donations in the magnitude of 4,000 so we are hitting things close to our detection limit.

This may be an outlier. It may also be in support of what Andy said where we have got donations that are really quite high in titer that are still antigen-negative. All of these are antigen-negative. This is the index donation that was positive for NAT only.

So, even if you don't necessarily count this 312,000, we do have several that are much higher than the 10,000 to 20,000 range and these are still antigen-negative. So it brings up some questions about the real robustness of the p24 antigen assay.

[Slide.]

So the conclusions; we have seen the reduction window period for those donors that are initially positive by NAT relative to the p24 an average of 5 days, the range being from 3 to 7 days. Relative antibody test; we have seen an average of 10.7 days with a range of 9 to 14 days. We haven't seen any false positives. As I showed you on the table, all the donors that we have been able to follow up, or we have follow-up information on from subsequent donations, we have corroborated that initial NAT reactive by some other HIV assay.

So I think these data support that pooled NAT is superior to individual donation testing for antigen.

Thank you.

DR. HOLLINGER: Thank you. I just had a couple of questions. You said you had 2.1 million donations. How many donors did that represent?

MS. MASECAR: We get a lot of applicant donations in there so it is probably in the range of 50,000 to 200,000 donors but I don't have that exact figure. I would say--

DR. HOLLINGER: 50,000 to 100,000?

MS. MASECAR: 50,000 to 200,000. 50,000 donors would be conservative.

DR. HOLLINGER: You initially, I think, said that you did pools of 96 and a 95 percent detection rate of 91 copies per ml, I think you said.

MS. MASECAR: No; in copies per ml at 95 percent, it is 10 to 20 copies per ml. 91 IUs.

DR. HOLLINGER: Okay. Again, explain to me; you had some samples in there that were 4,100 and 4,300, yet it seemed to have detected those?

MS. MASECAR: Yes, because our cutoff in copies would be 4,000. So we were detecting initial index donations right around that cutoff.

DR. HOLLINGER: So your lower copy level is what? How much is the copy level then from IUs?

MS. MASECAR: That would be a multiple. It is somewhat of a range. We have only done a couple of assays to correlate copies to IUs. It is a factor of about 7, I think. The scientist that did the work is here in the audience. I can get an actual number for you.

DR. HOLLINGER: And the IUs are from the NIBSC standard?

MS. MASECAR: Yes; that's correct.

DR. HOLLINGER: Anything else? Thank you.

The next presentation, Don Baker from Baxter Hyland Immuno.

Presentation

MR. BAKER: One of the great things about these meetings is the surprises. One of the biggest surprises you can get is to find that you are on the list as a presenter when you were not expecting to be presenting.

I had attempted to provide the committee with a written presentation which, I hope, was part of your presentation materials. If it wasn't, the fault is mine because I was on vacation and I got it late.

I will attempt, if it is not in the presentation materials, to summarize possibly the most pertinent point. Our PCR NAT IND was not constructed to directly address this question of the relative sensitivities of p24 versus PCR testing. Our test system was designed, our IND was designed, so that any donations which were--oh; good. I see the committee does have the materials.

In that case, I am going to be sure that you have all read it. And so are there any questions?

DR. HOLLINGER: Any questions? I guess there are no questions.

The next speaker is Sue Stramer from the American Red Cross. Susan?

Presentation

DR. STRAMER: Thanks, Blaine. I guess that was a hard act to follow.

[Slide.]

I represent the American Red Cross. We do collect plasma. However, we also are one of the two major whole-blood collectors in the United States. In our IND for NAT, we did include p24 antigen replacement. So my presentation today will focus on two areas, one, in collaboration with Mike's presentation earlier.

The first thing you will note in this slide is I included two dates because the topic of p24 antigen replacement by NAT was addressed in an earlier Blood Products Advisory Committee meeting on March 25. Unfortunately, I have a typo there. I will not be here presenting on Saturday.

[Slide.]

As part of background material; since the implementation of HIV p24 antigen screening in March of '96, only six antigen-positive window-case donations have been identified at the Red Cross. You will see later in the presentation--I read ahead--from ABC that they have identified four. So, in the national experience since March 14, 1996, the date of licensure, in the whole blood industry we have identified ten p24-antigen-reactive units.

Due to the low antigen yield and improved sensitivity of NAT screening for HIV-1, replacement of antigen with HIV-1 NAT should be possible.

[Slide.]

So, from the Red Cross side of the house, we have screened 27.5 million donations. You will see the same 27.5 million donations ironically enough from ABC, so we do have an even split. Of those screened, over 10,000 were tested by neutralization; that is, they were repeat-reactive. This represents 7,500 plus index donations totally to about a 0.027 percent repeat-reactive rate for the test.

We do donor reinstatement for p24 antigen, so, of these on index, about a third, 2,200, came in for follow up to see that they remained antigen-indeterminate and then were eligible for reentry. However, reentry is a relatively low yield proposition for antigen because you will see higher numbers as well. But, in our total study, 53 percent of antigens who do present a second time remain antigen-indeterminate. So there is a biological false positivity associated with the test.

Of this total, we have 316 confirmed positive donations; that is, 3.1 percent of repeat-reactives or 0.001 percent of the total screened.

[Slide.]

Of the 316 confirmed positives, this is the breakout. And this breakout goes until August 31 of this year. 158 were false positives. As Mike alluded to earlier, false positivity is commonplace in this test. We defined these as false positive based on RNA-negativity, RNA PCR testing performed by Mike Busch at Blood Centers of the Pacific, lack of seroconversion in these donors, and nonreproducible antigen-neutralization results.

In our SOP for donor counseling, we repeat the antigen-neutralization test because most frequently the false positives do not repeat. So we can assure the donor, "You are likely not to be infected." We could not even reproduce the antigen-neutralization result.

A subset of these 62 of the 158 were also tested for reverse-transcriptase activity which would indicate the presence of the retrovirus. I will show you there was data in detail, but none of those 62 were positive for reverse transcriptase by a CDC-developed AMP RT assay.

Of the 316, 152 were antigen-positive and six, as

I mentioned before, were recently infected seroconverting donors. So the yield for the Red Cross is 1 in 4.6 million. I should mention, one of these was an ABC donor, so if you add this one ABC to the four that ABC will show, that makes five from ABC, five from ARC, out of the same number of donations tested, so exactly an even split.

Of these six, one was detected following NAT implementation. We implemented in March of '99 and, since then, we did detect one donor who was both NAT an p24-antigen-reactive. I will also show you one donor that we detected on index that was NAT-reactive but not reactive by p24 antigen until we acquired follow-up samples.

As Dr. Busch showed, but I will show data also collected from the Red Cross, we did not observe a magnet effect with this test. And that was done by looking at increases in HIV antibody testing before implementation and post-implementation.

[Slide.]

If you look at the two populations I have just mentioned, antibody-confirmed positives and antibody-negatives, and look at their percent neutralization results for the 40 percent assay cutoff, you can see that antibody positives have a higher percent neutralization than to antibody-negative samples, or these are the false positives.

This subset here of 158 also includes the six

at

window-period donations that I showed you. So the median percent neutralization here was about 95 percent but you do see, and this represents one sample that we did have that was antibody-positive that did neutralize at a 40 percent neutralization.

Here the mean neutralization was much lower, at 51 percent.

[Slide.]

I mentioned before the false positives lacked evidence of other retroviral infection. Again, this was performed using and AMP RT test developed by the CDC. As controls, we included 28 positive samples by HIV-1 RNA that were also positive by the AMP RT test. These included antibody-positives and four seroconverters that we had from our yield samples.

The false-positive samples, which were all negative by HIV-1 RNA, were also all negative by the AMR RT test. So, again, these false-positive samples have no evidence of other retroviral infection.

[Slide.]

One other question that we tried to address, Red Cross in collaboration with the REDS study, is what is the meaning of antigen-indeterminate donors. Are these infected with HIV? So what we did was we took up subsets of the donation screened, so we started with about 7 million

at

donations screened, which resulted in 1,500, approximately, or 0.022 percent repeat-reactive indeterminate donors. There is no criteria in the test for a negative. You are either confirmed positive or indeterminate.

We submitted available samples for PCR testing and, of those samples submitted for PCR testing, there were none that were positive by PCR. So all indeterminates appear to be not infected with HIV.

Of those that we had, 38 percent, who did provide follow-up samples, those were antibody-negative and, in this subset, actually, a higher number than the 53 percent I showed earlier. 77 percent of these donors, on follow up, remained antigen-repeat-reactive but did not neutralize.

[Slide.]

The question of the magnet effect was addressed by looking at the antibody prevalence for the six months post-implementation of p24 antigen and comparing that to six months within the same--the same six months the year prior to. So we adjusted the data four times so that they were the same six-month periods looked at pre-implementation and post-implementation.

You can see the numbers of antibody-positives for first-time donors and for repeat donors. And you can see the rates here, 7.3 per 100,000 pre-licensure, 6.8 per 100,000 post-licensure, and there was no significant difference so there was no antibody test-seeking that we could determine.

[Slide.]

The other part of this presentation will focus on what studies we have performed to show the redundancy or lack of a need of p24 antigen. We have taken our antigen-yield samples and NAT-yield samples--actually, in the data I will show, there is only one, but we have subsequently added others. We have diluted these 1 to 128 in RNA-negative plasma.

The Red Cross IND had two phases. One was a phase in which we tested pool sizes of 128 and now we are testing pool sizes of 16. But all the work I will show you is a pool size of 128 which actually represents worst case because it is a eight-fold or greater dilution.

We have also looked at 25 commercial seroconversion panels tested neat and tested at 1 to 128 dilution. These were in our IND. As an additional control, with each run of NAT that we perform, we run a p24 antigen external-run control sample. Although we call this p24 antigen external-control sample, it is not reactive for p24 antigen. That is because of the way it is prepared.

We have taken three antigen-positive antibody-negative units and pooled then. They had an S to CO 1 to 2 by Coulter but were actually negative by the Abbott test.

The pool of these three was diluted 1 to 16, and at this 1 to 16 dilution, this pool was no longer reactive for p24 antigen. But it did have an RNA concentration equal to or greater, depending on the manufactured lot, of 2,400 copies per ml.

We do not accept a NAT run for release unless the p24 antigen run control is reactive. To date, at least to August 31, again, we have 6,674 runs. No run has ever not been accepted because of a failure of this external-run control. So, in all of these runs, the p24 antigen control has been reactive.

[Slide.]

Before I show you the dilutions, let me show you our NAT yield samples or I should say, in this case, our antigen yield samples. These are the first five antigen yield samples identified by the Red Cross. These are the days of follow up following the index donation.

This column is the RNA concentration and, hopefully, these are visible. These are p24 antigen signal-to-cutoff ratios. You can see, in gold, where p24 antigen is reactive. I have asterisked the peak antigen load. You can see that, in each case, it corresponds to the peak antigen signal. Even when antigen has a lower reactivity, you can see at least 10^4 and, in this case, 10^5 RNA copies per ml.

This is the percent neutralization. This is the antibody results and Western Blot results, again antibody always come up after p24 antigen and NAT.

[Slide.]

This is the six antigen yield samples, same scenario, maximum antigen concentration, maximum NAT concentration, first positive 10^5 copies per ml. This sample here is our first NAT yield sample. I show it because we did have follow-up samples here where this donor did undergo the p24 antigen reactivity and then reversion to negativity.

Here, the first antigen signal and an S to CO of 1 by the Coulter test did correspond to 4 times 10^5 copies per ml. Again, here are the antibody results and Western Blot results.

[Slide.]

If we took three of those samples, and actually one that we got from southeast Wisconsin, the first antigen-negative NAT-reactive sample identified since the NAT INDs for whole blood were implemented, this one detected in a pool of 24. What we did was diluted these 1 to 128 then to see if they were still reactive.

This is the initial viral load. This one first detected by NAT, therefore, having a lower viral load. But they were all detected at a pool size of 128 including the additional samples that we tested. Here you can see the signal-to-cutoff ratio by the GenProbe TMA assay. The one with the lowest viral load actually did have a little bit lower S to CO but, certainly, 7 is nowhere near the cutoff.

[Slide.]

This is the slide Mike showed, but I am going to show it for a different reason. Here, you can see the two dates prior, NAT pickup prior, to p24 antigen.

[Slide.]

This now adds the GenProbe NAT data, either a single unit testing, which is in the solid symbols, or as a pool size of 1 to 128. Here the qualitative test by TMA was a little bit more sensitive than quantitative PCR. However, quantitative PCR hit the same exact sensitivity as the pooled NAT at a dilution of 128 both of which were a two-day improvement over p24 antigen.

[Slide.]

If you look at all of the seroconversions that we looked at for our IND, which totaled 25 individuals having 92 seronegative bleeds, and you look at the question of assay reactivity by NAT versus p24 antigen--so, again, these are all the seronegative bleeds--the green symbols show you those that were positive both by NAT and by p24 antigen.

The yellow symbols here, the yellow circles, show you those that were only positive by NAT and could not be detected by p24 antigen. So, in this analysis, there were 24 NAT samples, samples that were NAT-positive but negative for p24 antigen, and there were no samples in the reverse category; that is, antigen-reactive but NAT-negative.

[Slide.]

If you take these samples and dilute them 1 to 128, you virtually get similar results, or identical results, with the exception that fewer samples remain NAT-positive. Here 21 samples were NAT-positive but p24 antigen, but this is NAT at 1 to 128 dilution. Again, no samples that were antigen-reactive and NAT-negative.

[Slide.]

If you look at all of the samples in the panel--that is, antigen-positive and antibody-positives--and we tested by two lots of the GenProbe reagents, we detected 162 in one lot and a 167 NAT-reactive samples undiluted.

Performing a dilution of 128, we detected subsets of this 162 of 145 or 90 percent and 148 or 89 percent on the second lot. If you look at the subset now that was p24-antigen-reactive, only 57 or 55 percent of these samples were p24-antigen-reactive. The data on this side just shows the similar relationship for ATL, that these tests are really antiquated and we have probably no need to be doing them.

[Slide.]

Now, to address the external run-control sample, the Red Cross, in addition to running the p24 antigen run control sample with each run also runs and HIV and HCV and a negative member. This is the concentration of our RNA NAT-reactive run-control samples. Of four lots manufactured for p24 antigen, using this same target for manufacture, we have a range of copies per ml for this external run-control sample of 2,400 to 6,800.

[Slide.]

This shows you the distributions of those four external run controls. Here are the negative samples. Here are the HCV-reactive samples. You can see that HIV, whether it is the NAT control or the p24 antigen control, virtually run very similarly. Actually, the p24 antigen control runs a little bit hotter.

[Slide.]

So, in conclusion, from that data I have showed, high RNA titers corresponding to equal to or greater than 10^4 copies per ml correspond with p24 antigen positivity. There was no p24-antigen-reactive sample that was found NAT nonreactive even using a pool size of 128. And we saw earlier detection and detection in HIV antibody-positives.

So NAT, even using pools of 128, which we are no longer doing, is more sensitive than screening with the currently licensed tests for HIV p24. According to these data, the antigen test could be replaced by the use of licensed pooled NAT.

[Slide.]

In combination with my data presentation, I am going to make the Red Cross position statement on the replacement of p24 antigen with NAT. Red Cross seeks to support its patients with a blood supply of the highest quality and safety. We also support the replacement of p24 antigen testing--that is, when the discussion emerges for whole blood--with NAT provided the following conditions are met.

We have a licensed test. The test is fully compliant with all cGMP features. It has full automation or at least the maximum automation that we can obtain, including positive sample ID and the test is at least as robust as the current licensed screening tests we use today.

Thank you.

DR. HOLLINGER: Thank you, Sue.

Any questions of Dr. Stramer? Anyone?

Sue, again, just for my information, the p24 antigen, what was, again, the range for the copies per ml for the p24 antigens that were positive and antibody-negative?

DR. STRAMER: From the six that we had? They were 10^4 to 10^5 copies per ml.

at

DR. HOLLINGER: So those were fairly high.

DR. STRAMER: Yes.

DR. HOLLINGER: But there were only six that you had.

DR. STRAMER: Yes; right. But that is why we did the larger sampling of the 92 seroconversion panels.

DR. HOLLINGER: And that ranged what?

DR. STRAMER: That ranged comparably to where we--well, actually, it covered the entire dynamic range because we started with antigen-negative NAT-positive samples. So it covered the entire dynamic range.

I didn't do the regressions as Mike did so I can't tell you what the antigen cutoff was, but I wouldn't be surprised if it paralleled the data that Mike showed pretty exactly.

DR. HOLLINGER: Thank you. Thanks very much, Sue.

The next presentation is by Dr. Schochetman from Abbott Laboratories.

Presentation

DR. SCHOCHETMAN: Thank you.

[Slide.]

As we contemplate whether to decide to continue or discontinue antigen testing in the plasma fractionation business, I would like to have you keep some things in mind. The first is that the current antigen assay is really not the ultimate in terms of sensitivity to which this assay can be driven. The second is that, as we contemplate this and since it may take a certain amount of time to implement any possible discontinuation, that we don't send negative or disincentive messages to manufacturers so that they will not put the effort in to develop more sensitive single-unit antigen testing.

[Slide.]

Our goal, actually, or our objective, is to develop more sensitive HIV antigen assays in the short term to make these assays comparable in sensitivity to pooled NAT testing and, in the long term, make this sensitivity equivalent to single-unit NAT testing.

I should say--I gave a talk somewhat similar to this at last year's BPAC meeting sort of giving the alternative view. I am getting to feel a little bit like salmon going upstream, and that is it seems like possibly a futile attempt, but you have this genetic urge that you must keep doing this.

[Slide.]

What I want to do is share with you some data on a research assay for our automated prism instrument that already has increased sensitivity. I should say that this is, by no means, as far as this assay can be driven.

As you can see here, this is our current antigen assay that is on the market. We estimate that the sensitivity of that antigen assay, using an internal Abbott standard, is in the neighborhood of around 7 to 10 picograms per ml. If one makes the assumption that a picogram is in the neighborhood of 10,000 copies per ml, then we are looking at the ability to detect a sample with somewhere between 70,000 and 100,000 copies.

At a dilution of 1 to 96, we would be looking at somewhere between 725 and 1,041 copies and, at a dilution of 1,200, we would be looking at 58 to 83 copies. The current research prism antigen assay is several-fold more sensitive already and is in the range of 1 to 2 picograms which would have an sensitivity, in terms of RNA copies per ml, of about 10,000 to 20,000 copies and a dilution of 1 to 96 would be somewhere in the range of 100 to 200 copies and 1 to 1200 would be somewhere in the range of about 8 to 16 copies.

[Slide.]

Assuming the sensitivity of a picogram being about 10,000 copies of viral RNA, then, if you look at a NAT assay with a sensitivity of about 50 copies per ml, then, at a dilution of 1 to 96, that sample would have to have a starting copy number of about 4,800. If you are looking at a dilution of 1,200, the starting copies would have to be in the range of around 60,000 copies per ml.

[Slide.]

What I would like to do is show you here some real data on early seroconversion samples from commercially available seroconversion panels. What you can see here is samples from each of those panels. The letter or the number after the slash represents the particular bleed.

You can see here we selected these because they were all negative by the current Abbott test. You can see here a signal to cutoff of less than 1 which would make it nonreactive. Yet, in our research prism antigen assay, these were easily detected. You can see here the published information on the NAT data for each of these specimens.

If you look at these specimens at a 1 to 96 dilution, the copies per ml range from about 122 up to about 1,000 copies per ml. But, at dilutions of 1 to 1,200, we are getting down into the range of 10 to about 80 copies per ml and it begins to push the sensitivity of the NAT assays as they currently exist.

[Slide.]

I think when one looks at an antigen assay such as the one we have now and are continuing to drive the sensitivity of that assay even further, that the advantages of such an assay is that it is already a fully automated system for antigen testing, and one gets really quite rapid results. There are already in place process controls for enhanced GMP compliance. There is really little or minimal sample preparation and, certainly, the issues of contamination or carryover that one potentially has with NAT testing are really virtually nonexistent.

The ability to confirm using a neutralization test exists. Certainly, for simplicity of implementation, there are no pools to dilute the sensitivity, no dissection of pools, no shipping of pooled specimens, et cetera, et cetera.

[Slide.]

On the final slide, I think I would like to leave you with sort of two messages. One is that the gap between individual antigen testing using more sensitive antigen assays and NAT for pooled samples really may not be as significant as we may like to think and that, as opposed to thinking about discontinuation, I think maybe one ought to really send a more positive message to manufacturers that they should be encouraged to develop ultrasensitive antigen assays with sensitivities equal to or greater than single-unit NAT testing.

Thank you.

DR. HOLLINGER: Questions? I have a question. Have you had a chance to compare this with actually many of the NAT-positive antigen-negative samples, a large number of the ones which have now been positive by NAT and negative by antigen, to see what percentage was actually picked up with

at

the new technique, the prism technique, and so on?

DR. SCHOCHETMAN: We are trying to obtain some of those samples and we understand there is little, if any, available. But we certainly would love to be able to look at those. I think maybe something that we would certainly like to do is actually try to set up sort of a head-to-head comparison going into very high-risk populations where the incidence would be extremely high, probably in the 7 to 14 percent range, and be able to look at many more early seroconverters and to do probably a better study.

But we certainly would be happy to look at any of the NAT-onlys. We have, so far, been unable to really get access to any.

DR. HOLLINGER: Thank you.

We have two more. Dr. Fang from Chiron Corporation?

Presentation

DR. FANG: Thank you, Mr. Chairman

[Slide.]

I am Chyang Fang from Chiron Corporation, the Blood Testing Division of Chiron Corporation. Chiron Corporation is a biotechnology company headquartered in Emoryville, California. We currently supply the NAT assay for blood screening on more than 70 percent of the nation's blood supply under an FDA-approved IND application.

at

The Chiron TMA HIV-1 HCV assay is a multiplex assay for simultaneous detection of HIV-1 HCR RNA in human plasma. This assay is developed and manufactured by GenProbe Incorporated located in San Diego, California.

Today, I would like to present data in support of the notion of discontinuation of routine HIV-1 antigen testing after a NAT assay is approved by the FDA and implemented for routine blood screening.

The first part of my presentation is to show that all p24-antigen-positive donor specimens from three studies were also positive with the Chiron TMA assay. The second part is to show the analysis of the Chiron TMA assay on different subtypes of HIV-1.

[Slide.]

I collaboration with Professor Anton Hans of the South African Blood Transfusion Service, we have conducted a study in South Africa. In South Africa, about 1 million blood donations are collected annually by seven blood centers; that is, one large center, two medium sized and four small centers.

About 90 percent of the donations are from they call it low-risk donors while the remaining 10 percent are from high-risk donors. The low-risk donors are many from the white and the Asian population and the high-risk donors are many from the black and colored populations. These are the terms used in South Africa.

The overall HIV prevalence was reported at 0.21 percent in 1997 and 1998. However, the rate among the high-risk group was more than 100 times higher than the low-risk group. In 1994, the risk of HIV transmission through transfusion in South Africa was estimated at 2.2 per 100,000. This number was much higher than the United States and other developed countries.

All donations are routinely screened for HIV-1, p24 antigen, HIV-1 to HCV antibody, HBsAg and syphilis serology with a double EIA strategy. That means, a second EIA test is used to confirm the initial reactivity instead of neutralization, Western Blot or RIBA.

[Slide.]

For the study, about 10,000 samples from low-risk donors and 10,000 samples from high-risk donors was collected and shipped frozen to Johannesburg Laboratory for TMA testing using the single-unit testing. Samples were provided by all seven blood centers, the contributions proportional to their collection volume. Routine laboratory results and donor demographic information was collected prior to the breaking of the linkage.

All serology and TMA-reactive samples have been shipped to Dr. Michael Busch's laboratory at the Blood Centers of the Pacific for further testing.

at

[Slide.]

A total of 20,620 donor samples have been TMA tested. Of this, 54 percent are from the high-risk group. 62 percent is from male donors and 15 percent from first-time donors. The higher percent of the first-time donors for the study population was mainly due to a higher proportion of the samples which were from the high-risk groups, particularly for the female high-risk groups. We suspect that there is some test seeking in this group.

[Slide.]

For the purpose of this meeting, there were seven HIV-1 p24-antigen-positive samples identified. Those are the TMA-positive and the p24-antigen-positive. That group is the TMA-positive, antigen-positive, but also antibody-positive as a total of seven donor samples.

All of the seven samples are from high-risk groups, six from males and one from females. Five are also positive for antibody and one was antibody-negative and antigen-negative--no; I'm sorry. One is also antibody-negative but antigen-positive.

Although the additional testing has not been completed yet in order to confirm this reactivity, but all seven p24-antigen-positive samples were also positive in the TMA testing. In addition, there is also one sample from a high-risk female donor which was positive only in the TMA testing.

[Slide.]

Data from this study will be presented at this year's AABB annual meeting in November by Dr. Nel. The preliminary conclusion includes that the role of routine HIV p24 antigen testing should be reevaluated.

[Slide.]

In a second study, Professor Jean Pierre Allain of the Cambridge University in U.K. has collected donor samples from Ghana and Durbin, South Africa, for single-unit and pooled TMA testing. Seroreactive samples were excluded from the study. However, some samples were identified as positive for HIV-1 RNA by the TMA assay. Most of the samples were purposely included as a positive control.

This included four p24-antigen-positive for the South African population and six antibody-positive from the Ghana population. Two additional samples from the Ghana group were TMA-positive but negative for p24 antigen and antibody. All twelve HIV-1 RNA-positive samples were confirmed by their in-house RT PCR methodology.

[Slide.]

A third study in the U.S. under the FDA-approved IND, the AIBC is currently testing about 35,000 donor samples monthly from 22-plus centers at the Citrus Blood Bank in Florida using the TMA assay.

at

From April, 1999 to August of this year, about 600,000 donor samples have been tested in pools of 24. Six samples were identified as positive for HIV-1 p24 antigen. All six samples were also positive with the TMA assay. All six were also reactive in the HIV antibody EIA, but only two were confirmed by Western Blot.

[Slide.]

A switch to the sensitivity. In terms of the TMA sensitivity for different HIV subtypes, we have compiled data from several assay performance evaluation studies around the world. Samples were from various sources and of different origins. Testing was conducted by six different laboratories. The viral load, or the copy per ml numbers, were provided by the testing lab or measured against the international standard.

This slide shows that the sensitivity of group M, subtype A, specimens. The data show that TMA could reliably detect type A specimens below 100 copies per ml.

[Slide.]

This slide shows type B specimens. Again, the TMA assay can detect all samples including those at the 100 copies per ml.

[Slide.]

This again is type B specimens to show that TMA can detect samples at a sensitivity less than 100 copies per ml. The samples have been arranged in descending order of the copy per ml number at testing. This column, basically, is tested once and then, again, positive results at the 1 to 100 dilution and so forth.

[Slide.]

This lists all the type C specimens. Again, TMA can detect samples at the viral load of 100 copies per ml or less.

[Slide.]

This lists all the type B samples that have been tested, down to less than 100 copies per ml.

[Slide.]

These are the type E specimens.

[Slide.]

This lists the type F, G and H. For type H, only two specimens were tested, once each by two different labs. Both samples had a viral load of higher than 100 copies per ml. This slide also shows that the TMA assay could detect group or specimens at 100 copies per ml or less.

[Slide.]

This slide summarizes the data from the previous seven slides. Overall, data from the six evaluation studies demonstrate that the TMA assay can reliably detect all HIV-1 subtypes at 100 copies per ml except for subtype H where insufficient data were available.

[Slide.]

Finally, one of these evaluation studies also included 30 HIV-1 specimens with different recombinant subtypes from around the world. The viral load of these samples were unknown. However, all samples were detected as positive by the TMA assay.

[Slide.]

In conclusion, we have shown that, from these three studies, all 17 HIV-1 p24-antigen-positive specimens were also positive in the Chiron TMA assay. Second, the Chiron TMA assay has a greater than 95 percent detection rate at 100 copies per ml for HIV-1 subtypes.

Thank you.

DR. HOLLINGER: Thank you. Any comments? Dr. Nelson?

DR. NELSON: We have seen all the various subtypes of HIV-1. How do both p24 antigen and PCR work for HIV-2?

DR. FANG: TMA has no detection of HIV-2.

DR. NELSON: Does p24 antigen? Will the licensed p24 antigen detect HIV-2?

DR. NELSON: Dr. Schochetman?

DR. SCHOCHETMAN: It can detect HIV-2 but not to the limits that it does HIV-1. We actually have some new reagents that will detect all the HIV-1's and the HIV-2's down to very low limits, now.

at

DR. HOLLINGER: An important point. Thank you.

DR. HEWLETT: May I add something?

DR. HOLLINGER: Yes. Dr. Hewlett?

DR. HEWLETT: I wanted to add that the licensed antigen assays don't have a claim for HIV-2.

DR. SCHOCHETMAN: Right.

DR. HEWLETT: So we really don't have a good sense how cross reactive they are with each other.

DR. HOLLINGER: I'm sorry. I can't hear you.

DR. HEWLETT: The licensed antigen assays are not labeled for sensitivity for HIV-2 so, at this point, I don't think we really have a good sense and good data to support cross-reactivity for HIV-2.

DR. HOLLINGER: Thank you.

The next presentation is by Dr. Bianco from the America's Blood Centers. Celso?

Presentation

DR. BIANCO: Thank you, Blaine.

[Slide.]

Thank you for the opportunity to present our experience here.

[Slide.]

America's Blood Centers is an association of 75 not-for-profit community-based blood centers that collect about half of the blood supply from volunteer donors.

at

[Slide.]

The results that I am going to present are summarized--these results were a survey or our centers. There were actually two surveys that were carried out in September. The period that they cover is from the introduction of HIV-1 p24 antigen. That was March, 1996, to August, 2000.

We received reports from 74 of the 75 centers in the survey about the detection or identification of HIV-1-p24-positive antibody-negative donors. This represents 27,525,000 collections. That is 98 percent of the total whole-blood and apheresis collections that these centers have for these periods.

I have more detail in the survey from 60 ABC centers.

[Slide.]

These 60 centers tested 22 million donations over the period. There were 3,425 samples that were repeatedly reactive for p24 antigen. Of those, 174 samples were positive on neutralization representing about one in each of twenty samples that were tested, that were repeatedly reactive, were positive on neutralization.

[Slide.]

This is just a pie chart representing more or less the same thing; that is, a very proportion of the samples

at

that are repeatedly reactive become positive on neutralization.

[Slide.]

This is a breakdown per year. You can see that it has been a more or less constant set of data. We have between 4.4 and 5 percent or 6 percent variation in terms of repeatedly reactive samples that neutralize. There were four among those centers' samples that actually were antigen-positive antibody-negative among those 174 neutralized samples.

[Slide.]

This is a bar graph just representing the same thing in the distribution per years. There was one sample identified in 1996, two samples identified in 1999 and one sample identified early this year.

[Slide.]

These samples came from all areas of the country, two from the Pacific, one from the Northeast and two from the Southwest. The last sample was detected after the introduction of NAT testing for HIV and so the sample was tested for both. The donor did not come back for follow up. All the other donors seroconverted, were neutralization-positive and two of them were PCR-positive.

[Slide.]

So the yield between March, 1996 and August, 2000, of over 27.5 million donations were four positive individuals for HIV-p24 antigen-negative on antibodies. So the ratio, the yield, here, is one in about 6,880,000 samples.

[Slide.]

In summary, HIV p24 was introduced as an interim step for shortening the window for HIV until molecular assays became available. And we heard very good summaries from Dr. Hewlett, Dr. Busch, and also from Sue Stramer. ABC identified one in 6,880,000 as window cases detected by the p24 antigen; thus, the yield is very small.

[Slide.]

In conclusion, NAT for HIV is much more sensitive than p24 antigen even when performed in pools of 16 to 24 specimens. NAT has been successfully performed under FDA-approved INDs and ABC centers support the elimination of the requirement for HIV p24 antigen screening when NAT for HIV is performed either under an approved IND or with an FDA-licensed test.

We understand that the position of FDA is for an FDA-licensed test but we would like to request that, for donor pools of 16 and 24 samples, that the same consideration being given to source plasma be given to whole-blood donations and apheresis.

Thank you.

at

DR. HOLLINGER: Thank you, Celso.

Any comments for Celso? Questions? Thank you.

This concludes the individuals who have asked to speak to the issues here, but I am just going to open it up for a second to the public for anyone else who wishes to present any information on this aspect of the question. If there is someone else that wants to speak about something, I will get to that in a minute.

Does anyone else wish to make a comment?

DR. CONRAD: I just think it is very important--I don't know if the issue of the false-positivity--it struck me as remarkable that that is the other side of this equation. I think that the polymerase chain reaction or TMA is very sensitive, but I also think the specificity shouldn't be overlooked and that some of the slides that Dr. Bianco and Dr. Stramer showed, there was an enormous number of people being told that they are potentially at risk for having HIV and that are not being confirmed.

I think many of those donors are permanently deferred. That is a pretty terrible position to be in. So it is not just whether or not NAT is more sensitive and imparts safety on the blood supply. It is also that it is probably more specific and imparts piece of mind on the donors.

DR. HOLLINGER: Thank you.

at

I want to get to just one final presentation and then we are going to take a break. Alpha 1 wants to make a presentation on the availability of therapeutic products. This is going to be Ms. Miriam O'Day.

Presentation

MS. O'DAY: Hi. First of all, I wanted to say that I am here today--I have changed my affiliation. I am Miriam O'Day. I am Director of Government Relations and Regulatory Affairs for the Alpha 1 Foundation. Thank you for the opportunity to comment today regarding the availability of therapeutic products for the treatment of alpha 1 antitrypsin deficiency.

The Alpha 1 Foundation is a national not-for-profit organization whose mission is dedicated to providing leadership and resources that will result in increased research, improved health, worldwide detection and a cure for alpha 1. The Alpha 1 Foundation works closely with the Alpha 1 Association to promote awareness and further advocacy goals.

AAD is a single-gene defect leading to the loss of one serum protein requiring augmentation therapy. Currently, the AAD protein is replaced with an intravenous plasma-derived product which is produced by a sole manufacturer. Because extreme product shortages have been experienced within the alpha 1 community, with supplies

at

decreasing to between 40 to 80 percent of the prescribed dosage, the Department of Health and Human Services Advisory Committee on Blood Safety and Availability passed a number of resolutions to recommend strategies for amelioration of this crisis.

On August 24, the Foundation provided a report to the advisory committee regarding the status of their recommendations which I will summarize for you. First, the advisory committee recommended that methods be developed to optimize and standardize allocation of available product in an equitable manner including the management of emergency supplies and programs that distribute products directly from manufacturers to registered consumers.

Distribution of Prolastin, the AAD augmentation therapy, was addressed by the alpha 1 community which included the Alpha 1 Association, the Alpha 1 Foundation and the Foundation's Medical and Scientific Advisory Committee.

In response to consumer requests, they have implemented a direct-distribution strategy. Prolastin is no longer sold to distributors. It is allocated directly from the manufacturer to the consumer insuring that the fully prescribed dosage is available to each alpha as long as there is product available.

All alphas enrolled in the program have received their full prescription at 28-day intervals since November of 1999 resolving the need for reduced dosages or increased intervals between infusions. In addition, over 200 consumers that did not have access to augmentation therapy prior to the establishment of this program are currently on product. Direct distribution of a one-product, one-manufacturer, community has resolved inequities and directly related to the decrease of the severe shortages.

The direct distribution from manufacturer to consumer has proven beneficial with regard to safety as well. Direct distribution has allowed for the swift and accurate notification of recalls and withdrawals. For example, information regarding the recent recall of CliniPads due to the confirmation of bacterial contamination within some lots reached all alpha-1 consumers within 48 hours.

I would like to note that the Foundation continues to support the voluntary patient notification system and per capita has the greatest number of consumers enrolled in the PNS. Second, the advisory committee recommended that industry explore with the FDA strategies for reallocating partially processed plasma materials from one manufacturer to another in order to optimize production of alpha-1 antitrypsin and other plasma derivatives.

The Foundation was able to work closely with industry on this issue and, on behalf of the alpha-1

at

community, we would like to thank the American Red Cross and Baxter for working with Bayer in a cooperative relationship to provide enough raw materials to reach full production capacity at both of Bayer's manufacturing facilities.

We caution, however, that this does not indicate that the end product or the throughput will satisfy demand nor does it resolve the uncomfortable feeling within the alpha-1 community of reliance on one manufacturer. This dependency leaves us vulnerable to possible GMP problems and accentuates the delicate balance between supply and demand.

Third, the committee recommended that NIH and industry should immediately evaluate alternative dosing schedules and alternative delivery systems for alpha-1 therapy including prophylaxis strategies and strategies for treatment during acute exacerbations of disease and accelerate development of gene-based products and gene-directed therapies for alpha 1.

This resolution supported the evaluation of new delivery technologies and expedited development of new and non-plasma-derived options including aerosol and inhaled delivery systems. Currently, there are three manufacturers in various stages of development with an aerosol product including one transgenic product.

The hope is that aerosol will prove more efficacious because it delivers the drug directly to the lung. It is also hoped that the aerosol products will provide increased access to a greater number of consumers, perhaps up to five times as many individuals with the same raw material and be more cost-effective.

In conclusion, the current situation with one manufacturer and one product causes concern about the inevitable situation when demand will exceed supply. Within the next few months, we anticipate a delay in dispensing causing a delay in serving all consumers enrolled in the direct-allocation program.

Providing the alpha-1 community with therapeutic alternatives should be a top priority for the FDA and the agency has been very cooperative in addressing issues relating to clinical-trial design and INDs. The Foundation hopes that this progress will continue and we are very encouraged that we have an opportunity for further discussions with the agency at a meeting that is scheduled at the end of the month.

Thank you very much for your time today.

DR. HOLLINGER: Thank you, Ms. O'Day.

We are going to take a break now until noon and we will meet back here for committee deliberations and to deal with the question. Thank you.

[Break.]

DR. HOLLINGER: We have most of the committee

at

members here, but not all of them. Is Dr. Hewlett still here? Is there anyone here from the FDA? Are we by ourselves? We can create anything we want now.

Dr. Hewlett, could you go over the question again, please?

Questions for the Committee

DR. HEWLETT: At this point, I am going to read the questions. The first question for the committee is, "Do the committee members agree that HIV-1 p24 antigen testing of source plasma may be discontinued if: a, it is demonstrated that a particular licensed NAT method can detect HIV at a level of 5,000 copies per ml or less in a unit of plasma even if the donor sample is tested as part of a pool, and b," as it is written here.

I am going to go ahead and read this question, but we have had some discussion about whether to rephrase it. The question, as written, is, "Comparative studies of the NAT method versus HIV-1 p24 are consistent with the hypothesis that the NAT method is of equal or greater sensitivity (including the ability to detect major subtypes)."

However, we would like the committee to focus on whether the data you have heard this morning are adequate for you to make a recommendation that it is acceptable to replace p24 with the NAT method.

The second question is, "If committee members disagree, please comment on an appropriate alternative."

Committee Discussions and Recommendations

DR. HOLLINGER: Except for fractionation being misspelled. I am going to open this up to the committee now--we have closed the open session--and ask the committee members to deliberate on this a little bit about the question, any comments that anybody wants to make, any discussions and so on before we actually vote.

Who would like to start? Dr. Simon?

DR. SIMON: I basically agree with the question. I had two comments I would like to make. Number one, at least from my point of view and I think, perhaps, we will hear this from several others, it would seem that we could go to both source plasma and whole blood. There must be a reason why the FDA wanted this restricted to source plasma, but it would be appear that the data would be very supportive of making the substitution for whole-blood donors as well.

Secondly, I did read--it was in packet, and there wasn't much more said about it--the Criteria for Discontinuation, Current Thinking. I just wanted to express a concern, given the data that we have heard which seems to be very strong, that the NAT will, if anything, improve safety over what might be now and allow us to eliminate a test that has caused us to lose many noninfectious donors, that the FDA not be too onerous in its requirements.

Some of the things here seem to go beyond what is in the question, for example about the reference to new clinical trials or clinical trials done by the companies. As was stated by at least one of the presenters, some of the companies didn't have this, per se, as a question in their clinical trials but their data show overwhelmingly that the NAT test is more sensitive in detection of potential window donors than is the p24 antigen.

So I would hope that data based on meeting the sensitivity limits set by the FDA as well as demonstrating in the various panels that the detection is possible would allow this approval to occur, because I think this potentially would be if not a significant advance certainly a step forward by eliminating a test that we do not need and utilizing the NAT technology that is coming along so well.

DR. HOLLINGER: Thank you.

John?

DR. BOYLE: I would just like a clarification on question 1 because I don't know enough about the test variability for NAT testing, but does detecting at 5,000 mean all, some or any?

DR. HOLLINGER: Can you rephrase that a little bit?

DR. BOYLE: In other words, "can detect HIV at a level of 5,000 copies." Does that mean in all cases, some cases or any cases?

DR. HOLLINGER: I would presume it would be in all cases. Dr. Hewlett?

DR. HEWLETT: At least 95 percent of the time.

DR. HOLLINGER: Dr. Fitzpatrick is not here, but he did send an E-mail and I would like to read into the record, at least his comments. It is fairly short. He says, "I agree with the recommendation of the FDA but would encourage the committee to ask the FDA, on record, to discuss each of the prerequisites for dropping the test that the FDA, itself, had outlined. All of these have not been addressed in the preexisting materials. One of the most important is a licensed NAT test for HIV. I know you all will follow up with them on the others," and so on.

His question just is, apparently, there are some reasons for dropping a test and one of them is that you would use another test for it like the NAT test. I think what he is just saying is since there are no licensed NAT tests at the present, how are you going to deal with that?

DR. HEWLETT: The issue that we would like to stress, one of the points that we would like to stress, is that replacement at the present time--our consideration is that it will not happen until a NAT method is licensed. So,

at

in other words, although data are being accumulated and certain manufacturers may, in fact, have analyzed their data to demonstrate utility or greater sensitivity of the NAT method, that particular manufacturer would get licensed and would be allowed to replace the HIV antigen test with their licensed NAT method when they get licensed for the NAT test.

So that is the current thinking at the FDA in regard to how it is going to be implemented. Obviously, the issue has been raised as to whether the industry as a whole can move towards a specific test. We need to recognize that the NAT testing, the pool testing, has been implemented in a somewhat different way than most of the other tests have. They have been implemented under IND primarily to gather data to support the efficacy of the test.

So in allowing manufacturers to do that, I think we have gone a long way. Another modification under IND I think would probably not serve us as well. So what we are looking at at this time is to go with specific licensed NAT methods and to allow manufacturers to replace p24 with their specific and particular method when it becomes licensed.

DR. HOLLINGER: Thank you.

Dr. Nelson?

DR. NELSON: I don't completely understand the licensure procedure given the fact that there is a lot of data on NAT testing. Have none of the manufacturers applied for licensure yet or, if they have applied, what is the procedure, what else needs to be done?

DR. HEWLETT: Obviously, our tongues are tied in a certain way in regard to what we can disclose, but I think you have heard from at least one manufacturer today that they have submitted an IND, they have submitted a BLA which is under review.

The review process, just to give you a two-minute summary of how it takes place; we review, we send them letters. We go through the deficiencies, do an inspection. So there is a lot of review and inspection and establishment issues that need to be addressed as well in addition to test validation and clinical-trial issues.

DR. HOLLINGER: At least in my opinion, I think it is clear that the nucleic-acid testing is clearly more sensitive than the p24 antigen testing. I don't think there is probably anybody here who would disagree with that. I think that the levels that have been selected are probably a reasonable selection. 5,000 copies per ml seems, at least to me, to be a reasonable one.

I would like to ask, just for the record because I know what the answer is, but I would like to have someone discuss from industry or from people who are doing the tests--we talk a lot about false-positive tests. That is not really the issue. The false-positive tests are no

at

problem. If you get a positive test, you look at the individual samples to see if any of them are positive or negative.

What is really important are the false-negative tests, and how often does false negative come into existence. The difficulties of potentially sucking up the DNA, the pellet, or something like this in which you will get a pool that is negative which is, indeed, truly positive.

So I would like somebody to discuss what things are being done, such as controls and so on. That is why I am really bringing it up. Also inhibitors, when you pool things, the potential for inhibitors that might inhibit a positive test that are in there.

Andy, why don't you go ahead and do that.

DR. CONRAD: For us, one of those manufacturers has submitted those BLAs. Of course, all of our samples that are actually tested, contain an internal control, and that internal control--I know that TMA has it, as well, and I know that the Roche system that Bayer uses as well. So all of the current manufacturers I think that are in the process of this all have internal controls which would indicate inhibitors either from an individual sample or in the pooled context--would indicate a preparation error where the nucleic acids weren't carried forward to the amplification reaction.

So the issue, just from our clinical experience, during our INDs which we have conducted with several manufacturers, there was not a single case in which a donor seroconverted where we had a prior donation that we did not find positive. So there were no false negatives.

There are individual donations that are not detected in pooled PCR but that is different than a donor passing through the system undetected. I am sure the story is true with TMA as well as the Roche system used by Bayer. So false negativity due to inhibition or extraction errors cannot be brought forward because the internal controls and the sensitivity prevents false negativity in the sense that truly positive donors are missed.

DR. FANG: Like Andy said, the TMA also has internal controls. TMA has been used for screening over 10 million donations. It is very rare cases that internal control may have a very low signal or suspect of inhibition, or whatever. But none of those samples can be repeated; in other words, it is all due to technical errors rather than due to inhibitions because when we retest the samples, the internal control was fine.

Secondly, because of pool testing, we also suspect that there may be some samples have an inhibitor and then, when you do the pooling, the inhibitor--and therefore you

at

can't detect it. But when you do the single-unit testing, you may have inhibition reaction and you missed it. So we take some of the pool reactive when we can and find any single samples in the pool that turn out to be reactive, we suspect whether there is any inhibitor and we do some testing.

Data, so far, is showing that there is no inhibition whatsoever. Data will be presented at the AABB this year.

DR. HELDEBRANT: In a similar system using PCR to screen HCV, because of the European requirement to test manufacturing pools for HCV, you have an independent confirmation as to whether there are any false negatives getting through the system. In our experience, we have had absolutely no false negatives when we have been screening for HCV with pools of 512 and then assaying the manufacturing pools made from that screened material.

So, as of yet, we have yet to see false negative get through the system.

DR. HOLLINGER: Thank you I would like to ask, also, the FDA whether--you say that you want to detect HIV at a level of 5,000 copies per ml. I wonder whether that ought to be modified somewhat to say at a level comparable to 5,000 copies per ml because I imagine that, somewhere down the line, you are going to go to an international unit,

at

most likely, whether it is WHO standard 1, genotype 1--I mean, I'm sorry; that is for HCV--or whether it is for HIV, some other standard.

Is that correct? Am I assuming--and is that an issue if we make that to say something at a level comparable to 5,000 copies per ml?

DR. HEWLETT: Yes; I think that is a reasonable modification. In fact, we are looking at moving towards the international unit. The CBER release panel is being calibrated against the international unit as we speak, so we hope that when those numbers come in, we will actually be able to adopt that in the future. But, at this time, we are going to stick with copies.

But adding the word "comparable" I think is a good suggestion.

DR. HOLLINGER: I think having a standard that all the manufacturers have to shoot for gets away a little bit about pooling and so on. One of the issues often is how low a test is, how sensitive a test is, can you detect 5 at 95 percent, can you detect 15 or 20 and so on.

The real issue is if you are going to use something like this, can you detect a particular standard or proficiency panel that is set out in a way that will reassure us that you are not going to miss any main samples.

DR. HEWLETT: I think we agree.

DR. HOLLINGER: Dr. Chamberland?

DR. CHAMBERLAND: I have a question for the FDA. It is a bit of a side bar to the main question that we have been asked to vote on. But if, in fact, industry elects--if there are licensed NAT tests available and industry elects to replace p24 with them, I was just wondering if there had been at least any preliminary discussion about would it be possible to entertain bringing back into the donor pool donors who had tested--would appear to be biologically false positives with the p24 antigen assay when you have a situation now where donors would be screened, let's say, with NAT.

I was curious if that was something that had come up for any preliminary discussion.

DR. HOLLINGER: Comments from the FDA?

DR. HEWLETT: I think that is a good suggestion. In fact, that is something we may want to look at as we go further along. At this point, for example, you are talking about reentry of false positives. That is obviously something that can be evaluated in clinical trials and so on in the future.

DR. SCHMIDT: Wouldn't the answer to that be there aren't any? I mean, we are talking about a dozen people and what would be the value of running a big protocol just for them.

DR. CHAMBERLAND: What I was referring to were individuals and maybe Sue Stramer could--I was speaking about individuals who were deferred currently from donation because they tested positive on a p24 antigen screen and neutralization and other workups seem to indicate that they were most likely false-positives. Sue, can you maybe give us some ballpark of numbers that we are talking about?

DR. STRAMER: For the Red Cross, we have seen, since March of 1996, 158 of those donors. The numbers are not high but if ABC has a comparable 158--174. Okay; not exactly, but the same. We already have a litany of test results that show that these individuals are not infected with HIV. But they are listed as permanently deferred in our donor-deferral registry.

DR. HOLLINGER: Dr. Kleinman?

DR. KLEINMAN: I think Mary's concerns should be broadened though because there is a whole group of donors who are p24 repeat reactive and don't neutralize that then fail the current reentry algorithm because they are still p24 antigen repeat reactive. That is a half of the repeat-reactive rate. What was the repeat reactive rate? About 0.027. So that is 0.013 percent of people, so about one in 10,000 donors are out because they can't requalify on antigen test.

So that would potentially be a very much larger yield for a reentry algorithm. So I would agree with your suggestion and encourage FDA to think about, once licensed occurs, different kinds of reentry algorithms for people who have been disqualified because of p24 antigen.

DR. HOLLINGER: Dr. Nelson?

DR. NELSON: When p24 antigen was replaced by NAT testing and then they came in again and were not tested by p24, wouldn't they automatically reenter? They would meet their criteria. They are on the permanent deferral. I see. Okay.

DR. HOLLINGER: Dr. Simon?

DR. SIMON: There is one other comment I need to make and it is not on point as to whether the committee should or should not support the question per se, but just to make people both from the agency and members of the committee and the public aware that if a single fractionater were to be approved and there were a plasma-donor collection center that supplied more than one manufacturer, and one of the manufacturers was approved and the others were not for their NAT to replace p24 antigen, then that collector might still be in a situation where they were doing p24 antigen in all their donations.

So there is a logistical issue here. Obviously, if all the manufacturers were approved, then that problem would go away. But as it is on a one-by-one basis, we would find at least a portion of the plasma industry that might have to keep doing the test.

DR. HOLLINGER: Also, Toby, just one question, is there a problem with eliminating the test in terms of Europe or other places also?

DR. SIMON: That was brought up also. My understanding is that Europe does not require it so one would think it would go away. However, it is in some of the filings that the companies have done for their product in Europe, so those would have to be changed, the various claims and filings and so forth.

So, unfortunately, while I support the question and would like to see us take this step and don't want to say anything discouraging, I just want people to know that there will be, then, an evolutionary process afterwards to actually implement it. It will be a little bit more difficult to implement than it would seem.

Now, if there is a manufacturer who is totally self-sustaining and gets all its donations from its own centers, that manufacturer could probably go ahead.

DR. HOLLINGER: Thank you. Again, as I say, we are talking about plasma here and, of course, plasma also has removal and activations procedures as well as another major safety. So I would like to make a motion, actually, for myself to change just that 1a), which would say, "It is

at

demonstrated that a particular licensed NAT method can detect HIV at a level comparable to 5,000 copies per ml or less in a unit of plasma even if the donor sample is tested as part of a pool."

Can I have a second to that? Mark?

DR. MITCHELL: Second.

DR. HOLLINGER: Any discussion? All those in favor of that change, raise your hand.

[Show of hands.]

All opposed?

[No response.]

Let's, then, go ahead and vote with that change in mind on 1a) and b) since they are interchangeable. Mark?

DR. MITCHELL: Before we do that, I have another question. If we vote on this, does this mean that the--we heard previously that there is an effort to improve the specificity of the antigen testing, itself, and that it may become similar to the NAT testing. Does voting on this mean that they would have to switch completely to NAT testing, or can they also have the p24 antigen testing if it is similar in sensitivity?

DR. HOLLINGER: I would think they would have to show comparable sensitivities. Dr. Epstein?

DR. EPSTEIN: I think the way we would look at it is that they need to be doing a test of equal or greater

at

sensitivity to the current p24. So the question is if they are approved for NAT, and that is determined to be a test of equal or greater sensitivity than p24, then they can discontinue p24. But we would not be saying you cannot do p24.

So, if a future test comes about which is, for argument's sake, equivalent to NAT or better than NAT, then we would simply argue that that becomes an acceptable test. What we are really trying to move toward here is a sensitivity standard for the HIV screen, the direct viral screen.

DR. HOLLINGER: So we will go ahead and vote on 1a) and b). Do I need to read it fully? I will read, then, 1a) and b). It says, "Do the committee members agree that HIV-1 p24 antigen testing of source plasma may be discontinued if, a), it is demonstrated that a particular licensed NAT method can detect HIV at a level comparable to 5,000 copies per ml or less in a unit of plasma even if the donor sample is tested as part of a pool and, b), comparative studies of the NAT method versus HIV-1 p24 are consistent with the hypothesis that the NAT method is of equal or greater sensitivity?"

The parenthesis you want in there or not in there? Parenthesis, "Including the ability to detect major subtypes." End of parenthesis.

at

With those in there, all those in favor of, the committee members agree, that it may be discontinued under those circumstances, please raise your hand.

[Show of hands.]

All those opposed?

[No response.]

DR. HOLLINGER: You realize this is a momentous occasion here. I think there should be music playing in the background now or something for this.

The consumer representative? Kathy Knowles?

MS. KNOWLES: Yes; I vote in favor of it, too.

DR. HOLLINGER: And the industry representative?

DR. SIMON: Yes.

DR. SMALLWOOD: The results of voting are as follows: there were unanimous "yes" votes of 13 votes. There were no "no" votes, no abstentions, and both the consumer and industry representatives agreed with the "yes" votes.

DR. HOLLINGER: Thank you.

[Applause.]

DR. HOLLINGER: Actually, we are finished fifteen minutes early. I don't know what to do. But we are going to take a lunch break until 1:45. This afternoon is going to be a lot of discussion here. I hope we finish somewhere near midnight, but we will move forward. I think it will be

at

an interesting session.

So we are adjourned until 1:45.

[Whereupon, at 11:45 a.m., the proceedings were recessed to be resumed at 1:45 p.m.]

at

A F T E R N O O N   P R O C E E D I N G S

[1:45 p.m.]

DR. HOLLINGER: Thank you. I think we are going to convene. I talked to somebody just before the meeting and they said you should always try something different. If you keep doing the same thing over and over again, you keep getting the same results. And he told me this story. So I will tell you another story.

It was about two avid moose hunters. These moose hunters, every year, chartered a plane to take them up to the Canadian back woods to hunt moose. This was an exceptionally good year this year. They got their moose within a few days, so they radioed the pilot to come and get them.

Well, the pilot returned and looked down there and saw these huge moose that they had gotten and he said, "You know, I can't carry those in my plane. They weigh too much." And the guy said, "We have had such a wonderful hunt this year." He said, "We really want to take these moose back. And besides, the pilot we hired last year didn't complain about the mooses' weight."

So the guy, after arguing a little bit more, he finally boarded the moose on board his plane as well as himself and the plane sort of took off, and it just sort of hovered in the air a little bit, lost altitude and crashed

at

into the mountain.

The two hunters sort of got themselves out from the wreckage and one of them looked at the other hunter and said, "Where are we?" The other one said, "About a mile farther than we got last year."

So maybe we can do the same thing here today, get a mile farther than we got a couple of years ago. Andy Dayton is going to give us an introduction and background on deferral, as blood or plasma donors, of males who have had sex with males.

II. Deferral, as Blood or Plasma Donors, of Males Who Have Had Sex with Males

Introduction and Background

DR. DAYTON: That is a rather inauspicious introduction, but I know it was not ill-intentioned.

We are going to reexamine the issue of deferral of men who have sex with men from giving blood. I am going to give a short introduction to remind the committee and the audience where we have been on this issue over the last several years. Then I am going to very briefly give the outline of the theoretical structure of my talk.

Then Mike Busch is going to summarize a lot of extremely interesting data in the field that is relevant. Then I will come back and, using data which has been provided to me by many tremendously cooperative people, both

at

inside the FDA and outside, and I will try to develop a model that will give us some idea of the consequences of projected changes in this policy.

I should also begin by thanking the audiovisual staff. They couldn't solve the slide advance problem, so I have a little slide projector thing. I push it and it makes a noise and he advances the slide for me. I think this is tremendous dedication and greatly appreciated.

[Slide.]

The HIV epidemic in the United States is generally recognized to have started just after 1977 and, as a consequence of that, currently, MSMs, or men who have sex with men, are deferred for MSM behavior which has occurred even one time since 1977.

In 1997, to this committee, the FDA presented an analysis of the potential effects of relaxing the MSM deferral policy to either one year or five years; in other words, the one year meaning if you have had any MSM activity within the last year or the five-year meaning if you have had any within the last five years.

At that time, we were only able to take the analysis so far. The summary of a very complicated analysis was that blood-banking errors of various types were poorly quantified at the time, but they were considered the most significant risk to changing the policy. The conclusion we

at

were able to reach at that point was that we don't know, in terms of exact numbers, what exact risk we are taking.

We get roughly 10^3 HIV-positive units into the blood banks which are then interdicted by tests. We calculated that, under some MSM relaxation standards, we might double or triple that number of units which got into the blood banks and which we hope to be interdicted by tests.

And we didn't know the failure rate but we estimated that we would be tripling whatever the risk was that we were willing to take, given the knowledge at the time.

[Slide.]

In November of 1998, we hosted a workshop on donor suitability. It was a fantastic workshop and a lot of very good data on incidence and prevalence was presented. In December of 1998, we summarized that data for this committee and I will rely heavily upon that data during my presentation.

[Slide.]

Why are we reexamining this policy at this time? Well, the widespread adoption for nucleic-acid testing for HCV and particularly HIV provides, amongst other benefits, redundancy. Now, I am not talking about the reduction of the window period here. The NAT tests are run as entirely separate tests from the ELISA tests, for instance, and the idea is, let's say you have a pipetting error.

If you have two tests and you make a pipetting error in one of them, it is unlikely you will make a pipetting error in the second of them. So the two are redundant. That gives you a tremendous protection against errors of certain types.

The other reason we are reexamining this issue is that we have not a complete but at least a better understanding of blood-bank error rates, their types and their frequencies, and I do hope that at least some of that improved understanding was, at least in part, the result of the highlighting of blood-bank error rates in the 1997 BPAC presentation on MSMs.

[Slide.]

I am going to go through, briefly, the logic behind my subsequent presentation which will come after Mike Busch's talk. Diagrammatically, this is how bad things happen. This is how errors are made. Infection gets into potential donors and then, from potential donors, it gets into the blood supply.

Now, our primary line of offense against bad things getting into the blood supply are blood-screening tests. However, there are ways that the system gets--or bad things circumvent the tests and happen, or agents can

at

circumvent the tests and get through to the blood supply.

Examples include undetectable strains which can't be picked up by the tests; blood-bank errors, which I have already mentioned; primary test failure, which is more of a theoretical problem than a practical problem because the tests--it doesn't seem to be a problem. And, finally, there are window-period donations in which no current tests are able to pick up certain agents very early in the infectious process.

So we build redundancy of another sort into the system and we try to stop infections from getting into the blood supply by the introduction of a questionnaire. That is to reduce the number of potential donors that even have their units taken, and those units are sitting around in the blood supply waiting for test.

So it is very much like a forward chemical reaction in the sense, and the math is--it really is just arithmetic--is the same. Of course, questionnaires are not perfect. They can be bypassed by several mechanisms including ineffective risk identification which means we have not really identified correctly the risks that should be screened out. Test-seeking behavior; for instance, somebody may know they are in a high-risk group but they may seek to get a test anyway because they want to know what their serostatus is.

at

Peer pressure; that is an obvious one. And misunderstanding of questions, which is a major problem in designing a questionnaire. Finally, we also have at this position here self-deferral which is mediated by appropriate education.

[Slide.]

Most of what I am going to focus on today is in this second half of the equation and the consequences of getting people to this step and what might happen. We can break the relevant issues into prevalence issues and incidence issues. I will talk about them somewhat separately in my talk.

The prevalence issues involve errors allowing undetectable strains or blood-bank errors or test-failure issues, and I will go through this in more detail later on. Incidence includes primarily the window-period issues.

[Slide.]

To summarize where I am going to go; if we consider changes in the deferral policy which result in a new group, a new population, a new set of donors appearing, we want to know how many errors--in other words, in this case, how many infected units could conceivably get through the system with a new population of donors.

The way we are going to calculate that is the new errors--now, this means the new units that get through that we don't want getting through the system--in other words, infected units that might sneak through all of our testing and questionnaires. That is going to equal to the size of the newly donating population--how many new donors do we have coming in--times the overall error rate.

I will just briefly show what that equation looks like. It is really quite trivial. The newly donating population is merely the change in population. The new errors I just simply wrote here as delta errors. And then there is a term here, and I will go through that in more detail in my later talk, which calculates the error rate. All that is is the prevalence issues plus the incidence issues.

[Slide.]

At the end of my second talk, and when the committee is ready to approach questions, we are proposing that this be the question the committee entertains. Of course, it will probably be rewritten. But, as it stands now, what we are going to be asking you is do the available scientific data support the concept that men who have sex with other men, MSMs, can be deferred from donating blood for a period of five years following MSM activity rather than being deferred for any MSM behavior since 1977.

That is the end of my introduction. Now we are going to have a very interesting talk from Mike Busch.

Mike, I don't know if you have the neat slide connection that I have arranged, but you are welcome to it.

Epidemiology Presentation

DR. BUSCH: Thanks, Andy. Those were great slides, by the way. I would really like to get some of those E-mailed.

[Slide.]

What I was asked to do sort of evolved over the last couple of weeks but was to present kind of mostly the impact of new testing to give you confidence that, with these new tests, we really understand risk and the risk is exceedingly low, which gives a certain level of comfort in terms of relaxing the deferral criteria as discussed.

But I have also been asked to add to the talk discussion of HHV-8 as kind of a prototype new and emerging virus that is prevalent in the gay community. So I will review some data that has been generated by several groups looking at HHV-8 and the potential for its transmission by blood transfusion and a preliminary study to document the prevalence in the donor pool.

Finally, just a couple of days ago, I was asked to address some new data, recent data, from San Francisco on the increasing incidence of HIV in gay men. So I will present a colleague's talk addressing that topic at the end.

[Slide.]

Basically, this is just the historical risk. The committee--I apologize; I did E-mail the slide presentations recently, but you received some handouts, some chapters and stuff. There is a slide like this in there. Basically, this is just to emphasize really the very dramatic reduction in risk that was achieved from the mid-80's through the mid-90's through the discovery of several of these viruses and the introduction of progressively enhanced antibody tests.

[Slide.]

Basically, as Andy indicated, what we have tried to do in the last five years or so was to understand risk in the context of the element sources of risk. We have dissected the risk into the four what we think are relevant activities or issues. One is the window-phase donations. The second is viral variance. These are divergent viral strains that the test could miss and, as we talked about earlier, it is critical that we understand the prevalence of these variants and fix the test to detect them.

A fourth source is what have been termed atypical or immunosilent infections where people do not form antibody or the markers we are screening for even though they are persistently infected. This is more of a red herring because, although they do exist, they are extraordinarily rare and a lot of the concern around immunosilent carriers arose from early studies that really were reporting false-positive PCR results. So these are extremely rare for all of the major viruses.

Then, finally, testing error which is, as Andy indicated, is an important contribution to the potential impact of relaxing the deferral criteria because if test error occurs on an increasing frequency of prevalent infections, it can allow these prevalent infections to sneak through.

So, with the REDS group, and particularly Steve Kleinman and myself, what we have been trying to do is to quantify each of these sources of risk. What this table does is to actually put hard numbers on the risk per 10 million donations. So this is essentially the frequency that infected units would potentially enter the blood supply per year, because we screen about 12 million.

You can see, this is actually post-p24-antigen but pre-NAT. We will talk about, in a minute, the impact of NAT. So you can see that, for HIV and HTLV, the two retroviruses, we are dealing with risks in the range of 1 in 750,000. For the two hepatitis viruses, pre-NAT, we were dealing with risks in the range of 1 in 100,000.

You can also see that most of that risk, we believe, is due to window-phase infections.

[Slide.]

I wanted to just spend a moment on the test-error issue because that is, I think, going to be a major driver in the decision here. There are two recent studies that have estimated error rate in routine blood-bank screening. One of them is a study that we did in the REDS group that was published six months or so ago.

In this study, what we did was to track serial donations from routinely screened blood donors. What we looked for was donations that were initially scored as confirmed positive where the donor gave another unit. That may seem to be an odd thing. Why would a donor be allowed to give again after they had given a prior confirmed positive donation.

But that is allowed for autologous donors. So people who are giving for themselves can give sequential donations even though they were positive because the blood is exclusively used for that person. So, through this analysis, we were able to quantify the rate of error by looking at the frequency of negative subsequent donations where we had over 2,000 donations that had previously been confirmed as seropositive.

On first cut, we had eleven donations that tested non-reactive on the follow up. It turns out ten of those were actually early test configurations like first-generation HCV tests or HTLV-1 tests that were missing--they were actually subtype problems where they were missing HTLV-

at

2 or relatively less common subtypes of HCV, where the initial sample was borderline reactive and the follow-up bleed that tested nonreactive was actually just below the cutoff.

So these were not actually what we would construe as test errors. These were actually problems with subtype detection that have since been fixed. But we did detect one frank test error which, when put over the denominator, yielded an error rate of 5 per 10,000. We then applied, in the paper, that error rate times prevalence to calculate, as on the previous slide, the frequency of risk due to errors.

It turns out they are a very small contributor to risk. But this is one of the estimates that we have on error rate.

The second actually comes from the NAT screening. This is data from Sue Stramer's NAT program where over the first approximately six months of NAT screening, in the Red Cross system, they detected three donations that were antibody-negative for HCV but scored NAT-confirmed positive.

As they routinely do, when they reflex tested these samples again to confirm the antibody-negative status, in three cases, the antibody was actually positive. It was a testing error where the initial screening serology was incorrect. That yielded--over that period of time, they had about 2,300 HCV seropositive donors. So that illustrates an independent measure of error rate in routine serology of about 0.1 percent, so, similar to this other estimate.

In fact, two points. The Red Cross has not seen any more test errors in the subsequent almost year of screening. So if we updated this, we would estimate that the error rate is probably more comparable to this 0.05 percent because the denominator would go up about three- or four-fold.

Another point is this is the redundancy that Andy spoke to. This is NAT picking up a sample that was a test error serologically. So this is really a good illustration of the important advance that having these two highly sensitive redundant technologies offers in terms of test error.

[Slide.]

As we talk about the major risk, though, it is window phase. An important distinction here; a lot of those risk estimates that we report for window period assume that individuals are infectious from the point of exposure and infectious exposure to when the antibody becomes positive. But what we have come to understand, through various studies, is that there are actually two phases of the window period, the pre-seroconversion window period.

There is what we call the eclipse phase, which is the period after an infectious exposure but before one can detect any evidence of the virus in the body by any nucleic-acid tests or any methodology. Then there is the phase where we can detect viremia and where clearly people are infectious.

So our risk estimates, because we want to be conservative, assume that this entire phase could be infectious when we calculate out the pre-seroconversion window period. But, as I will show you, actually we have pretty good evidence that this early phase is probably not infectious, so our risk estimates are, perhaps, overestimates.

Then, obviously, with NAT, we are essentially eliminating this pre-seroconversion viremic phase.

[Slide.]

Just a little bit of data on the overall window period from exposure to seroconversion. That is derived from modest studies typically of parenteral exposures where you have an individual who has a discrete exposure who is then sampled at some modest frequency until they seroconvert.

For HIV, the best data comes from a CDC compilation of HIV-infected healthcare workers who acquired the infection from a needle-stick accident, so you have a discrete date of exposure. Then these people were sampled at irregular intervals and, through an analysis by Glen

Satten, he estimated that the time from exposure to seroconversion with sort of early-generation assays was about 46 days.

We know that contemporary, particularly blood-screening assays, have closed this antibody window by about two weeks so we typically talk about a 25-or-so-day period from exposure to antibody seroconversion on the new third-generation assays that we screen the blood supply with. And you see the confidence bound.

For HCV, from post-transfusion hepatitis cases, the data supports, from several groups, approximately a 70-day incubation period from exposure to antibody. For HBV, on very limited data, it is 59 days. For HTLV, again, fairly limited data, about 50 days.

So this is where we get our number that we use for the exposure to seroconversion window period.

[Slide.]

For each virus, I am going to just show you a summary slide of what is an enormous amount of work to characterize the durations of the viremic and other characteristics of the window phase. We talked about HIV earlier today. For HIV, as I indicated, it is about twenty-five days before the high sensitivity antibody tests detect infection.

Viremia is detected by high sensitivity NAT about ten days prior to antibody with this doubling-time parameter reflecting the rapid ramp-up of viremia. And then p24 antigen comes in about halfway through. From this data, we can calculate the overall potentially infectious window period as well as the portions of that window period that can be closed through adding antigen or RNA testing, as discussed earlier.

[Slide.]

With HCV, it is quite a different dynamics in that the virus ramps up very rapidly--the next slide will show it--but the very rapid doubling time of well less than a day and then reaches a very high-titer plateau phase that is readily detectable either with single-donation or minipool nucleic-acid testing.

This lasts, as a plateau phase, for, on average, almost two months, about sixty days. And then antibody become detectable.

[Slide.]

Through analysis of large numbers of these panels, we have derived similar summary curves that again show that typically, within about ten days of exposure, you develop rapid ramp-up viremia doubling time about 17 hours and then there is a plateau phase that lasts about 57 days prior to antibody seroconversion.

[Slide.]

at

HBV; I am not going to go into it. We have talked about it at the last meeting. But the summary statistics are here so the number of cases that have been studied, plasma-donor panels for HCV, we have this unique phenomenon of this high-titer plateau phase.

We have these different doubling times ranging from 17 hours to 20 hours to about three days, so a slower ramp-up for HBV. Another interesting observation recently is prior to the what we call definitive ramp-up phase, in a proportion of these plasma panels, we do detect very low-level transient viremia a week or two prior to the ramp-up.

This is a subtle point when we talk about the impact of single-donation NAT but studies are ongoing to understand what this is and is this pre-ramp-up transient low-level viremia infectious.

[Slide.]

So, as we talk about the decision on NAT, we can use those numbers to estimate the window-period closure that could be achieved by moving into minipool versus individual-donation NAT. You can see that, for HCV, because it has that rapid ramp-up and plateau phase, we are going to dramatically--or we have dramatically--reduced the potentially infectious window period by almost two months through introduction of minipool NAT.

We would only add a modest, about three-day, further window-period closure by moving to single-donation NAT assuming that that intermittent low-level viremia is not important.

For HIV, minipool NAT relative to antibody closes about nine days. You would close another four days. For HBV, if you have a NAT assay and minipool which has about 1,000-copy sensitivity, you would close by about six days. If we theoretically moved to single-donation NAT with 50 copy sensitivity, we would close about 17 days.

[Slide.]

We have introduced HIV and HCV NAT, as you heard from Sue Stramer. This is a first-year experience of whole-blood screening, over 10 million donations screened for HCV and almost 7.6 million screened for HIV. The yield was 42 HCV viremic seronegatives and 4 HIV. That yield observed yields of 40 per 10 million and 5 per 10 million for the minipool screening.

I would just juxtapose this, and this is a slide from Steve Kleinman's analysis, with what we predicted the yield would be based on the those model projections. You can see that the predictions were that we would pick up something in the range of 50 to 80 for HCV--we picked up 40--and something in the range of 7 to 8 for HIV--we picked up 5.

So the yields are a little bit lower but, really, very consistent and, in fact, our projections were conservative. We slightly overestimated the observed yield. So I think this is very important to give us confidence that the model bases of estimated risk are accurate, that we are proving out essentially what we predicted based on these models.

In addition, for HCV, these cases can be broken up into whether they were window-period yield, test-error yield or immunosilent infections. What you see down here is that essentially, as predicted, 84 percent of these cases that were evaluated turned out to be window period seroconverters. 12 percent, I mentioned earlier, those three cases that were test error, and one case proved out to be a long-term nonseroconverter.

So, again, not only the overall yield but the distribution of types of risk were quite consistent with the model projections.

[Slide.]

In terms of the risk now, and these are Steve Kleinman's slides, for HCV and HIV, we projected the impact of NAT on reduction of risk. As I have summarized, we think the current window estimate was about 70 days. We believe that NAT has closed it something in the range of 42 to 58 days, probably more in the range of 58 days, and so a 60 to 80 percent reduction in the window period

at

When we run that through the incidence rates, the new risk estimate would be in the range of 1 in 250,000 to 1 in 500,000 for HCV. That assumes that the entire pre-minipool NAT-positive phase, the so-called eclipse phase, is infectious. So this is almost certainly a worst-case scenario that is an overestimate of risk.

[Slide.]

The same for HIV. We can look at the overall window and we have closed it by adding minipool NAT. So, if we calculate out the new risk for HIV based on a residual, about a ten-day theoretical window phase which is mostly, probably, noninfectious eclipse, we estimate the risk at around 1 in 750,000.

[Slide.]

So we are really dealing with extremely low risks now for HIV and HCV as a result of NAT screening. There is a lot of discussion about, in the very near future, and some of the plasma industry has already implemented HBV NAT, again probably not indicated in the whole-blood sector as discussed by your committee but, certainly, in the plasma industry, again, there is a move to add B19 and hepatitis A.

There are studies looking at the potential role of NAT screening for these other agents that we are currently aware of and are concerned about.

[Slide.]

at

But, perhaps, more important is the availability of a direct-virus screening platform like NAT as we uncover new agents. This is important because most of the new viruses that are being discovered are being discovered by genome-based methods. I will talk about that in a moment. So, therefore, it is often a year or two between the time where we have nucleic-acid assays for these newly discovered viruses before we have a serologic test that can detect antibody or theoretically antigen.

So if we need to move quickly, the assay that would be available will probably be a nucleic-acid-based system well before there is a serologic test. More importantly, the nucleic-acid testing strategy is much more rational. It directly detects infectivity rather than a serological response. For many of the new agents, like hepatitis G and TTV, when people seroconvert, they have usually cleared the viremia.

So, to detect the infected donor, a direct virus NAT system is much more appropriate. So we can avoid the problems with antibody testing by going straight to a nucleic-acid test system.

[Slide.]

This slide is actually from Jean Pierre Allain which was a nice sort of capsulization of how, over the last several decades, the discovery of viruses has really tracked the evolution of new technology. So HBV was discovered through electron microscopy, HTLV through culture and early PCR, HIV mostly through PCR, and then HCV through immunoscreening strategies.

Then, over the last few years, new methods, like representational-difference analysis, PCR screening strategies, have led to the discovery, every year, of a new putative blood-borne agent. So we have to anticipate that this is going to continue.

We have to have rational strategies for detecting and assessing the importance of these new agents.

[Slide.]

What I will be using in the moment is HHV-8 as an example of how these studies need to address these key issues. We need to determine the prevalence of the agent. Since we are not screening, it is really the prevalence which will tell us how many units are actually being transfused from infected people.

If we start screening, and also just in general, we also want to understand the incidence because that is the critical factor that tells us the rate of new infections and addresses the issue of window-period risk.

Optimally, we would like to understand these parameters both today, but also in the donor pool and in general historically because that gives us a sense of whether this is a new and spreading infection which could be, like HIV, creating a huge problem in the blood supply and transmitting to recipients.

If it is a virus that has been prevalent forever and we, obviously, don't have a lot of transfusion recipients dying of unknown diseases, then it is probably not as significant a factor. So both studying historical repositories as well as current donors is a critical issue.

Obviously, the question of transmission is critical both by looking at linked donor recipient samples, high-risk patients like hemophiliacs who have been exposed, and also animal-transmission studies. If transmission is observed, then the question of disease is important and, as we will see, a number of the recent "hepatitis agents" have turned out to not be disease-causing at all. They are transmitted by blood but they have proven to be not associated with any disease. So, obviously, we don't need to screen for a background nonpathogenic agent.

Finally, if we do want to screen, the approach to screening needs to be understood.

[Slide.]

This is just a summary slide that looks at several of these new recently addressed agents, hepatitis G, HHV-8, TTV, SEN-V through in variant CJD in terms of the question of whether these established persistent infections in asymptomatic donors. The answer is yes.

There is data on the prevalence rates of these agents in the donor pool. Some of them are really quite prevalent and we will focus on HHV-8 in a moment. But some of them are 10, 20 percent of our donors are actually PCR-positive for several of these viruses.

Many of these are transmitted by transfusion, the so-called hepatitis viruses, but, in contrast, as we will talk about in a moment, HHV-8, the evidence at this point does not support transmission by blood transfusion. There is highly debated transmission of the CJD agent.

In terms of pathogenicity, again, several of these hepatitis viruses turn out not to be pathogenic. Clearly, HHV-8 does cause disease and then what screening strategy would be appropriate, I think for all of these agents, actually, a direct virus or pathogen assay makes a lot more sense than some indirect marker of exposure.

[Slide.]

Moving directly to HHV-8, this is the virus that causes Kaposi's sarcoma. That has unequivocally been established. It also causes several other disease, lymphoma and several other phenomenon. It is typically prevalent either in gay men or in certain endemic populations around the world such as SubSaharan Africa and in southern Italy.

This summarizes prevalence estimates for HHV-8 based on antibody assays, either immunofluorescence of ELISA-type assays. You can see that, in patients with Kaposi's sarcoma, 80 to 95 percent test positive for HHV-8 antibodies. In HIV-positive homosexual men who do not have KS, it ranges from 30 to 65 percent depending on the population in the assay; HIV-negative homosexual men, 15 to 30 percent.

In injection drug users, the rates are really much lower, background rates. In general, there is not a lot of evidence, and we will review some recent CDC data for injection drug-use transmission of HHV-8.

In women and donor donors, the rates are quite low, again, depending mostly on the assay. We will look at some hard, new data on this issue. In southern Europe, the rates are quite a bit higher, again, in, I think, Spain and Italy. Quite a bit more of this virus is present in these populations. Also, some studies from the Middle East and from Africa that show very high rates.

[Slide.]

This show prevalence in gay men in San Francisco by year. You can see here that, in gay men, the rates increase over time from younger individuals fairly dramatically reaching rates of 30 to 40 percent whereas in other populations, there is no evidence of increased prevalence over time in women or injection drug-using men.

[Slide.]

Also, we know that this virus has been around for a long time in gay men. This is a study from Jeff Martin collaborating with several of the cohort studies in San Francisco that look back at samples collected from gay men in the late '70's, in the mid-'80's and in the mid-'90's. You can see that the prevalence rates of HHV-8 are essentially identical over time, both in the HIV-positive and HIV-negative.

So this is not a new emerging agent. This has been around in the risk population at a fairly stable rate over time.

[Slide.]

In terms of the virus in the body, this slide summarizes data on the prevalence rate in different body compartments. This is direct virus detection now with PCR-type assays. What you can see is the virus is present peripheral blood lymphocytes. It is a herpes virus so it is predominantly a cell-associated virus in leukocyte compartments.

In KS patients, at least half of KS patients have circulating virus in their leukocytes. In non-KS patients who are seropositive, in the range of 15 to 25 percent have positive PCR on their mononuclear cells. That does not necessarily mean that this is an infectious compartment of

at

the blood. It could be a noninfectious circulating viral byproduct.

Also, this is a fresh PCR analysis which doesn't necessarily reflect infectivity. We will talk about that.

Interestingly, several studies, including studies from our lab and in collaboration with Jeff Martin in the San Francisco gay cohorts, have documented very high rates of HHV-8 virus in saliva, much higher than are present in semen. So there is probably a significant mode of transmission of this virus through salivary virus shedding.

[Slide.]

This is data comparing, again in our San Francisco studies, the prevalence of HHV-8 in peripheral blood leukocytes in different populations, in blood donors including a small number of HHV-8 seroreactive donors. We have never detected HHV-8 by RPCR studies in healthy blood donors.

In HHV-8 seronegative gay men, again, no evidence of peripheral-blood PCR positivity. In HHV-8 seropositive gay men without KS, peripheral-blood lymphocytes are positive about 24 percent of the time and, in KS patients, in our studies, 43 percent. So there is evidence in seropositive and, particularly, symptomatic patients of virus in the blood, in the lymphocytes.

[Slide.]

But is it transmitted by blood transfusion? There are several studies that address this. The problematic study was published from Jay Levy's group in Lancet three years ago. What happened here was they were actually getting blood from our blood bank from healthy donors to use as controls and as seeded cultures for trying to grow HHV-8.

So they had 72 donors who were being studied over a period of several years just getting buffy coats. Low and behold, what happened was they were stimulating these cells and then adding cells from infected patients to try to transmit the virus from HHV-8-infected patients but they would also grow the normal donor cells in the absence of seeding in infected patient samples as controls, as sort of background lab controls.

Lo and behold, they detected one donor who, on coculture, or on stimulation and then coculture with additional normal cells, they indicated had evidence of infectious HHV-8.

Now it turns out, and this was reported in Lancet, it turns out we have brought this donor back on a series of occasions and the donor was actually seronegative and HHV-8-negative on follow up. So my personal bias is that this was actually a contamination.

But the bottom line is this paper alluded to the potential risk of transmitting HHV-8 from a healthy blood donor because they could demonstrate in vitro transmission from a donor through another donor's lymphocytes. So this is the paper that has raised the greatest concern about blood safety.

On the other side of the coin, if you look at the epidemiologic data, there is no evidence for transfusion transmission of HHV-8. KS is virtually unheard of in HIV-positive transfusion recipients or hemophiliacs. The seroprevalence of HHV-8 in a variety of studies is virtually identical or essential background levels in hemophiliacs and transfusion recipients versus blood donors from the same regions tested using the same assays.

In studies from the TSS, none of 14 recipients who got transfused with blood that was HIV- and HHV-8-positive, none of those recipients developed HHV-8 infection even though 13 of the 14 acquired HIV. There are several other studies that have looked at transfusion recipients. There is a total of about 30 transfusion recipients who have gotten known HHV-8-positive blood who have not become infected. So this data supports, albeit in small numbers, lack of transmission.

[Slide.]

There is, however, evidence for transmission by allograft transplant. So renal transplantation studies, a paper in the New England Journal a few years ago, showed that a series of transplant patients had significantly higher HHV-8 seroreactivity following transfusion than before transfusion, and the seroconverters predominantly received blood from donors--organ donor; the kidney, itself, was HHV-8 positive.

So it is, I think, clear that kidney transplants do transmit this virus. Of course, kidneys have a lot more than blood, and this virus is predominantly--may have a significant compartment in vascular tissue as well as lymphocytes.

So all of this has led to a study which Phil and I are leading to look at the prevalence of this virus in healthy blood donors, and then some other studies are planned to look at the potential transmission question.

This study has just completed a first large phase of testing which involved a panel of 1,040 specimens going to six different laboratories with extensive published expertise in HHV-8. The panel included 40 positive control patient samples, known KS-positive patients, and all of the labs picked all those up. Essentially, there was one QNS sample that was missed. So all the labs demonstrated excellent sensitivity.

But when we then looked at 1,000 normal donors, what we saw was dramatically different rates on identical samples ranging from labs that reported only a handful of positive EIA-reactive results, 0.5 percent rates, to labs reporting 7 percent of these healthy donors being seroreactive.

It turns out only one of the samples was positive in all of the laboratories. We asked, then, how many samples were positive in two labs, three labs, four labs. We sort of made a cut that if at least two labs called a particular donor sample positive, we would at least consider potentially positive and do PCR, et cetera.

So, overall, there were 3.6 percent of the donors that were reactive in at least two labs. All of these samples were PCR-negative. So, in truth, these are probably false-positive serologies. There are studies underway to further characterize that.

[Slide.]

The last data on HHV-8 is actually Phil Pellett's data, who is here, in a cohort. This is important because it is sort of the newest data that raises another small concern regarding potential transfusion or parenteral transmission. This is a cohort called the HERS cohort which is a cohort of women, 871 HIV-positive and 439 HIV-negative women.

They have been followed for over six years or up to six years with six-month sampling. They have extensive sexual behavior and drug-use history. They have also had a variety of laboratory studies conducted.

[Slide.]

The important new data with respect to parenteral transmission is in this population of women who are obviously not at risk for male-male sex transmission where there is a major driver for transmission of HHV-8. In these women, there is a highly significant correlation between drug use, parenteral exposure, and HHV-8 seropositivity, with 35 percent of women who share drugs regularly being HHV-8 seropositive as opposed to 12 percent of those who deny drug use so about a four-fold increased relative risk associated with high-level drug use, no significant association with a variety of other parameters such as crack cocaine use and other characteristics.

[Slide.]

In addition, when they looked at these samples from these women and correlated the HHV-8 status with other markers of either blood-borne or sexually transmitted infections, it turns out that there were significantly higher rates of HHV-8 reactivity in persons who were anticore-positive, HCV-positive, in particular.

And these are blood-borne viruses. So this laboratory association supports that there may be, in this population at low risk for male-male sex transmission, there seems to be a hint of a parenteral association, that you can potentially transmit this virus through use of drugs and it is correlating predominantly with blood-borne viral infections, not with HSV-2, which is an STD, so suggesting that, in this population, a blood-borne transmission may be important.

Most important, if the analysis was restricted to women who denied any commercial sex, any prostitution, and who were negative for these other STD markers and said they had very few lifetime sex partners, so sort of the lowest-risk group with respect to any sexual transmission, the association with drug use held up. So they continued to see a significant increasing prevalence of HHV-8 with increased use of drugs.

So this is a disturbing finding supporting an association of HHV-8 with parenteral risk exposures.

[Slide.]

So the summary of Phil's work is that both laboratory and self-reported risk data support an association of HHV-8 with I.V. drug use in populations that have low risk of sexual transmission. This does not appear to be an artifact of confounding and is also observed in this lowest sexual risk population.

So this is somewhat disturbing and has led to additional interest in expanding the studies about HHV-8 association with blood transfusion. Perhaps, that will be discussed later.

[Slide.]

So we remain concerned, albeit that the data does not, at this point, demonstrate there has never been a documented case of HHV-8 transmission by blood transfusion. So that is the story with respect to risk in terms of the blood supply and HHV-8. Again, I was asked a few days ago to add one last bit of data so I have just a handful of slides from my colleagues, Sandy Schwartz and Willie McFarland in San Francisco. They are at the Public Health Department.

Over the last six months or so, there has been increasing concern about what might be called relapse in high-risk behavior in the gay community. This has led to some very public meetings and press interest in the potential relapse of infection.

[Slide.]

I just wanted to share that this is--this is actually a curve that should peak up here and come down here. This is the estimated number of new HIV infections per year in San Francisco. It shows that in the late '70's, early '80's, the percent of at-risk people infected per year went up dramatically. Over 8,000 people per year were becoming infected in the early '80's.

This dropped dramatically. This curve shows that, in the mid-'90's, the frequency of new transmissions further declined from 1,000 to 500. But the figure actually sort of dovetails out at the end here because we are not sure, and there is some suggestive evidence, that there is an increasing transmission going on.

I will summarize data from AIDS surveillance cases, risk studies and, most important, incidence data that supports a concern that there has been some increasing--so you can see that you have got these potential modeled estimates of incidence.

[Slide.]

So this is their surveillance data. Overall, AIDS cases have dropped in the last few years as a reflection of HAART and the rate of death due to AIDS has also dropped. This is attributable in great part to the introduction of highly active antiretroviral therapies.

But one of the down sides of highly active retroviral therapies, if you will, is it has resulted in a plateau, an increase in a plateau of infected people in the population. There is a concern that some of these people, because they are on HAART and healthy and often virus-negative by viral-load test, that they may be engaging in increased high-risk behavior and there may be increased transmission of the virus.

[Slide.]

at

This has been documented in a series of studies looking at STD frequency in various clinics. You can see that, in the last few years, there has been a significant increase in the rate of STD diagnoses in clinic settings.

[Slide.]

Rectal gonorrhea rates have gone up again in the last three or four years fairly consistently, so we are seeing increasing rates of both general STD and gonorrhea, particularly.

In some street-outreach analyses, again, of gay men, the rate of condom use has begun to drop and the rate of frequent multiple anal-risk exposures has begun to creep up through survey studies.

[Slide.]

Then data coming from several populations; the Young Men's Study has formally documented risk behavior and, again, rates of increased high-risk behavior have gone up in these studies, both unprotected anal sex and multiple partner unprotected anal sex, a small trend upwards.

[Slide.]

And then, from several studies, I will show incidence rates that, again, have documented dramatic declines in incidence, either measured formally through prospective studies or using what is called the detuned assay, or the less sensitive HIV incidence projection model.

at

[Slide.]

A series of studies from San Francisco have shown this same phenomenon--I will just bounce through them--that, over the last three or four years, the curve has begun to come up. So there is evidence that there is increased incidence occurring in these cohorts.

[Slide.]

This is overkill. Why don't we stop here. This is just a summary. Basically, there are a variety of sources of data that are of concern but I think, as Andy will play out, these are very, very sort of small, barely statistically detectable trends toward increased incidence and that, in the blood supply, there is such a small fraction.

Of course, we are talking incidence. So we are talking about people coming in, potentially, during the window period which we have got all these tests now and we are not talking about eliminating deferral for recent behavior. The consideration is a much more lengthy, five-year or one-year deferral period.

Thank you.

DR. HOLLINGER: Thank you, Mike. Any questions of Mike before Andy continues?

DR. CHAMBERLAND: Mike, I may have missed this because it went by pretty fast, but when you were talking

at

about your findings of these pre-ramp-up viremic blips, were those consistently detected by minipool or single-donation NAT testing methodologies?

DR. BUSCH: Yes; again, these are fairly recently uncovered as we have tested back on these very early bleeds that are now being identified. As a result of NAT screening, we have these early bleeds available. We have done a series of studies where, after initially seeing these samples, we recoded samples and sent them to two or three--or processed them through two or three different NAT systems including the TMA assay, PCR assays, Andy Conrad's high-input.

They have been confirmed, so they are observed consistency and they are very low titer. So, although we have not done a lot of formal pooled study on them, they tend to have copy numbers in the less-than-100 to 200 genome equivalents per ml.

They were initially missed, actually, by the routine pool screening as done in the plasma industry. So our expectation is that these low-level viremic-phase observations are only detectable by single-donation nucleic-acid testing.

However, even if you factor them in and assume that they are infectious, they are really a very, very small source of risk because they are very transient and they only are observed over the several weeks prior to ramp-up viremia.

DR. PELLETT: Phil Pellett, Chief of the Herpes Virus Section at Centers for Disease Control. First, I want to thank Mike for a nice summary of the data. It was just a very nice overview. I think if I ever get invited to give a seminar on the study that he described, I can just defer it to Mike because he actually covered our data quite nicely.

I need to credit someone named Michael Cannon, part of our group, who actually did the analyses on the HERS study. I have a couple of minor points and then I have a couple of points that I would like to raise relating to the deferral issue.

One minor point is the name of the virus. In some rooms I am in, the language is human herpes virus 8. In other rooms I am in, the language is predominantly Kaposi's-sarcoma-associated-herpes virus, or KSHV. I have become bilingual on it, but my native tongue is HHV-8.

Mike talked about the presence of the virus in bodily fluids. I think it is important to make clear that the bodily fluids that the virus has been found in have come predominantly from individuals who are HIV-positive, not just people who have healthy immune systems and are seropositive. You very rarely find the virus.

So I had four questions I wanted to raise relating to the MSM deferral that I think maybe relate to some of this. One question is, is the virus in the MSM population. Mike showed clearly yes, 15 to 30 percent of the population does have the virus.

Then, importantly, is the virus concentrated in this population. The answer there is clearly yes, relative to the U.S. blood donors, 2 to 4 percent positive. The 15 to 30 percent really is a different number.

Other than MSM in the United States, the injection-drug users who are high-frequency intravenous drug users are the other easily identified group. I think it might be a smaller group. There are other places around the world that have relatively elevated levels of the virus; Mediterranean Europe, some portions, as well as some parts in Africa. So there is a very significant concentration of the virus in MSM in the United States.

I think a big question is is the epidemiology of virus distinct from that of HIV; that is, would HIV screening eliminate the virus from the pool. The reality is, yes. As Mike showed, the prevalence of the virus was high before the HIV epidemic and in a substantial proportion of men who have sex with men, the virus is actually present.

So elimination of people based on HIV status would not actually eliminate the virus. Then we get to the important question of is there a risk for transfusion transmission. I think, obviously, the answer is we are uncertain on that right now. The CD19-positive cell presence, the study of Blackbourne who actually, and I think I will disagree with Mike a little here--I think in 7 out of 11 occurrences over a year and a half period, that person was culture-positive for the virus, which it makes it hard to ascribe to contamination.

There is our injection drug-use data. Mike talked about zero for 30 of individuals who have gotten blood from HHV-8-positive donors have become positive, so it is clearly a relatively low risk for transmission, and the fact that in the U.S. blood donors, of those who were seropositive, none of them were PCR-positive argues, again, on the side of low probability of transmission. But, at this point, overall, it remains uncertain.

So thank you.

DR. HOLLINGER: Thank you.

Questions of Mike for right now? Dr. Simon?

DR. SIMON: What about the associated issue if you do transfuse it, does that mean you transmit disease. If you transmit the virus, does it also mean that the virus will cause disease in the recipient?

DR. BUSCH: One issue is, in terms of transfusion, even if you were PCR-positive, what we know is, as with CMV and HTLV, these cell-associated viruses, the cells probably have to be viable to actually proliferate and transmit. So, with blood that is stored, the transmission rates for CMV and HTLV plummet over the first week of storage.

So, even though the virus is in the blood, it may not transmit by a stored blood component. But, if it does, the issue is really you need to have HHV-8 probably plus some immunosuppression to manifest disease.

So that is why KS and these lymphomas are virtually seen, at least in the United States, in HIV-infected, highly immunosuppressed or moderately immunosuppressed patients. So the disease, it seems to me, in addition to the virus, needs to be associated with some type of immunosuppression.

DR. SIMON: So it is a story much like the CMV story.

DR. BUSCH: Right.

DR. NELSON: Lisa Jacobson, from the MAC study, has done some very interesting studies looking at the timing of the infection with KS related to the immunosuppression. The data that she has suggests that if the HHV-8 preceded immunosuppression, there is often no clinical--the error rate of clinical, but of patients who are severely immunosuppressed and then receive HHV-8 and become infected, the likelihood of progression to KS or some clinical manifestation is much greater.