the questions.

DR. NAKHASI: Yes; I think since the time is short, we will move on. Maybe at the end of the talks, we will have some discussion.

The next topic is the Trypanosoma cruzi, agent of Chagas' disease, to NAT or not to NAT, Dr. David Leiby.

Trypanosoma cruzi, Agent of Chagas' Disease

to NAT or not to NAT

DR. LEIBY: As we heard in the last day or so, there has been a rush to NAT virtually everything out there. So maybe now I will provide a little dissenting view or at least maybe a framework in which to think about things in the future that we might consider to NAT or not to NAT.

[Slide.]

The one I am going to talk to you about today is Trypanosoma cruzi which is the etiologic agent of Chagas' disease first described by Carlo Chagas from Brazil in the 1900s. It is a small protozoan parasite that has an

at

intracellular and extracellular phase in humans. This is the extracellular flagellated state seen. You can see it is comparable to the size of a red blood cell.

It causes a chronic asymptomatic and untreatable infection. Those are all very important issues when you think about whether or not we should be testing even at all since we don't even screen for Chagas at this point, but for NAT testing at all.

The fact that it is a chronic infection is something that I am going to come back to. It is endemic to portions of Mexico, Central America and South America and there have been several autochthonous cases reported in the U.S., the most recent one just a couple of years ago in Tennessee.

Now, transmission occurs by several routes. The most common one or, actually, the natural transmission, is vectoral transmission. I will show you a picture of the bug in the next slide. It also occurs by blood transmission and there have been six documented cases in the U.S. and Canada. Certainly, there have been several other cases or many more cases that have been missed.

Lastly, I want to point out something that is sometimes lost, the idea of congenital transmission, congenital transmission, mother to child. That occurs probably through one generation but, perhaps, passed down

at

through several generations. That has implications in the U.S.

[Slide.]

This is a good picture of what the reduvid bug, the vector, looks like. In fact, there are better pictures that are much more colorful than this. They are really rather pretty bugs. They are hematophagous so they feed on blood. When they defecate, the parasites are passed in the feces and those are either rubbed into the bit wound, or in the case of this young Brazilian girl, into a mucosal surface, being the eye.

In this case, she has a local reaction, a Chagoma, where the parasite has entered. But the real issue with Chagas' disease and how it affects individuals is when the parasite lodges in the cardiac tissue. These are amastigote stages found in the cells of the heart. This is the place in which most of the disease pathology actually occurs leading to problems with arrhythmias and also to sudden death.

[Slide.]

How does this all get to play in the United States? If we say natural transmission is extremely rare in the U.S., why is it a concern to the blood-banking and transfusion medicine. It all comes back to issues of immigration.

at

So when one wants to talk about emerging infections and how they may play a role, emerging infections can be ones that are newly discovered but they can also be ones that have shifted in their location through the immigration of individuals. Things like Chagas' disease, malaria and other organisms fall into this category.

[Slide.]

Over the last twenty or thirty years, there have been large numbers of individuals who have immigrated to the U.S. from Latin America, Central America, South America as well as Mexico. In the census in 1990, when they asked legal residents--and I say "legal"; keep that mind--where their country of birth was, almost 4.5 million said Mexico. Almost 1.5 million said Central America. About 1.1 million said South America.

The Hispanic population in this country continues to grow, as you can see from some recent census data from the 2000 census in which the population of Hispanics in this country has gone from 22.4 to 35.3. That is not to say that 35.3 million individuals are at risk for transmitting Chagas, but it does show these numbers are growing.

In part, those numbers are growing from people who have immigrated to the U.S. The other issue for

at

individuals who are in this country and who are infected, the potential for congenital transmission cannot be discounted.

[Slide.]

How big a problem is Chagas or what is the real risk in the United States? I put together some numbers. If you want to try to figure out what the nationwide risk is, if we go first with the idea that there are approximate 12 million blood donors per year in the country.

We did a survey several years ago in which we determined individuals who are risk. We found that about 2.5 percent of the individuals in this country are at risk, at risk meaning those who are born or have spent extensive time in an endemic country, that being Mexico, Central America or South America.

That leads you to have about 300,000 at-risk donors in the U.S. Through many of our studies looking at seroprevalence, we know that approximately about 1 out of 625 donors who are at risk will confirm as seropositive. So, out of those 300,000, there are approximately 480 seropositive donors in the U.S.

If each of those, on average, donates 1.6 times per year, then there are 768 seropositive donations per year in the U.S. From those, if we make two components per unit, we have slightly over 1,500 potentially infectious

at

components in the U.S. each year. It is not a trivial amount and certainly something that is worthwhile addressing.

[Slide.]

What about nucleic-acid testing in Chagas' disease, or T. cruzi, the parasite, itself. I posed these three questions, and these three questions can be used, I think, not only for Chagas' disease and T. cruzi but for any other emerging agent which you might want to address.

First of all, is there anything there to measure? It is not worthwhile doing NAT if there is nothing to find. In many of these parasitic agents, you have to keep in mind that they live in relatively sequestered environments. Some parasites are found only in brain tissue, or they are found, as I just showed you, cardiac tissue.

If they are not circulating in the peripheral blood, it is not very likely you are going to be to measure them.

How sound is the technique? We can measure DNA. We can measure RNA. We can talk about reverse transcriptase. There are many different options. Unfortunately, for many of these emerging pathogens, some of the development of tests are still in their infancy. So how sound is the technique and does it really pick up? How sensitive is it?

at

Lastly, and something we are going to talk about, is there a benefit over serologic testing? Just because you can do a NAT test doesn't mean, in each case, that it is better than serologic testing. I hope to explain some reasons why this might be the case.

[Slide.]

This is some data from a study that we did in Los Angeles and Miami. It was over four or five years. It is in the final stage of review in Transfusion. We hope to have it out very soon. I just want to talk to you briefly. I am not going to go through these numbers. Some of you have seen these before.

I want to talk a little bit about the Los Angeles data which involved the study of over 1.1 million donors in L.A. of which using a radioimmunoprecipitation assay we confirmed that 147 of these were positive. They were seropositive. They had antibodies to Chagas' disease.

That is the other thing to consider; just because they have antibodies doesn't mean they clear the parasite. Actually, anyone who works with Chagas will tell you if they have antibodies, they more than likely have the parasite. The seropositivity rate in L.A. was about 1 in 7500.

We asked that very question of these 147 donors, if they had antibodies but they also had the parasite.

at

[Slide.]

So we went about it in several ways. These were actually our transmission studies, or as part of parasitemia testing, we were trying to find if they had parasites that could be likely transmitted by blood transfusion.

We did two things. We brought in the donors, enrolled them in a study. We interviewed the seropositive donors for risk factors. That is quite important and I will come back to that in a moment.

We also drew whole-blood samples for testing for both PCR and hemoculture. Now, I highlight whole blood because, as we begin to think about these emerging agents and how we want to test for them, if we want to use NAT, we have to get beyond the idea of using plasma or sera.

We have intracellular agents some of which are circulating in the blood cells, so we have to figure out what kind of blood sample you will use. In this case, it is whole blood. If you are going to lyse the whole blood, what does that do to the sample? Then you have to lyse the cells, themselves, to get the parasites out. So these issues become more complex as you move into these ideas of emerging agents.

For PCR, there is actually a very nice PCR available that identifies a 330 base-pair product of the

at

kDNA minicircle. T. cruzi, as well as its cousin, Leishmania, have what is called a kinetoplast which is an extra chromosomal chunk of DNA. Each parasite has 30,000 to 40,000 copies of the sequence that you can target.

So, based on that, the sensitivity has been calculated not by me but in the literature as one parasite per 10 mls of blood. So, in essence, it is a very sensitive PCR. But the problem is that there are not that many parasites in the unit of blood that is drawn.

So you also have to consider, when you take your sample, how much sample you are going to test. There may only be one parasite in the unit of blood, so if you test that sample, you may very well likely miss the infection. But, as for the Rickettsia and other agents, one parasite can transmit disease.

Hemoculture, I really won't go into, but it is a blood-culture method not quite as sensitive but what it nicely does it you can see the actual parasites swimming around. So it gives you indisputable evidence of the presence of parasite.

[Slide.]

We had 52 donors enrolled in the study. When we tested them by PCR, 33 of those donors, or 63 percent, were parasitemic or they had PRC-positive results. That is a pretty large number, I would say.

at

One thing to keep in mind here is not all these donors were identified as PCR-positive for one test. Some of these, you had to test more than once. That gets back to the idea of the intermittent nature of parasitemia as well as the fact of low numbers. So a single test alone is not going to identify all the individuals. We may have to do more tests than just three, even.

There are some issues with the hemoculture. It is less sensitive. We had issues of shipping the samples from L.A. I won't go into those. But all three samples are hemoculture-positive or also PCR-positive.

[Slide.]

We looked at some correlations between PCR-positives and PCR-negatives. Is there any way to judge why some would be PCR-positive and some others would be PCR-negative. One of the ideas that comes to mind is that, perhaps, they had more recently immigrated from their endemic country and had circulating parasites swimming through their veins.

So we looked at a number of risk factors. We looked at their age, as I said, years post-immigration, living in substandard housing, recognizing the vector or being bitten by the vector.

What we observed for all these characteristics for the PCR positives and negatives was that there was no

at

significant difference for any of these. But what I wanted to point to here is mean number of years post-immigration. PCR positives, eighteen years post-immigration. The same with the negatives, an average of 20.

So what it tells you is these people are quite long removed from their initial exposure. They were probably exposed when they were young children. So what we are dealing with is a very chronic infection. We are not dealing with active transmission which you see in a lot of the viral agents. So, in that case, there probably isn't a window period to measure. So that makes a suggestion that, perhaps, NAT testing may not be the way to go. But we will come back to that issue.

You might say what good would NAT testing be? Well, it was quite useful and I think it continues to have a role certainly in research and certainly looking at some clinical cases.

This was the Miami transfusion case which we published in The New England Journal a couple of years ago. This involved a multiple-myeloma patient--we just heard about myeloma patients--who was transfused with a platelet unit that later confirmed as seropositive.

Once this had occurred, we followed this individual, or asked permission to follow this individual, through blood samples using both serology as well as PCR

at

and hemoculture to determine if this individual actually became infected with parasite from the transfusion and what was the natural history of the infection.

I should point out the donor who was actually implicated in this infection emigrated from Chili 33 years ago. The first sample we received was at 43 days and was only a serosample which we tested for antibodies using Abbott's Chagas kit. It was virtually near baseline at zero signal-to-cutoff value.

The next sample we received was at 57 days. Once again, the serology was negative but we had a whole-blood sample that we tested by PCR and also hemoculture and it was positive. Every sample subsequent, or thereafter, was positive by PCR and hemoculture.

What we observed as the titers finally began to rise, we didn't see seroconversion until 100 days. So, certainly, there is some type of window period. One has to keep in mind, this is a myeloma patient so we are not sure how she may have been affected. Her immune system may have been compromised, but there certainly is a window phase in there that one could use to measure.

I would also like to say both the donor and recipient not only were serologically positive but they were demonstrably parasitemic.

[Slide.]

at

You could also use NAT testing because it was quite useful in this case. We used restriction fragment-length polymorphisms to actually nail down the relationship between the donor and recipient. These are a number of primer sets, the DNR of the donor and recipient. The T was a totally human reference strain. You can look, particularly in this one, it is very nice, the pattern of the donor and recipient are nice matches and quite different from the other ones.

[Slide.]

So, to summarize, to NAT or not to NAT. First of all, NAT, at least for T. cruzi and, perhaps, other agents, is actually very good at identifying circulating parasites, if they are there. So it is quite good for identifying active parasitemia but we get into those questions about how high are the numbers, are they there, and so forth.

As I just showed you, it is good for using it for donor-recipient matches. It is also highly specific and sensitive, but there are those questions, then. What about the sample source is an issue. Are we going to use whole blood? How are we going to treat the blood? How are we going to get the parasite out? Is there enough there to measure, and so forth. That is the same thing; whole blood versus sera or plasma.

at

This is a relevant point I think that needs to be brought out for other emergent agents as well, whether it be Ehrlichia, Babesia or any other agent which we might test for down the road.

Lastly, I would say that it does have, at least in this case, limited benefit over serologic testing. First of all, there is very rare active transmission in the U.S. so what we are looking at is a chronic infection in individuals who immigrated here years and years ago. So, for that case, in Chagas' disease, they have lifelong, very high, antibody titers. Based on that, serologic testing along is probably sufficient for Chagas' disease.

Thank you.

DR. NAKHASI: We have time for a question or two.

DR. GALLARDA: Why aren't we screening for Chagas' antibody?

DR. LEIBY: Why aren't we?

DR. GALLARDA: At those prevalence rates; yes?

DR. LEIBY: That is a good question. That is something that is under active consideration, I think, by the Red Cross and other organizations. Certainly, with the transfusion cases, the chronicity of infection, it is something, I think, that needs to be considered. Perhaps in the near future, we will see it.