just for the record say that there has been no transmission, no documented transmission of hepatitis B virus by a plasma derivative since 1987 and that the inactivation removal steps at the present time are pretty good.

DR. KOERPER: No, I agree, but there have been episodes of slip-ups in GMP, so that is where my concern comes from. I agree that the present methods are satisfactory as long as they are applied appropriately and there aren't any slip-ups.

DR. BOYLE: I would like to respond to that prior comment. Based upon your statement, would you agree that we could take off hepatitis B questions from the donor screener, since part of the issue before us is why do we have donor screener questions if we have got the treatment methods that have made it a very safe and--

DR. TABOR: I think you are getting into a very complex field when we talk about that question, and there is a lot that could be discussed about that question. We probably should put it off until another meeting.

DR. NELSON: There have been no outbreaks certainly of hepatitis B, but I just wonder if we can be sure that there has never been a transmission. There has been no recognized. You know, proving a negative is difficult, I guess.

DR. BIANCO: Celso Bianco, America's Blood

Centers.

In my statement during the public session, I suggested that we vote against two separate standards, however, after hearing a lot of arguments particularly during our break, I see some logistic advantages at this time in having two separate standards, like facilitating the introduction of these more sensitive tests for the whole blood while the plasma industry, that has set up already large pooling schemes for the application of HBV NAT, already having, at least in their system, a control for that load of virus that is added to a pool that is going to later be inactivated, that is not going to exceed a certain limit.

So, I think that in the short term, while the ultimate objective that I think that we all want, is to have a single standard that is the highest possible standard. I think that I would modify that position to say that at this point, the two standards would be appropriate.

DR. SCHMIDT: It is not only the question of donor screening questions, but if the manufacture were done properly, you wouldn't have to do any testing at all.

[Laughter.]

DR. SCHMIDT: So, you either do the best testing or none at all, and that is kind of an open and shut, I think.

DR. BUSCH: I think that we need to be cautious

because, as indicated, there actually haven't been B transmissions despite the fact that the surface antigen tests have improved, and obviously, we haven't done NAT, and, in fact, the plasma industry doesn't even do anticore, and there are some prospective follow-up studies of hemophiliacs, et cetera, that have not failed to document B transmissions.

The reality is the plasma industry has introduced HBV NAT, and by virtue of their methodologies, which involved concentrating, once they build these pools, which may be 500 or 1,000, they then pellet the virus from anywhere from 3 to 5 mL's, and then they do very sensitive analytic PCR on those pellets.

So, the factors we saw is that the plasma industry is head to head comparable in sensitivity. They are achieving sensitivity on a per-donation level in the range of 500 to 1,000 genomes equivalents per mL, so the truth is what they have put in place today, and is being used in every plasma components, is an extraordinary sensitive system.

My concern is not that we don't have two levels, but rather that we not set the whole blood level lower than that because we can't achieve it. Instead, I would say, if anything, if you want two levels, the probability is that you could set the plasma industry level much higher than

they are actually achieving, which to me seems ludicrous.

So, I don't see a rationale for two levels because the whole blood side, I don't think can actually do it.

DR. EPSTEIN: I think the real implication of the question has to do with logistics and implementation. What is being said here is correct, that if we were to establish a lower standard than that which is represented by the assays that you saw, it won't be achieved, at least in the short run, for whole blood. It may not be necessary for source plasma, but it certainly won't be achieved for whole blood.

Therefore, the implication of that is that there will not be an era of minipool NAT for hepatitis B, at least until there are more sensitive systems, and those may not come about with minipool testing. That may delay this implementation of NAT until there is single unit testing.

On the other hand, if we were to establish a comparable standard consistent with the capabilities of current assays, then, we will create an era in which we can contemplate HBsAg testing roughly equivalent to minipool NAT.

In that scenario, what we would like to be able to do is say either one is acceptable, but before we draw that conclusion we are going to have to look very, very carefully at what happens in the chronic infections where you have a

big amplification due to excess of antigen and where the HBV NAT may be falsely negative because of very, very low levels of Dane particles, and we don't right now know whether those units are infectious or not.

So, we could end up with the situation where if we accept the current standard, we end up arguing that we need to implement both tests, that we will have minipool NAT and antigen with roughly comparable sensitivity and everybody has to do both.

I think what FDA is trying to get at is maybe that is undesirable.

DR. NELSON: So, are you arguing for or against the question?

[Laughter.]

DR. EPSTEIN: Okay. Well, I am trying to be neutral because I am trying to get advised by the committee. I think that if the committee feels that the minipool NAT at the sensitivities that are seen would be reasonable to be implemented for whole blood screening, then, I think you should argue that we keep what is de facto a current achievable standard. We haven't set a standard, but we would set it consistent with the technology you are seeing.

The implication of that is that we would have to try to minimize the impact on the system, because it could end up causing implementation of NAT, as well as a new

generation of antigen, with not clearly an additive benefit.

If, on the other hand, we say no, we don't really want NAT implemented until there is a more sensitive NAT, we will be delaying the implementation of NAT in whole blood.

Now, what Dr. Dodd was pointing out is that there are pressures to implement NAT anyway. These are coming from Japan and from Europe where there are regulatory bodies that are considering requiring that there be NAT-negative tests of plasma used for fractionation.

If that situation obtains, then, recovered plasma, which comes from whole blood, if it is to be sold for fractionation, would have to also comply with NAT testing even though the NAT testing being done might not offer any detection advantage over HBsAg.

Now, I am saying that based on the estimates of comparable sensitivity although I have to point out that in Sue Stramer's data, there was additive benefit of the two assays, in other words, there were antigen positive DNA negatives, but there were DNA positive antigen negatives roughly in equal measure compared to current rates of detection.

So, I am really not trying to argue this one way or the other. I am just trying to make clearer to the committee what is at stake. What is at stake is that we may have an era of implementing minipool NAT offering no safety

advantage over HBsAg.

That may be desirable because it keeps the market open for recovered plasma, but we shouldn't kid ourselves if it is not a real safety advantage, and it comes at a price. I mean it is a whole other technology being implemented now.

Now, one could argue that maybe that is a good transition because it will make the next transition to better NAT easier, so maybe that is worth it, but I am just saying that there are a lot of practical implications of the answer to the question that may not be apparent.

DR. NELSON: But the committee has already voted that as the technology, you know, as it is licensed, et cetera, that either/or, or both, could be implemented at a better sensitivity. It doesn't say that it has to be either surface antigen or NAT.

DR. EPSTEIN: Well, actually Question 1 was only concerning HBsAg. We have not set a sensitivity standard for NAT. I think your remark is well founded, that FDA should consider setting the sensitivity standard for licensed NAT at essentially the same equivalent level, at least in seroconversions, as antigen.

I consider that rational and we would presumably seek to do that, however, it is, in fact, an open question at the moment where we should set the sensitivity for minipool NAT.

DR. NELSON: I guess that is right because the current standard applies to HBsAg.

DR. EPSTEIN: Only.

DR. NELSON: You could interpret this, that if there is an equivalent with NAT, that that could be applied like in plasma, in another--

DR. EPSTEIN: Let me remark parenthetically that current regulations require the HBsAg test, however, the FDA proposed a regulation in August 1999 concerned with donor testing which would have changed the paradigm from identifying required tests to identifying the agents for which one must test.

Under that regulation, which we hope will become final fairly soon, there would be a requirement to test for hepatitis B, but the agency could, through guidance, indicate which tests were deemed appropriate at any point in time.

So, we do think that in sort of the same time window during which NAT may become an approved licensed test, we will acquire the authority to become technology neutral. Right now we are not. The regs require HBsAg. Of course, we can always do variances to the regulation.

But I think once again what is at issue here is whether to create an approval standard for HBV NAT consistent with the data that you have seen for the current

generation of assays and thereby permitted to be an alternative to HBsAg at the sensitivity level.

Whether they could be used exclusive of each other is still an open question because we haven't quite focused on what happens in the chronic phase of the carrier. You know, we think we pick up the carriers with the anticore, but we would really have to sort this out.

DR. KOERPER: I am sorry, I don't quite understand the relationship between this question and what you were saying about setting NAT levels of detection. I interpret this question to say, you know, do we have the same standard of NAT for both whole blood and source plasma, or do we have different levels of detection. So, I need a little help with clarification.

DR. EPSTEIN: Once again, what you have seen is that the current assays for source plasma and whole blood have comparable sensitivity. So, if we take the current state of the art and set a standard that recognizes that level of sensitivity, then, the screening tests as they will be approved would become available for screening whole blood, and that would be true at a level where they are not clearly better than screening for antigen with tests available in the pipeline that presumably at some point also may become approved.

So, is that what we want to happen? It is really

that simple, is that what we want to happen. It has the virtue that it would enable the whole blood system to comply with external requirements that may necessitate testing by NAT. It has the detriment that it may cause an era where you implement NAT testing without--you know, you have dual testing for antigen and NAT without any real safety advantage of doing so.

DR. FITZPATRICK: Under the new proposed rule, the plasma industry could make the case, because they are already doing minipool HBV NAT, that they don't have to do surface antigen.

DR. EPSTEIN: Yes, they could. Since they are not doing anticore, that issue would be moot.

DR. FITZPATRICK: So, that is a practical application on both sides. For whole blood, there is the practical application that we would not have to institute minipool NAT because there is a comparable sensitivity surface antigen test available, and on the other side, they would possibly not have to change to the more sensitive HBsAg because they are already doing minipool NAT.

DR. EPSTEIN: But I am trying to be very careful and not prejudge that question because we know that for the seroconverters that the equivalence looks very good, but we are not so sure about the chronic carriers.

DR. FITZPATRICK: You are into the core question

then.

DR. EPSTEIN: Right, and you are not doing core on source plasma. In whole blood, you might be able to argue that you capture them all with anticore, and hopefully, as Harvey Alter pointed out, we really need to find out. For source plasma, where you are not doing anticore, would you be missing infectious units because you are not doing antigen and you have a false negative rate with minipool NAT.

DR. FITZPATRICK: Right.

DR. EPSTEIN: Because again, there is a major amplification factor in the chronic phase with antigen excess, many, many logs antigen excess.

DR. FITZPATRICK: On the other practical side for GMPs, though, now you are placing a GMP burden on both industries. If we say we can have two sets of sensitivity levels, we have manufacturers under consent decrees because of GMP problems, so now we have manufacturers manufacturing tests with two different sensitivity levels and having users needing to make sure that they are getting the right tests with the right sensitivity level to do the screening on their donor, and that complicates the GMP issue also.

DR. EPSTEIN: That is a down side of dual standards. I mean generally speaking, dual standards are anathema, but I am only pointing out that if we have one

standard, we are really talking about minipool NAT fairly soon when it is not clearly better than the emerging antigen tests. That is just a practical implication.

DR. SIMON: Let me see if I understand the practical implications correctly. If we vote no to this question, it could mean that the source plasma industry would have go to smaller pools than they are now using, and whole blood would have to institute minipool NAT, is that the implication?

DR. EPSTEIN: I am sort of looking at it the other way around. I would say that we would set the standard consistent with the current state of the art, in other words, a less sensitive than desirable minipool would be used in whole blood.

DR. SIMON: I see.

DR. EPSTEIN: Because we couldn't realistically set a standard for what doesn't exist in the pipeline unless we just want to put off the whole era of NAT, you know, some indefinite number of years.

DR. SIMON: And if we voted yes to the question, and you followed that advice, then, you could set two standards based on your assessment.

DR. EPSTEIN: Yes, and then the implication of that scenario would be that we would be leaving the system in place for source plasma and sort of putting on hold

ruling out minipool NAT to whole blood.

DR. CHAMBERLAND: Jay, unless I misunderstood something, whole blood, recovered plasma, Red Cross, et cetera, they are going to want to sell that to entities that require minipool testing, correct, folks in Japan, potentially European Union, so, in essence, external forces are dictating, if you will, a point in time where we are likely to have both minipool and antigen testing occurring.

Please tell me if I am not understanding this correctly.

DR. EPSTEIN: I think the industry should answer, but the implication would be they would have to find some other way to--

[Laughter.]

DR. STRONCEK: Mr. Chairman, isn't this time for the committee to have discussion, and not for the industry to discuss? There are still questions from the floor from committee members.

DR. NELSON: Yes. Okay. Do you have a question?

DR. STRONCEK: Yes. It is easy to say that it is safer just to vote one standard, but in this case, I am going to vote that for this question that we have two standards. I think that, first of all, NAT testing is clearly in transition. We don't know where--well, we know where we are at, and it is not where we want to be a few

years from now.

So, if we can have the regulations flexible, so we can move to where we really should be, the best position, then, I think that is going to be of benefit for everybody and everything.

Second, these are really different products, fresh frozen plasma and whole blood, and we really do have different standards for other biologicals. We don't treat bone marrow and blood the same way, and we don't have to necessarily treat plasma and whole blood collections the same way. There are quite a few differences.

Third, sometimes I think if we are flexible, we actually increase safety rather than decrease safety because it gives the industry, the plasma manufacturers and the whole blood manufacturers more flexibility to streamline their operations and do what is best to make their products the safest.

DR. MITCHELL: I had a couple of points. One is that if we vote for the question, that there could be two standards. That doesn't mean that the standards don't have to be the same. To me, it gives the FDA flexibility of saying we can have one standard for all of them or we can have two standards that are the same or different.

So, it gives them the flexibility of having different standards or having the same standards, you know,

depending on what they deem appropriate. I agree that it is clear that we are not where we think that we will be in five years and that there needs to be the flexibility to move toward that standard in the future.

My question was that previously, you said that there has been no hepatitis B transmitted through plasma, but the question was whether there has been hepatitis B transmitted through whole blood in recent years.

DR. NELSON: Yes. The estimate is 5.1 per 100,000.

DR. MITCHELL: I just wanted to make sure.

DR. NELSON: There is still an issue of how much of that would be prevented with these tests.

DR. MITCHELL: Right. I just wanted to make sure that was on the record.

MR. RICE: Just to basically echo Mark's remarks, as well as Mary's, I think both of those issues were tied into a question that I had, which was on the recovered plasma side, obviously, you are going to want to bring that into the fractionation process.

Is there really a difference that we need to address in order for that to occur even in this country, not so much in having them sell the recovered plasma even in other countries?

The other question or just statement was that we

have always had some differences between whole blood and source plasma. For instance, the same screening questionnaire, history of hepatitis, has been applied differently to those who are donating for whole blood and those who are donating for plasma, which I think in retrospect I feel was a mistake not to have the same screening question apply equally, but I see this as just another place where there may need to be two standards, and how you reconcile the recovered plasma and the source plasma ending up in fractionated products.

DR. BOYLE: I am confused. I think I am confused by the preamble to the question. The preamble to the question leads me to believe that what we are talking about is a lower standard, if you will, for the plasma because it is better protected, but what I am hearing is it is quite the contrary, that to impose the same level of DNA testing on whole blood would be an unreasonable burden.

Am I wrong in that?

DR. EPSTEIN: No, you got it right the first time. What we are saying is that if we were to set two standards, we would set a higher standard for whole blood, in other words, we would leave plasma for fractionation as is with the current state of the art, but we would hold out for a higher standard for whole blood, which would mean postponing its implementation for whole blood, because it is not there

now. I mean what you saw is that the current standard isn't better than what could be achieved by existing antigen.

Now, the other point of view would be the FDA should be technology neutral, and if we can achieve a comparable sensitivity by NAT and antigen, just approve it now at a comparable sensitivity, and that is, of course, a very logical thing to do, you know, personally, I like it. But there is a practical implication, which is that you are going to have a whole era of minipool testing which is not clearly better than current technology.

I mean you are talking about new instruments, new pooling systems, and so forth, that will permit recovered plasma to be sold for fractionation, but really won't make transfusions safer than antigen alone, the added burden of another test. So, what is the better part of valor here, is it just to be technology neutral right now and say that what has been developed is okay at the state of the art, or to say that we can go ahead and approve it for source plasma, but let's hold out for better for whole blood?

DR. BOYLE: But there are two pieces. One piece is the higher standard for whole blood, but the second piece is the delayed implementation until it is possible.

DR. EPSTEIN: Well, the reason those are linked is that you have heard it stated that we are not there yet for whole blood, and I accept that as true. That doesn't mean

we couldn't have another iteration of the product development.

I do think we need to hear what the industry says would happen with recovered plasma, because I don't know the answer. I think it is worth hearing.

DR. NELSON: There is somebody that has been standing for a while.

MR. BULT: I have been standing all the time, and I am very patient. I am Jan Bult. I am the president of PPTA. I think it is important that we should not forget that the whole discussion about NAT started in '95, and this industry is working in a global environment, so we have to listen to the advisory committees in the states, we have to listen to Japan, we have to listen in Europe, and this industry has made a commitment to introduce NAT for the three viruses.

We made that announcement. In addition to that, we have started a certification program where, with the help of independent inspectors, companies are inspected to see that they have really implemented this. We will continue in doing that.

Now, when we talk about recovered plasma, that is one of the questions that came on the table, we will also use the same criteria for a certification program, which means that it has to be NAT tested for hepatitis B.

I do believe that this industry has made a very strong point that we will manufacture by the single standard, we cannot allow it to have dual standards, we will continue doing that, but having said that, I think in this particular case it is very worthwhile to listen carefully to the transfusion specialists in this regard, so we are not going to come up with a recommendation, but I just want to reconfirm the commitment that we have made to manufacture with one single standard that includes the introduction and implementation of NAT for hepatitis B in all parts of the world.

DR. STRAMER: Just to address the recovered plasma issue, I think to be consistent with everything that is said, we need to reach one standard that assures safety. That standard can be achieved by NAT or HBsAg at the current level of technology. That is the point, doing two tests may have some additional incremental value, but certainly there are other down sides that may introduce errors because of all the other implementation issues relating to NAT.

I think the argument can be made for recovered plasma if there is a standard required, and that standard is one by NAT, we would have to make the same argument, that we can achieve equivalence through HBsAg testing, and the final product that they receive will have a reduced viral load, whether that reduced viral load was achieved through removal

of HBsAg positives or DNA positives, it shouldn't matter, the final product will be DNA negative and the recovered plasma versus source plasma will be equivalent post-inactivation, et cetera.

DR. BUSCH: I have a problem with the two-standard concept if, as Jay alludes to, the idea would be to set a current standard for the plasma industry at the currently achieved levels, which might be something around 1,000 genome equivalent, and a lower standard for the whole blood side, higher standard, but let's say 100 copies or something that could only be achievable with dramatic enhancement of sensitivity, probably transitioning essentially to singe donation NAT.

I have a problem for two reasons. One is I think implicitly it says that we are not doing something that should be done, that we should be screening with whole blood with an assay that has 100 copy sensitivity, but we can't do it, and I just think that puts out a bad message to the public. If the FDA stipulates that here is the standard for whole blood screening, and we can't do it, that to me is not a good situation to be in.

In addition, it drives the industry to single donation NAT, which I think may evolve and may be justified, but I think we should we be very clear that we are making that decision based on an increment of HBV window closure

that, to me, buys very little in the big picture, and we need to be very clear that we are setting that standard with that implication.

In terms of the recovered plasma side, to me, what I hope the committee can address is whether they believe that with current technology, HBV minipool NAT should be implemented for whole blood product release because that is really the crux of the short-term issue, is if we have to add HBV minipool NAT, buying us very little benefit over good surface antigen, and add it into the system in on-line screening mode, that means every product needs to be NAT negative before it is released, and that is a huge burden on the whole blood industry.

It actually brings us back to an earlier committee decision that HBV should be viewed as a product release virus, whereas, you have recommended that hepatitis A and B-19 can be process control tested in the context of NAT, and to me, I think surface antigen buys us close to equivalent sensitivity to minipool NAT for component release and that one option would be to view HBV NAT for recovered plasma more in the context of process control, such as HAV and B-19.

In that context, the HBV NAT that could be done which would meet the European plasma, whoever's requirements, could be done in a different strategy, such as

Sue was alluding to. We could do it on large pools downstream or we could test the anticore reactives to make sure that the bDNA positives from those were not coming in.

But again, to me, a question that I hope the committee can speak to is whether the data justifies HBV NAT for whole blood release in and of itself, and then secondarily, you know, how we as an industry deal with the interface with the recovered plasma side. I think there are options that if it is not justified for blood products release, that there are options that could be worked around in terms of the recovered plasma.

DR. KLEINMAN: Steve Kleinman from the REDDS study. It is a question for Jay. This Question No. 2 here, to me, when Jay was discussing the difference between plasma and blood, implies that FDA will set a standard for minipool NAT in source plasma screening, not that the industry will do it. We know the industry is doing it, but FDA will make it a requirement.

Is that the case, Jay, because they are not doing anticore testing, is that a given?

DR. EPSTEIN: If we are going to approve any HBV NAT system, we would have to do it against a standard, there has to be a standard. We have already decided that we would regard HBV NAT as a donor screen, and so our concept is that there should be lot release control and therefore some

minimum sensitivity standard, yes.

DR. KLEINMAN: But my question is rather than set a standard that says there is a detectability level that is required, meaning you can achieve it with a more sensitive surface antigen or with HBV minipool NAT for the plasma industry, what I am gathering from this question is you are going to say we are going to set a standard for HBV NAT, but are we going to require the plasma industry to use it.

Could they not achieve--I know they are all doing it, but it is sort of a conceptual issue of mandating that NAT move forward for HBV in any situation now, given the fact that surface antigen tests seem to be comparable. That is the data we heard today.

So, I am a little mystified about why you are going to sort of impose a requirement on the plasma industry for HBV minipool NAT, when you could equally as well say you have to detect so many copies per mL and you could achieve it through surface antigen, which is I thought what the data was showing to begin with.

So, that is my confusion, because I think we are debating this question based on the fact that you will set a standard for plasma, and a lot of your clarifications were what is the impact of that on the whole blood sector, but I am not sure why we are setting the standard for plasma.

DR. EPSTEIN: What you are distinguishing is

whether we would recommend or require, based on interpretation of regulation, HBV NAT as opposed to would we set a standard for approved tests.

DR. KLEINMAN: Right.

DR. EPSTEIN: We weren't having that discussion today. What you are really doing is begging the question of whether we ought to be recommending or requiring HBV NAT to begin with, but nonetheless, there are candidate assays that will seek approval and we have to have a standard if we are going to approve them, so it is a separable question.

To the extent that industry is doing it anyway, we probably would decide that it's GMP as a voluntary industry standard, but it is an open question, and I guess we are not prejudging it, we are just assuming the world is moving that way and we want to be able to approve products.

DR. NELSON: Okay. Each has their own question that they are voting on here. We will all have to write an essay explaining our vote. I thought this was a simple question.

[Laughter.]

DR. NELSON: Are we ready to vote or do you want to have more discussion?

Robin, do you want to read the question again.

DR. BISWAS: Inasmuch as products from pooled plasma undergo validated viral inactivation/removal steps

during their manufacture, whereas whole blood and components are not subject to such steps, should FDA set two separate standards for the lower limits of detectability of HBV DNA in individual donations: one standard for plasma for further manufacture and a different standard for whole blood and components?

DR. NELSON: This one does use the word HBV DNA, so that is not surface antigen, right? Okay.

So, if you vote yes, you are voting for two separate standards, and if you vote no, you are voting one standard, and if you abstain, I don't know what you are voting for.

Do you want to vote now?

DR. MITCHELL: No, I had a comment. It would make it clearer to me if FDA should have the ability to set separate standards, because again, I see this as evolving, and I think that the FDA should have the ability, but that they shouldn't necessarily set separate standards for whole blood and inactivated products.

DR. NELSON: Then, you should vote yes, I guess.

DR. MITCHELL: I was suggesting that we change the language.

DR. NELSON: Would you put that they should have the ability?

DR. MITCHELL: Yes, FDA should have the ability to

set two separate standards for the lower limits of detectability.

DR. NELSON: I see what you mean. I think it doesn't modify the question too much. Paul?

DR. McCURDY: It seems to me, listening to the discussion, that what is going on in other parts of the world kind of mucks this up, and it certainly would be desirable from my perspective anyhow that there be harmonization with what is going on at least in the developed countries around the world.

We don't have that. We certainly should have that, but my feeling is that we ought to vote this on its merits or demerits for the U.S., and recognizing that the market may drive from other parts of the world things a bit differently.

DR. NELSON: Are we ready to vote?

How many will vote yes to this question, should there be two separate standards? You wanted to differentiate between whether or not we definitively ask the FDA to set two separate standards or whether we would give them permission to do so, I guess, is that it?

DR. MITCHELL: Right, and I wanted to hear from the other members as to whether we thought we should make that distinction.

DR. NELSON: Jay.

DR. EPSTEIN: I appreciate Dr. Mitchell's remark, however, we have the authority to do either, and the question is not our authority, it is what are you advising us to do in this case.

DR. MITCHELL: I understand that.

DR. NELSON: We are advisory, and sometimes not, but--

DR. MITCHELL: I understood that, I was just trying to make a nuance, so that if our advice is that there should be versus our advice is that there can be, I think are two different things.

DR. NELSON: All right. I would think we could vote on the question the way it is and recognize that technology and other things may drive the FDA's decision.

So, how many would vote yes to this question?

[Show of hands.]

DR. NELSON: And "no"?

[Show of hands.]

DR. NELSON: And the undeclared or abstentions?

[No response.]

DR. NELSON: Industry?

DR. SIMON: I would vote yes.

DR. NELSON: And consumer?

MS. KNOWLES: Yes.

DR. SMALLWOOD: The results of voting on Question

2, there were 7 "yes" votes, there were 7 "no" votes. Voting strength is 14. There were no abstentions. The consumer representative agreed with the "yes" vote, the industry representative agreed with the "yes" vote.

DR. NELSON: With that mandate that was almost as clear as the last presidential election, I think we will break for lunch.

[Laughter.]

DR. NELSON: We will return at 1:30.

[Whereupon, at 12:20 p.m., the proceedings were recessed, to be resumed at 1:30 p.m.]

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

[1:30 p.m.]

II. Implementation of NAT for HCV and HIV Testing Algorithms for Donor and Product Management

DR. NELSON: The first issue for the committee to consider is implementation of NAT for hepatitis C and HIV, testing algorithms for donor and product management, in other words, considering what to do when there is internal inconsistencies.

Dr. Andrew Dayton from the FDA is going to give an introduction and a background for the issues to be discussed.

Introduction and Background

Andrew Dayton, M.D., Ph.D., DETTD, OBRR

DR. DAYTON: Thank you.

[Slide.]

We are going to be going over some algorithms primarily involving with test resolution today for HIV, HCV. The most complicated issues are the test resolution issues which have immediate implications for product management in particular, which is where most of us will be today.

[Slide.]

The algorithm that is going to appear on this screen here is going to be very hard for those of you to read from very far away. What I recommend is that committee

members refer to the algorithms that they were given in their packets. This will just show you where we are from the algorithm. For those of you for whom it is harder to read, this is going to be the first algorithm we will discuss.

The FDA is developing draft algorithms for the implementation of NAT screening of blood and plasma for HCV and HIV in anticipation of eventual licensure of these methods. Today, we are going to focus on portions of the algorithms dealing with test resolution, as I mentioned.

At a later date, we will bring the topic of reentry issues to the BPAC, and I should emphasize now that the recommendations we are discussing today are intended for eventual implementation in the post-IND phase after issuance, first, presumably as draft guidance, so we are not making these recommendations for immediate implementation, the recommendations we will make will be published in draft form and will be subject to comment and further modification.

At the present time, most, but not all of NAT screening done under IND is being done on pooled donor samples because the current NAT methods are so labor intensive. To resolve a reactive pool into reactive and non-reactive individual donations necessarily leads to at least two layers, if you will, of testing the master pool

and either individual donations or subpools as you go down from a positive master pool and try to figure out which are the reactive individual donations.

Generally, we consider several approaches to resolving discrepancies between the layers of testing. Now, of course, this means you have got a positive master pool and at some point you lose the trail as you go through deconstructions.

There are about six major points that I want to address from a global perspective before I go into the details of the algorithms. These are basically choices which come up time and time again as you get to various points in various algorithms, and ask, well, what shall we do at this point.

One possibility, again in general terms, when you have got a master pool that is positive and then as you deconstruct it, somewhere you lose the trail, one possibility is to retest the positive pool, the positive master pool or subpool in replicate.

Now, the premise for this approach is that the false positive result is most likely to have come from contamination during the assay. Although this approach would not result, a false positive result due to contamination that occurred during pooling, a negative result must be construed as justifying release of all units

in a pool. This will be a major question for the committee is are you willing or, if ever, to accept this.

The problem that remains with these effects, however, you can get Poisson effects at low viral load, so, for instance, you might just get lucky on your master pool and just detect true positives that you are only going to detect 1 out of 10 times because you are at the borderline for detectability of the assay.

One way around this, of course, is when you do the replicate testing, to do multiple replicates, but, of course, this begs the question of how many retests should be performed if you go this route and what do you do if one or more of them is reactive.

Now, the second global point that I want to discuss, again, a point that comes up time and time again in resolving discrepancies in the testing, you can do repooling and retesting of the positive pool.

So, if you have a master pool which is positive, and then you lose the trail as you deconstruct, you might say, well, let's go back and really test this master pool carefully, we will repool it in case contamination occurred during pooling, and if we get a negative result, then, we will take that as evidence that everything is okay and that it is really a false positive.

Now, this approach has the same drawbacks as

simply retesting a positive pool, the first point I discussed except that it expected to resolve false positive contaminations that result during the pooling process, and not just to contaminations that occur during the performance of the assay.

A third possibility that routinely comes up in trying to make decisions on how to resolve discrepancies is the possibility of considering, well, the individual unit testing is the gold standard, and this approach would allow non-reactive results, possibly even in replicate, from individual units testing to outweigh any reactive results encountered during deconstruction.

So, you might get a master pool that is positive, you may even get a subpool that is positive. Then, you get down to the individual donations and whoa, they are all negative, what do you do?

Well, we may be able to make a recommendation--we may not be--we may be able to make a recommendation that, well, the individual test is the gold standard.

Now, two other possibilities that I think are problematic, but they always come up, and I feel we should discuss them. One is the often discussed possibility of retesting the negative layer using a different NAT method for the same virus.

This certainly has an appeal for patient

management, for individual counseling, but in terms of protecting the blood supply, it is problematic, because of the following logic. If the initial test is picking up a true positive sample in the master pool, then, it clearly is using the primers and probes capable of detecting the culprit virus, the infecting virus.

So, if you now switch away from those primers and probes, which is basically what you are doing in an alternate NAT, you really are statistically biasing yourself away from positive results.

For that reason, we generally feel that an alternate NAT is not a very good way of doing things except at certain points in the algorithm down at the level of discriminatory testing and fairly down the road.

Now, another often discussed way to resolve discrepancies, again, one that we don't feel comfortable with, but it always comes up and it certainly merits discussion, is to test diluted individual donations or subpools using the same NAT method.

The rationale behind this is in the master pool, individual samples are very highly diluted, let's say, down at the individual donation level, individual samples are not highly diluted, there is the theoretical possibility that there is some kind of contaminant in the individual donation that at high concentrations that you run into when you are

doing individual donation tests inhibit the assay, but if the dilutions of the master pool or subpools, the inhibitor is diluted up and doesn't inhibit the assay, allowing a true positive result.

Well, we haven't seen any evidence that this happens. I would be very interested if today's speakers do have any evidence that that happens, but also there is a very good theoretical reason why that shouldn't happen and that all NAT tests have an internal control, and if there is a failure of the assay to amplify and give a readout, the internal control is designed to pick that up, and I am told that it is quite efficient at doing so.

So, we don't, in general, feel that it is a good idea to expect to be able to resolve problems by going the dilution route, although if we see evidence to the contrary, we certainly will rethink the matter.

Finally, the last of the global points, there is the possibility of simply accepting a negative result from the lowest level of deconstruction and releasing all the individual units on that basis, the idea being that most of these contaminations, let's say, most of these false positives, let's say the master pool, are actually due to assay contamination, and if you get down to the subpool levels and you get a good negative read, there is reason to believe, I am not saying we should accept this, but you can

make a strong argument that that says, well, this was really a false positive.

One of the questions here today will be, well, are we willing to consider situations like that as documentation of false positivity, allowing us to release the results.

These various global points will come up time and again at the various points of the algorithms, and I think it is very possible that we may have different answers at different point in the algorithms for some of these questions, maybe, maybe not, but I think at this point we should now go to the algorithms, the specific algorithms.

Let's start with the one that is present on both screens. I will have to take this one down in a little while on the easy-to-see screen, so I can put up some of the questions.

The first algorithm goes directly from testing the master pool to testing individual donations, so we expect this to be more applicable to the whole blood screening industry than to screening source plasma, although it could be used for either at the discretion of the blood establishment.

Obviously, this is something in which the master pools, this is a situation in which the master pool size is fairly small and it is not considered terribly burdensome to immediately go to individual donations.

[Slide.]

Now, I am going to take this algorithm off and I am going put up what we intend to be the first question for the committee, and all of these questions are wedded to specific algorithms. I will try to organize the discussions of the algorithms around the questions. When we actually get to the voting on the questions, I will try to keep the same organization.

[Slide.]

Just to run through an easy process, what is going to happen when we come down this side of the algorithm, and this we feel is fairly noncontroversial, but I think it is a good way to start out discussing the algorithms.

In this case, you start out with a positive master pool. I should say that in all these cases that we are discussing, all the donations in the master pool are seronegative, so what we are discussing today is when you are flying blind by serology and all you have got are the NAT results.

So, the master pool is positive. In this case, elected to go directly to testing individual donations using the same NAT method. Well, over here on the left, again you can't read it on the slide up there on the screen, but you can see it in your individual handouts, you see this possibility here is you get some of the individual donations

are reactive donations and some are non-reactive donations, and this is exactly what you would expect if everything is working normally.

In this case, you would go to a release of the negative donations because presumably, you have tracked down the positive donations, and then coming down that orange arrow, you would go to discarding the unit and associated product management and discriminatory testing, et cetera, et cetera. So, that is what happens when things are simple.

[Slide.]

What happens when we come down the other side of this algorithm? Here is where we get a problem. In this case, you have had a positive master pool, but all of the individual donations are non-reactive. So, what do you do?

The questions are going to be in this case, well, should a single negative test on the individual donations be sufficient for release, in this case, can the individual donations are considered a gold standard, have you ruled out positivity, in which case you would be releasing all of the samples without ever tracking down the culprit.

We are going to be asking whether there are other possibilities in this scenario. For instance, if it is not sufficient just to release all the individual donations, should you go to additional testing, and if so, is it sufficient to retest the master pool in replicate, in other

words, do you just go back to the master pool and retest it, and this time if it is negative, do you assume that it was a contamination the first time, and then release everything on that basis, or should you go back and retest the individual donations, not diluted now, but just retest the individual donations with the same NAT, in other words, replicate testing of the individual donations.

The way we are going to phrase or propose these questions is that they are not going to be mutually exclusive, so, for instance, you--of course, you could always change the questions--but as we have them designed now, you could say yes, it is sufficient to retest the master pool in replicate and go ahead, or you could say it is also sufficient to retest the individual donations and go ahead, but the two don't have to be mutually exclusive, and if you do this, you are basically giving the option to the establishment of what to do.

The third and fourth sub-options here, is it sufficient to dilute the individual donations and retest using the same NAT method? This is the dilution phenomenon that I mentioned early on. Another possibility is would it be sufficient to retest the individual donations with an alternate NAT method using a different technology or different set of primers, again, one of the global issues that we discussed.

[Slide.]

So, we have just discussed here, and another animation.

[Slide.]

We have also come down here.

[Slide.]

The next one. This is basically a rehash of the questions I just went through here.

[Slide.]

Now, let's move to the second major algorithm

[Slide.]

In the second algorithm, which also contains a separate sub-algorithm, over here on the right, uses tests of subpools to resolve discrepant result. This obviously is applicable to people or would be preferred by people who do large master pools, 500 and 1,000, and which it is very burdensome to go directly to individual testing of 500 or 1,000 samples.

The desire, of course, is to go from a master pool to various levels of subpools and the various different scenarios, and presumably to resolve it at that level.

[Slide.]

The first difficult issue here arises when all subpools test non-reactive after the master pool has tested reactive. Now, this takes us to a sub-algorithm.

[Slide.]

Again, this arrow here on your figure 2 of the algorithms, really just leads you into this page 3 sub-algorithm here, in which all subpools are non-reactive. With respect to this, I would like to put up the relevant eventual questions for the committee.

[Slide.]

Again, just to remind you where we are, the master pool positive, and then all of the subpools are non-reactive, so now you have got a discrepancy. Of course, you run into very much the same set of questions that we just ran into.

Should all units be released is going to be the first question. In other words, have you gone through a retested the subpools, are you happy now that they are all negative, that that means that everything can be released.

If not, if you are not happy with that, again, we suggest a similarly structured set of questions which are not mutually exclusive and which are largely what you just ran into. In this case, is it sufficient to retest the master pool in replicate or possibly after repooling? Is it sufficient to test individual donations using the same NAT method, of course, releasing those that test negative, in other words, is the individual donation the gold standard?

Or 3 and 4, should you dilute the subpools looking

for a dilution effect and test with the same NAT method, or should you check the subpools with an alternate NAT method using a different technology or a different set of primers?

I have noted the objections to alternate NAT and the dilution retesting, so I would submit that the real choice seems to be release versus testing individual donations or testing the master pool.

Obviously, establishments that use pool sizes, such as 512 and 1,200 are going to be very reluctant to retest an entire master pool using individual donations.

Let's go back to the main algorithm, which is figure 2 in your handouts.

[Slide.]

That is this one here. Now, you get a different situation, although analogous. If one or more of the subpools has tested reactive, now, the last one we just looked at, master pool positive, all subpools negative.

In this case, master pool positive, one or more of the subpools is reactive, again, this arrow just shows what happens if everything works normally. Even I can't read anything on that screen. That arrow just shows when you come down this portion of the algorithm, and you test subpools, some are reactive, some are unreactive, but these reactive ones, you test the individual donations using the same NAT method.

If you then get reactives and non-reactives, which is the normal situation, what you would expect, you go through a fairly normal process.

Joe, give me the other animation. What happens when you test the individual donations and you come around to here, and all the individual donations are non-reactive?

Now, you might say that you have now had a history of two reactives, the master pool and a sub-pool. Now you have gotten to individual donations that are non-reactive, but you might say, well, you have got two reactive results. This sets up alarm bells. The extent to which one believes that the history of two reactive testing results implies a reproducible reactivity largely determines one's commitment to retesting in this situation.

However, most of these situations arise when subpools have been contaminated during assay runs, so that the history of two reactive results carries less weight than it otherwise might.

Obviously, you never get to testing a subpool until you have had a reactive master pool, but still you get false positive from contamination during the assay run from true positives, so it is not that unlikely a situation and the history of two reactive results may not carry as much weight as you otherwise would think it would.

[Slide.]

So, the questions that will come up with respect to this portion of the algorithm are largely as follows. So, again, master pool positive, subpools positive, but no individual donations are positive. In that case, should all units be released? Basically, the same set of questions.

Do those reactive individual donations tell you, you are safe, or should you go on to additional testing? I would point that again we have some similar questions as the last one. It is more complicated because we have elected to take into account the belief the two positive results maybe sets up alarm bells, and that is why these questions, they are basically the same as the ones for the other points in the algorithms, but we have taken this possibility into consideration, and that suggests some other possibilities which we have suggested here.

Again, the first possibility, if you decide to go to additional testing, is it sufficient to retest the master pool or subpool, positive subpool, in replicate? That is one possibility.

Another possibility is again these are not mutually exclusive. Is it sufficient to retest the individual donations, that is, without dilution, with the same NAT and release accordingly?

So, in other words, again, you have already had one set of individual donations that tested all negative.

Should you go back and repeat that to make sure they are all negative?

Now, in 3 here, we get into the question of whether or not this subpooling was an independent event from the construction of the master pool. What do I mean by that? Well, the logic is as follows. Let's say the contamination actually occurs during pooling, so you have sequential pooling, you make a subpool and then you put them all together to make a master pool.

If you contaminate that subpool, well, it is not unreasonable for the contamination to be carried into the master pool. So, when you then do the two independent tests, master pool and subpool, they really aren't independent.

So, one possible approach for this is to say, well, when this is a possibility, we should recommend a repooling under the idea that a repooling is unlikely to cause another contamination event during the pooling process.

So, that logic gives rise to the structure in 3 and 4 here. If the subpool was an archived pool from the construction of the original master pool, in other words, it is not an independent repooling, it was just made on the way, in that case, is it sufficient to test a freshly made subpool with the same NAT, repool and retest, proceeding

with further testing only if the fresh subpool is reactive and releasing all units at the fresh subpool is non-reactive, so should you do a repooling event, and if it is negative, then, can you let things go?

Again, is it sufficient to do that? Mind you, this is not mutually exclusive from any of the other first two possibilities. Then, again, we have the possibilities of dilute and retest individual donations or test individual donations using an alternate NAT.

Now, the other possibility, 4 here, in distinction from 3, if the subpool is freshly made, if the subpool was independent from the master pool, now, that means that you really did have two independent positive events unless the contamination occurred during the assay runs.

In this case, is it sufficient to dilute and retest or is it sufficient to test individual donations using an alternate NAT?

One other consideration that we might want to take into account, too, as we consider these, a lot of these events we have been discussing are fairly common, and a lot of them are fairly rare. We would like to have a perfect answer for every possible event that comes down the pike, but we can also survive if the extremely rare events aren't totally nailed down as for what to do.

So, we should remember when we are discussing this

that if an event happens once a year in the entire blood industry, we may be able to discuss it when it happens.

That is all I wanted to present.

What do we do next, do we go to questions or the next presentation?

DR. NELSON: I think we have questions at this point. I had one that came up, and that is--and maybe Dr. Simon could help me with this, too--my understanding was that in the source plasma industry, it is common to wait, and in people who are donating frequently, to wait and hold a lot until a subsequent negative sample on that same person, one that is donating weekly or many times weekly.

It seems to me that under that circumstance, you might have some additional data, in other words, if everybody who was in that pool had subsequently tested negative, or one had tested positive, you might have your answer as to whether or not the initial pooled positive was likely to be a contamination at the time of pooling or whether, in fact, it was a person that really was seroconverting.

Now, is that true or would there be times when you wouldn't have these data?

DR. SIMON: Well, this is the old discussion. There is an inventory hold, in other words, if a person happens not to come back in the 60-day inventory hold, it

would still be released, however, obviously, the vast majority of the donors would be donating once or twice a week, and you would have subsequent samples, but it is possible that there would be a donor you wouldn't.

DR. NELSON: You could have a scenario where you had subsequent negative on all of the people in a pool, or you had subsequent testing, one or more of whom the donor was subsequently positive, and that obviously would answer the question.

DR. DAYTON: I guess the real question you are asking--and correct me if I am wrong, and I don't know the answer, and I hope industry can provide this--has industry ever shown that a so-called false positive, which was possibly resolved by various means we have suggested in the algorithms, has it ever turned out to be real positive as determined by a donor who seroconverted by the next time he came in or whose seroconversion was detected by the next time he came in.

I think that is really what you are getting at, isn't it?

DR. NELSON: It could be a seroconversion or subsequent NAT--

DR. DAYTON: I mean NAT conversion.

DR. SIMON: I believe the answer is no, but I would like to defer to--is there someone from the industry,

perhaps the Red Cross, I think he is asking about plasma donors who donate multiple times a week, because I think the answer is no, but I obviously didn't look at the data before I came. I have never heard of such a case.

DR. DAYTON: Obviously, we are looking for this kind of data, if anybody has it now, of course, we want to see it, but if it comes out during the comment period of subsequently, that is helpful, too.

DR. NELSON: Are there other questions for Dr. Dayton? Everybody got all those algorithms in their head now?

MR. HEALEY: I am Chris Healey with ABRA. I am sorry, but we don't have data on that today. As Dr. Simon said, I don't think there has been an occurrence, but we can certainly look into that.

DR. DAYTON: I would appreciate that. Thank you.

DR. NELSON: Next is Dr. Stramer, and she warned me that she has got a huge amount of data which she is going to present in a very short period of time, but if you need to take a little more time to make it clear, that is okay.

Susan Stramer, Ph.D.

DR. STRAMER: Thank you. I hope to add clarity to what we have just heard by going through the algorithms again and presenting some data supporting the fact that we don't have inhibitors, the false positives are just that,

false positives.

First, I want to address the issue of flying blind or losing the trail. Hopefully, in the whole blood industry, and I am sure the case for the source plasma, we have not done that. In an implementation of NAT, that has been clearly our goal not to.

[Slide.]

So, when we first introduced the concept of NAT or NAT loomed on the horizon, what the industry did was got together under a number of different groups. The first group formed to ensure that we had standardization in the industry was the AABB Interorganizational Task Force on NAT, so that the entire industry could get together and come up with standardized and unified concepts prior to any testing occurring.

Some of the issues that we dealt with in these sessions were defining the risks and impact of NAT for these agents, understanding the technology, that is, the test performance, how we should do our pooling algorithms, what our options were, the FDA perspective from a regulatory standpoint, and how do we validate, and then we brought the source plasma industry in to hear their experience and we could learn what already had been done.

Following the AABB group, there is another group that Mike Busch chairs and helps keep us together, and that

is the Nat Study Group which was referenced this morning, and that really deals with the next phases of all of the issues above - post-implementation prior to any test getting an FDA license.

[Slide.]

I wanted to acknowledge all of the members on the AABB Task Force for NAT implementation, and you can see by looking at the names and their associations, that we pretty much had everyone in the industry or every organization in the industry covered including Canadian Blood Services, College of American Pathologists, et cetera.

[Slide.]

This slide was shown earlier this morning, again by Mike and it represents the NAT Working Group that we are all continuing to work with. It includes the blood centers, government agencies, the different test kit manufacturers including source plasma manufacturers, and the source plasma industry.

[Slide.]

I want to say that there are two major INDs that are occurring for whole blood in the United States. One falls under Roche Molecular Systems, that is the test that is used, the test is used in pools of 24, it is polymerase chain reaction or PCR, and there are two separate tests, one of HIV and one for HCV.

So, after pooling is done and samples are extracted, they go into separate test systems to test for each virus. There are 13 centers who test by the Roche system, and you can see them listed, and the total volume covered is 4.5 million donations annually.

We have all been doing this--I probably will say this again--but from March to June of 1999 is when all of these programs kicked in, so we are close to celebrating our two year anniversary of doing this testing and having met with FDA to determine what the best algorithms for testing were. Hopefully, those are the ones we have implemented.

[Slide.]

This is the other program that is going on, the Gen-Probe test that is distributed by Chiron. We test pools of 16 using transcription mediated amplification. This is not two different independents tests as in the Roche system, but it is what is called a multiplex or a combination test. So, when we do screening, we screen both for HIV and HCV.

Now, the part that Andrew alluded to with discriminatory, only occurs in this test because we test it as a combination initially as part of screening, so we have a screen reactive. The next phase obviously has to be discrimination into HIV reactivity or HCV reactivity.

There are five major groups using the Roche test including the Red Cross, BSL, and two centers in Florida,

and Blood Center South East Wisconsin. The Gen-Probe users test about 8 million donations annually.

I also want to note this does not include 100 percent of the whole blood industry. There are other INDs that I am aware of, and also there are some hospital blood banks that currently do not do NAT testing.

[Slide.]

In the October issue of Transfusion, there was an article by--and I forgot to acknowledge my collaborators on the first slide--the other principal investigators for all the NAT programs and I got together and we summed up our first year of testing experience for North America.

You can see from the different programs here the number of donations screened. This was our yield for HIV and our yield for HCV.

In the next slide, I have really summarized that, so you don't have to add them all up, although there is one discrepancy.

[Slide.]

For HCV, we have had 62 in over 16 million for the first year. That is a yield of about 1 in 250 to 1 in 300,000. For HIV, if you look at just NAT in the absence of p24 antigen, the yield has been 4 or 1 in 3,150,000. There were also two p24 antigen samples detected, but they were also detected by NAT, so the combined yield of p24 antigen

and NAT is just over 1 in 2 million.

[Slide.]

All of the issues that Andrew talked about really have one cause, and that is false positivity, so actually the majority of my talk now covers false positivity, the sources of false positivity, how we deal with it, and data demonstrating that there is false positivity.

If you look at the same programs out of that same Transfusion article, number of donations tested, and look at the number of false positives we have based on deferred donors, there is some variability, but generally, it runs at about 1 in 25,000 even though the sum here was 1 in 15,000. Through the learning curve and us getting more comfortable with the assay, the false positive rates have decreased and now they are about 1 in 25,000.

[Slide.]

One very important issue that I want you to all understand is NAT is different than serology. The cause of false positive results is very different than in serology.

In serology, we deal with specific biological false positives, that is, where the sample and the test components interact, and we know that from persistent p24's on HIV-1 western blot, from nonviral bands on HIV western blots that we have talked about at this meeting, and other causes of biological false positivity.

In NAT, we are really dealing with a first generation technology in which the technicians must become proficient, so there is a learning curve associated with doing this that results in false positives.

These are amplification technologies that are very sensitive, so we can have aerosolization or splashing that occurs from test well to test well, or it may be random. This is intra-assay contamination, that these contamination events lead to false positives.

These techniques have many manual pipetting steps. They have steps where you remove cover seals after vortexing, which may cause aerosolization. There are manipulations of samples on different arrays of the samples in open systems. So, there are very many opportunities for contamination, and it also should be noted that we test the EIA reactives, so we get products out as quickly as possible. The EIA reactives, which are frequently NAT-reactive, are also tested in the same pools and runs, and these are actually the source of our false positives.

[Slide.]

Just to look at the issue of false positivity, these are data from Blood Systems Laboratory, and if you look at the position of a false positive relative to a true seropositive, NAT-reactive seropositive, 44 percent occur right adjacent to, side by side. Another 16 occur either

from or back, behind or in front of the false positive in the run. Twenty-six percent occur within the same test unit, the same configuration of tubes that the reactive test is contained in, and only 14 percent are random.

[Slide.]

To show that the technique is very technician dependent and very user dependent, you can see again data from BSL looking across 16 different technicians, the number of false positives that occur from technician to technician vary. So, it is a very user dependent assay.

[Slide.]

Relative to the learning curve, these are more data from BSL showing the post-implementation of the assay, then, bringing on a lot of new technicians and having one major process change, you can see that the number of false positives were high, and then over time they decreased.

The manufacturer made a substantial change in the wash system here or the method of washing, which certainly decreased false positive rates, but you can see overall the trend here, and the users have to become experienced and really gain knowledge with this assay.

[Slide.]

What do we do to prevent contamination? I just want to read our list. We have extensive training by the manufacturers and then we have retraining on-site. We have

a unidirectional work flow. We have separate air handling and separate rooms for sample accessioning, pooling, amplification, and detection. These may vary by site.

We used disposable, single-use equipment. We decontaminate the laboratory between every shift. All the technicians are fully equipped with PPE, labcoats, booties, face shields, gloves, which are worn at all times, and we are very persnickety about changing gloves between each and every step.

[Slide.]

As Andrew mentioned, we run an internal control in every test by both manufacturers. The internal control again is included in every test. It detects the omission of a reagent or if you improperly perform the assay, such as improper vortexing, if you discard your DNA pellet. A negative result in an assay may not be released without a valid internal control.

The S to CO, at least in the Gen-Probe system, is set at a very low level to be a very sensitive indicator of sample or assay validity, such that the issue we have talked about before, do samples have inhibitors, well, if a sample had an inhibitor, we wouldn't generate a valid result because you wouldn't have a valid internal control.

[Slide.]

This shows you for about 20,000 data points, and I

don't know why that is off, but these are four different master lots of the Gen-Probe reagent, and you can see this is an S to CO of 2, how reproducibly the internal control runs. I don't know why on the computer that this happens. Hopefully, it won't be a reproducible event.

[Slide.]

We have all operated in the industry using some unifying concepts for which to manage our testing and our algorithms, knowing that we are a very diverse industry. We have developed resolution algorithms to ensure recipient safety.

We define a confirmed positive or a yield sample, that is, the seronegative sample that is NAT-reactive, based on one of three criteria - either that the sample confirms by an independent NAT assay, which we refer to as "supplemental NAT," and for example, the TMA users use PCR to confirm their reactivity.

We confirm using an independent sample, and when we get this independent sample, which is frequently a plasma unit, we repeat the NAT, we repeat alternate NAT, the supplemental NAT, and we repeat serology to make sure the results are accurate.

We also enroll all NAT-reactive donors into follow-up studies, and for HIV, depending on the IND, they range from 3 to 6 month follow-up, or HCV, 6 to 12, until

the donor seroconverts.

Recipient tracing of prior collections is generally based on a confirmed positive result. A false positive pool is one that does not resolve to a single reactive subpool or individual sample, and again this is caused by intra-assay contamination, and these are the issues that Andrew has addressed in the questions.

We believe that the undiluted sample is the absolute gold standard. That is basically what we have been serology since the seventies on, so we know that what is in the true sample is what is reality.

A nondiscriminated result, at least in the TMA assay, are also false positives, and again are caused by intra-assay contamination. What a non-discriminated result is, is in the Gen-Probe system where we have the multiplex test, we do have the opportunity to have a multiplex reactive result and then neither of the two discriminatory tests test reactive.

[Slide.]

I am not going to go through the algorithms, you have had enough of that, but on the red side here, what happens if something is reactive. Let me just summarize to say the products are destroyed and donors deferred.

The question is what happens if you have a reactive that doesn't resolve to individual donation level

or if you have a nondiscriminated reactive result, multiplex reactive, but neither discriminatory test is reactive.

[Slide.]

Let's skip this.

[Slide.]

The same in the Roche algorithm. The previous algorithm I showed you was the TMA algorithm for pools of 16. The Roche algorithm actually goes through a subpool step, so they have two opportunities where a pool may be reactive, but all subpools test negative, and then is additional testing required, which they currently do in their IND, but then if you have two pools reactive, the master pool and the subpool, what happens then if all donations within that subpool are all now negative, and there is additional testing that currently occurs under the Roche algorithm, but I will show you some data to address whether these are real or not.

[Slide.]

Let's skip that one.

[Slide.]

In the Roche algorithm, which deals with testing pools of 24, 24 donations are pooled into one pool, and that pool is tested. At the same time, an archive plate is prepared, so that all resolution can occur from independent samples pipetted at the time that the pools were initially

pipetted.

[Slide.]

So, if there is a reactive pool, you go back to your archive plate and create four, six-member pools and test those four, six-member pools. The way the algorithm is supposed to work is then you have a reactive six-member pool which then resolves into a single reactive donation, and the products are discarded and donors are deferred.

[Slide.]

But what happens now if you have a reactive pool and all your subpools are negative, they all test negative? In the Roche algorithm, you retest the master pool twice more, and when you pipetted the master pool initially, there were two other master pools that were pipetted and just held in reserve, if you will. Well, these are two are tested, and if they test negative, then, product is released.

Data that I got from Puget Sound for the period of 4-99 to 12-00, through the end of last year, show 18 pools that have this kind of reactivity, where the master pool is reactive, but no subpools were reactive. Of those, when they retested the master pool times two, all 18 were negative and product was released.

[Slide.]

This is the next level of that algorithm. Let's say you have a reactive master pool now, and you also have a

reactive subpool, but all six of these individual donations test negative. Well, these are retested, and if they are negative, even so, the products are discarded because they had two reactives, and the donors are put into surveillance. But from data that I also got from Puget Sound, there were two instances during this year and a half period of time where this phenomenon occurred, and of those two pools, that comprise 12 donors who were in surveillance, 9 of the 12 donors did come back to donate again and all were subsequently seronegative and NAT-negative showing that there were false positive.

[Slide.]

Now, these are data from BSL showing, in the pools of 16, what is the meaning of a pool that does not resolve. In testing of close to 60,000 pools, there were about 2.6 pools that were reactive, but all of these pools resolved to single donations, so we are not going to talk about those, but there were 155 pools that did not resolve to single donation, and the BSL algorithm at the time, they did two things simultaneously.

They repeated the pool in duplicate and they tested all 24 donations individually. So, of those 155, 149 tested negative when the duplicate pools were retested, and all 24 donations were NAT-negative. There were 6, however, that showed some reactivity in one of the two retests,

however, no individual sample was reactive, and all pools were negative when repooled and retested whether they were combined with the 24 members together or just diluted 1 to 24 in negative plasma.

[Slide.]

Those data are shown on this slide. In yellow here, you can see the results of the initial retesting. Then, new pools were created and 5 out of 6 were negative. When all the constituent 24 individual donations were tested, there were not reactives. When each of the 24 constituent donations were then pooled, 1 to 24, and tested again, all reps were negative except there were 2 replicates here that were reactive by the multiplex test, however, they were false positive as neither was reactive by the discriminatory tests.

[Slide.]

We have similar data from Blood Center of South East Wisconsin. In their algorithm, they had 10 reactive pools. You can see relatively low S to CO values, and when retested in duplicate, all of the pools tested negative.

[Slide.]

Looking at Red Cross data, hopefully, this will be in large enough data set to put this issue to rest, over the period of time from 9-8-99 to 2-25 of this year, we had over 4,000 reactive pools. The pink bars here show you those

pools that resolve to single donation tests and had a NAT-reactive individual donation that was either a true positive being sero-reactive or a NAT yield sample.

The S to CO mean of these samples was 9.84. You can see for those pools that didn't resolve here, that the S to CO's were lower. If we take a closer look at what those low S to CO values of those 1,212 pools were, the data are on the next slide.

[Slide.]

Of the 1,212 pools, this included 19,392 donations, that is, 16 times 1,212. 17,232 donations were from Red Cross regions, so we could do further investigation, and the further investigation was to see how many of these donors we then accept, came back and if in subsequent bleeds, they showed any seroreactivity or any NAT reactivity, to answer the question I think that Ken asked earlier.

Well, we had 7,666 donors who did return at a median time of 87 days with a range of 3 through 457 days. All 7,666 donors were NAT-negative and none confirmed positive by serology. I will say that we had some false positives, as you would expect. There were 3 donations, two of which tested repeat reactive by p24 antigen, 1 tested repeat reactive by antibody, but none confirmed, so those were false positives.

We had 28 of these 7,666 that did test reactive again in a pool of 16, but then when tested individually, were all negative. Eight of those 28 have subsequently come back and re-donated and have been negative even in pools.

So, we believe that these data do say that NAT unresolved pools do not contain samples from HIV- or HCV-infected individuals.

[Slide.]

Now I want to deal with the issue of what does an undiscriminated or nondiscriminated result mean, and I will start by using the Red Cross data to demonstrate this.

In our program of testing pools of 16, and this data go to January 14th, we have had 437 donations that were NAT-reactive at the individual donation level; 32 of these were real, 30 were HCV positives, 2 were HIV positives, but 405 of these were false positives.

[Slide.]

As I mentioned earlier, we enroll in follow-up studies and in the follow-up studies we retest the EIA's, TMA, and PCR. We also retrieve the plasma unit, and we repeat all testing on the plasma unit. That is the index plasma unit.

Other centers do the same thing. They may not have the plasma unit, but they will retest the index tubes that they have again for EIA and TMA.

[Slide.]

This slide shows the resolution of some of these 437 into those that resolved to HCV based on discriminatory testing and those that were HIV-reactive on discriminatory testing, and these are the yield samples, but what this shows you, and I don't want to belabor this, is we have false positives in that type of testing scenario, as well.

[Slide.]

We also have the scenario where we don't have enough sample to complete discriminatory testing, but what I want to focus on are the 279 discriminatory non-reactive samples and are they positive or negative.

265, we had supplemental information, supplemental NAT. They were negative. 181 also tested negative in the index plasma unit by all testing I showed and in follow-up testing.

[Slide.]

This slide gives you those details for the 265 in total. For 84, we only had one result, 66 were NAT-negative on the index donation, 12 were negative in plasma, and 5 in follow up. So, here we had independent samples confirming NAT negativity.

For the 181 listed down here that were multiplex, they had multiple tests. We had 161 here whose index donation tested supplemental NAT-negative. Of those, we had

an addition 72 we confirmed negative in plasma, an additional 22 who we confirmed negative in follow up, and lastly, an additional 62 who we confirmed negative in plasma and follow up.

For 20 of these donors we didn't have supplemental NAT results on index, but we had plasma and we had follow up, again showing that none of these, or if we combined all of these that we had independent results on, 198 were confirmed false positives.

[Slide.]

We have the same data from the ABC sites, BSL, and Blood Center of South East Wisconsin combined. Here, they had 155 samples that, on follow up, 154 tested negative, 1 again repeated with a nondiscriminated result, but on the second follow up was negative. So, here, we add another 155 false positives.

[Slide.]

If you believe nondiscriminated results are false positive, you have to know that the discriminatory test and the multiplex test have the same sensitivities. So, this just shows you that you can have a multiplex reactive and discriminatory tests all have the same level of sensitivity, and this is about 50 percent cutoff at 8 copies per mL.

[Slide.]

Lastly, you see the same thing for HCV, so it is

not a sensitivity difference that we would see a multiplex reactive and a discriminatory test negative because all the tests have equivalent sensitivity.

[Slide.]

So, I am happy to say in conclusion, let me read it directly, and I only have one page of conclusions, NAT implementation in the U.S. has followed an interorganizational approach so that the best interest of donors and recipients could be achieved.

The major issue with NAT is contamination through intra-assay contamination events.

The IND process has provided a mechanism to collect ample data to support rational policies.

Pools that do not resolve to individual donation are false positive and products are safe for transfusion based on retesting and follow-up data. I have shown you 20 from Roche, 154 from BSL, 10 from the Blood Center of South East Wisconsin, and the 7,666 that came from the 1,212 reactive pools at the Red Cross.

Nondiscriminated reactive samples that are multiplex reactive, discriminatory HIV and HCV non-reactive are false positive, and these donors should not be deferred.

Thank you very much.

DR. NELSON: Thank you for efficiently presenting quite a lot of data.

Are there questions for Dr. Stramer? Yes, Jeanne.

DR. LINDEN: Could you please elaborate on how the internal control would detect inhibitors, is this a test sample that is spiked with a known positive? Can you just explain that a little better?

DR. STRAMER: The internal control, at least I can in the Gen-Probe assay, it is another sequence of HIV that is not the same sequence. It has a different primer pair than the target sequence we are looking for in the assay, so it is an independent sequence of HIV, it is added at the same time we add the reagents for the assay, so everything, sample, target capture, and internal control are all added at the same time.

So, if this HIV sequence doesn't amplify, then, we know there was some inhibitory event that occurred during amplification. Interestingly enough, if we have had an inhibited sample and we have rested it, we have never had an inhibited sample repeat as inhibited, so it has really proven to us that we have never seen anything like an inhibitory substance.

DR. FITZPATRICK: In the pooling process, the instrument that pools has an error rate in sampling or not sampling the right tube, how is that handled?

DR. STRAMER: There are two different instruments that are used in the different programs for pooling. We

believe sample errors due to the pipetters are very, very rare events, but what we do, and the Gen-Probe users, we weigh all of our pools, and if the pool weight is outside that, a fraction of one sample not being pipetted, the pool is invalid. We just had one last week that was less than one-third of one sample, one sample weight of 0.3 grams, and that was an invalid pool. I mean we have them infrequently, but we do, and that indicates that something potentially is wrong with the pipetter, so we do have a QC check to ensure that every sample has been pipetted.

DR. FITZPATRICK: Is there any chance that a sample could be sampled twice and a sample not sampled?

DR. STRAMER: Sure, and there is the same error that when we run a CV antibody test or HIV antibody test in a screening lab, that the same pipetters could have easily missed those samples, as well.

DR. FITZPATRICK: Okay. Just one other. On the last two slides or almost last two, where it is analytical sensitivity of HIV and HCV, in the legend, the discriminatory is labeled HIV on both?

DR. STRAMER: Well, if so, then, there is a typographical error. Yes. These are not my slides, but anyway, I proofed them 5 million times, and I should have picked that up, so shame on me. Yes, the legend is incorrect, and for HIV discriminatory lot A and HIV

discriminatory lot B, those should be HCV. Please, TGMP, correct your copy.

DR. FITZPATRICK: Thanks.

DR. STRAMER: Thank you for pointing that error out.

DR. NELSON: Any other? Yes.

DR. SIMON: Is anybody holding first donations anymore, first time donors, holding them until you get serological results, or are those all going in right away?

DR. STRAMER: All testing occurs simultaneously. First time donors, repeat donors, I mean we don't know, the testing labs don't know that. All tubes are the same and handled the same.

DR. SIMON: Because initially, a few people were holding them.

DR. STRAMER: Oh, I see what you mean, in the algorithms, yes, BSL was doing that, where first time donors went into a different pool than repeat donors, similar to the way the source plasma, that is no longer done.

DR. SIMON: So, the contamination issue, presumably most of it comes from first time donors.

DR. STRAMER: Well, it could come from a repeat donor who is positive, as well. I mean of our yield samples, exactly one-half of them have been repeat donors, and those are the ones who are pretty high titer.

DR. NELSON: Thank you. Yes, Andrew.

DR. DAYTON: I appreciate you pointing out that the vast majority of false positives or contamination, but I want to make sure. Have you ever seen, even once since 1977, an individual donation which reproducibly tests reactive, and it wasn't due to contamination of that sample, was there ever a specific false positive result in NAT?

DR. STRAMER: You mean a biological false positive?

DR. DAYTON: Yes, the way you get in the serology. I mean you have never seen that.

DR. STRAMER: Never once, even once since 1997.

[Laughter.]

DR. NELSON: We are a big behind, in fact, about an hour, but this is sort of a complex issue, and I think we really need to discuss it.

The next speaker is Dr. Chuck Heldebrant from Alpha Therapeutics. I would ask the subsequent speakers, if they can be brief, or if something is already covered, to be brief.

Charles Heldebrant, Ph.D.

DR. HELDEBRANT: We can go straight to the next slide and get going here.

[Slide.]

The plasma industry, through their QPP and Qseal

initiatives, have been working to increase safety including donor and inventory management issues with qualified donors, inventory hold, the viral marker standards, and a donor deferral database, and programs which have been going on since 1997 for the NAT testing of three viruses.

[Slide.]

All of these industry safety initiatives contribute to a continuous reduction in the risk to patients throughout the entire chain of plasma production, product production, and patient treatment.

[Slide.]

Our NAT experience began in 1997. We have tested well over 20 million donations. We have well-established algorithms for donor and donation management, and call it prozones, call it inhibitors, or the like, we haven't seen them. We don't know if they exist.

[Slide.]

There are some basic principles we feel that should be built into any algorithm that you use. The first is that only single donation positive results should be communicated to a donor. A positive result communicated to a donor, even in the context of we need to do further investigation, is a life-changing event and should be done with extreme caution.

On the other hand, only NAT-negative donations

that are qualified should be used. In simple terms, the donor is innocent until proven guilty, the unit is guilty until proven innocent.

All positive results must be resolved to a donation or otherwise accounted for by an SOP, and the SOPs that you use should be tailored to the specific test system that you are using. As Dr. Stramer so eloquently pointed out, there is no one way to do this, and all of us in the plasma industry have as many different ways as you do in the blood industry.

Again, it is important that no donations be released until discrepancies are resolved.

[Slide.]

We would propose a slightly simplified algorithm. Again, for just the sake of argument, anything here on the left side in red and green is exactly what Dr. Dayton presented. When it works the way it should and you account for all your positives, you are fine, but the first question you need to ask when you go and do your testing at a subpool level, at every level you ask a first question, are all my positive signals accounted for.

If the answer is yes, then, go ahead and proceed down your algorithm as you normally would. Once you get a no and you have not accounted for all your positive signals, then, you need to go and resolve it by your SOP.

Again, in this algorithm, we will go down and test subpools, we will move to positive individual donations.

[Slide.]

The second half of the algorithm which is we test the suspect positive individual donations and again we ask the question are all the expected positive signals accounted for. If yes, we go on down, we take the reactive donations, and we do the appropriate things.

If a test is used that is a combination test, you must do discriminatory NAT prior to notifying the donor. You have to quarantine the reactive donation. You have to defer the donor and refer them for appropriate medical follow up. You have to quarantine any prior and subsequent collections and notify any consignees.

Once again, if you fail to get the number of positives accounted for, you must go to a resolution SOP, and not release anything until you complete it.

[Slide.]

Individual donor testing is not always necessary to resolve a discrepancy. When you have a test on a pool that is adequately sensitive, where the pool is small enough, that will be adequate.

In one study by one of our members of NAT-negative subpools, and these are small subpools, associated with a larger, NAT-positive pool, when all of the small subpools

test negative, they went through and tested in these cases over 7,000 individual samples from these subpools, they tested them all, and each and every one of them was negative on individual testing. You will hear a little bit more about that later from the specific individuals involved.

[Slide.]

Another study shows us that NAT results are definitive. In one study, we had 301 HCV suspect positive donors that were pointed to by a positive master pool and the intersection of positive primary pools.

These 301 donations were individually tested and found negative. We followed all of the greater than 2,900 subsequent donations from these individuals, and in each and every case when the donor was called negative based on the individual PCR test of the index unit, every single subsequent unit was HCV negative all the way through.

[Slide.]

With respect to the questions that Dr. Dayton answered, with respect to figure 2, if the master pool is NAT-reactive but all subpools are non-reactive, then you go off to figure 3, which is that sub thing.

Option A was there should all units be released, and our answer is based on our experience, no, we believe that resolution according to a user-specific and appropriate SOP is required.

[Slide.]

If not--this is the second part of the question--there is Option B and Option C, and the like. Again, I will go back to our simple answer, which is there is no obvious "one size fits all" way to resolve a discrepancy. We should hold all the donations involved until the discrepancies are released after resolution according to the user-appropriate SOP.

[Slide.]

Now, the resolution of test results--and please appreciate that people in the source plasma industry have an advantage that people in the whole blood industry do not have in terms of the ability to have time series testing of donors in a time window that is appropriate to do donor history evaluations and the like--but, nonetheless, given your particular resolution algorithm, your algorithm elements may include, as appropriate, donor history evaluations, additional testing, review of sample handling, and evaluation of contamination in the sampling pooling extraction and testing part of the systems.

[Slide.]

In summary, we believe that a simplified source plasma NAT testing algorithm provides a more comprehensive approach to the diverse implementations of NAT tests that we will see in the next years.

NAT testing of source plasma, in conjunction with the QPP and Qseal voluntary standards, assure the highest level of source plasma safety that we have ever been able to achieve, and we look to make it better yet.

Thank you.

DR. NELSON: Thank you very much. Are there questions for Dr. Heldebrant? Yes, Mary.

DR. CHAMBERLAND: Just referring your summary point then, the simplified approach, so each individual licensee, either source plasma, whole blood industry, under your recommendation here, would have to then submit, if you will, their own algorithm? Is that what you are proposing?

DR. HELDEBRANT: Yes, I believe that is appropriate given the fact that while you may buy the test kit with its particular package insert instructions, reagents, and the like, the test kit manufacturer, by and large, does not control your sampling, does not control your pooling, does not control significant portions of the system which can lead to the high incidence of contamination, as Dr. Stramer showed in her presentation, for example, due to the learning curve.

So, I feel it is appropriate for any responsible user, who is moving up from the level of sophistication of serology to the level of sophistication of PCR, to bring the level of their own internal quality systems up to the point

where they can do appropriate investigations themselves.

DR. SIMON: I hope I can get clarification, so I am trying to combine Dr. Stramer's presentation with yours. It would seem to me that on these Option A questions, Dr. Stramer is answering yes, all units could be released, am I understanding that correctly, and you are saying no, but is it fair to say that you are not saying that that is not an acceptable algorithm, it is just that it should be individually evaluated with each submission?

DR. HELDEBRANT: That is absolutely right. I don't believe we are at the point yet where we know enough about the systems and have enough experience to give a blanket answer yes, go ahead and do it. I think it is appropriate to be a little more reasoned in our approach to handling discrepancies.

DR. BOYLE: On the four elements you had for the standard operating procedure, are you saying that a standard operating procedure should incorporate all four elements or at least one of those four elements?

DR. HELDEBRANT: It could incorporate those four and others as appropriate to your system. Some of them may not be appropriate, for example, a donor history evaluation looking at contemporaneous donations in a whole blood setting is largely useless to determine if there was window period infection.

Again, you need to consider how you are using it and the kinds of donors you are using and how you operate.

DR. BOYLE: But you wouldn't be proposing that just one element, for instance, the donor history, would be sufficient?

DR. HELDEBRANT: No, I believe there is a coordinated approach that must be followed.

DR. FITZPATRICK: In that four, when you say additional tests, are you suggesting just serologies or an alternate NAT?

DR. HELDEBRANT: I believe in general we look at these situations in terms of the potential for contamination, but there is also the potential for labeling errors and other things that may occur.

I believe that you should stay with, if you will, go with the girl who took you to the dance, and stay with the NAT that got you in trouble, and resolve your problem there, don't go looking for another NAT to get you into a second set of problems.

I think appropriate serology does help you. If you are in a situation where let's say, for example, your robotic pipetter went out to lunch and it happened to be a high-titered window period sample, serology will be of no help, but you need to find that out yourself.

It is most appropriate when you investigate these

things as you do the testing. You will learn where the failure modes are, and you will make your systems better, and that is what we really want them to do.

DR. NELSON: Thank you.

DR. EPSTEIN: I just want to comment that from a regulatory point of view, there is an advantage in standardization, and the challenge here is to figure out whether we can have general scheme which is broadly applicable.

I am willing to entertain the notion that because of the different logistics involved with large pools, and therefore breaking down to medium-sized subpools, that there may be some specifics in the recommendations that should address that situation different from whole blood where you basically start with a small pool, not unlike a subpool.

So, that said, I think there is some room for difference. On the other hand, I think many of the issues that you are raising really have to do with proficiency and integrity of the operation.

FDA recognizes, and has recognized for years, the need to consider invalidation of results when there are identifiable errors, whether they are errors with the reagents or with the handling or any other aspect of the assay and the SOP.

But I think that that is really a fundamentally

different question, in other words, what do you do when mistakes have been made or reagents have failed can be distinguished from what do you do when you have got reactive results and there is no apparent system failure or reagent failure, and it is the latter that we are really discussing here because we know that there is some inherent false positive rate with these assays, just like any other assays. Sure, they probably have underlying causes, but those causes are not always discernible, so what we are looking for to the extent possible is a standardized approach to what to do with those reactivities.

DR. HELDEBRANT: I appreciate that. I think unfortunately, there are commonly assignable cause scenarios that will lead you to a reactive master pool or reactive primary pool in a negative individual sample, which through an appropriate SOP-based resolution are resolvable and assignable, and these are errors that are--they randomly occur, they will happen.

I share your goal of trying to have an algorithm that is standard and suitable. I am just not sure that at this early stage of bringing this new technology in and beginning to hand it out to a tremendously wide variety of people who are going to implement it, let's face it, with a history of having implemented serology assays successfully, I think there is another level of sophistication they need

to be aware of, and I don't think it would necessarily be a bad thing to take it in a two-step approach, to go ahead and put it out there, but ask people to be a little bit more circumspect and a little bit more thoughtful until we do gather a substantial amount of data.

I think the way to really resolve this, Jay, would be at some point, perhaps a year after these are in general use, is to have the FDA convene a workshop and then come back together and talk about the experience in a rational way and try to develop some way to generate this.

I think it is just too early for us to be all knowing enough to be able to get it down on paper right now.

DR. STRAMER: Not to disagree, but most IND studies, as we have all been doing, test a finite number of samples, 10,000, and we find our sensitivity, we find out specificity, and we find all of the policy issues associated with managing that new test - lookback, deferral, everything.

We have now had, and you have had a lot more experience than we have had, but in the whole blood industry, we have had two years of testing now. We have tested nearly 25 million donations in this industry, and that excludes source plasma. I am not sure we are going to know a whole lot more with another 12 or another 25 million donations than we know right now.

Anyway, that is my comment. Now, my question. Just for my clarification to the question Toby asked, because I was not paying attention and I said yes, for Question 1(a), should a single negative test on the individual donation be sufficient for release?

In the NGI algorithm, which I am sure we will see, the cube, if you have a 512 pool that is reactive when you test the layers and the X, Y, and Z rows, layers, and columns, if they test all negative, those 24 tests, you consider those 512 donations and product for release, is that correct?

DR. HELDEBRANT: No. What we consider is we have failed to account for the number of positive signals, and we go to an investigation and a resolution algorithm.

DR. STRAMER: And what does that involve?

DR. HELDEBRANT: Basically, you take them as individual cases. You begin by going back and retesting and then you follow the trail wherever it leads.

DR. STRAMER: But through all the trains, not to go back the Andrew, the lost trails, have any of those been found trails that would help educate us on what the causes are?

DR. HELDEBRANT: I think so. I think everybody in the industry has them, and I think a workshop is probably the best place to do that, where we can sit down and talk

about our experience with anomalous result resolutions.

DR. STRAMER: Thank you.

DR. SIMON: Is the inherent problem here or is there an inherent problem in the difference between the plasma and whole blood with regard to the size of the pools and what that involves, does that account for it, between the two of you, the size of the pools?

DR. HELDEBRANT: I don't think it is the size of the pools necessarily. I think many of the things that happen will happen whether you use a big pool or a small pool. If they are pre-test events that are involved in generating contamination, if it is a bad practice for a small pool, it is a bad practice for a big pool, you are going to get in the same kind of trouble.

DR. SIMON: But in terms of whether a single agent, these questions, in other words, are these questions different for a large pool or a small pool?

DR. HELDEBRANT: Well, not for the question of is a single unit test definitive. I mean we consider the individual test of a sample to be definitive.

DR. FITZPATRICK: I think, Toby, both industries are doing similar things, are calling it something different. I don't think the Red Cross is going to release a unit without resolving that that unit is safe, and that unit is a single lot. The plasma industry is going to

resolve their lot before they release it, and so they want an answer before they release that lot as to what caused that false positive, and I think what we are hearing is just an application of GMP to release that lot, be it a unit of blood or a lot of plasma product.

So, they have incorporated into their algorithm the requirement for a GMP review prior to lot release as a resolution of the positive, and what they are suggesting is that we require that same sort of GMP review of the results and procedures before that unit of blood is released, and I think both organizations do that, it is just a matter of making it a principle and a policy.

DR. BUSCH: I think we saw data that Susan summarized from the whole blood programs that supported, to my mind, the firm conclusion that a reactive pool that does not yield either individual or subpool reactivity represents false positive results.

In the current program, certainly the Gen-Probe program releases all the donations from a reactive pool if you do not get individual donation reactivity, and I think what we heard from Chuck is that their programs add a layer of further investigation, trail following.

You know, to me the data that Susan summarized during the early phases of the programs, and still in the Roche programs, there has been further testing. We did

retesting of the pool, repooling, dilutions, as has Roche continued to do retesting. None of those efforts have identified any basis in terms of true infection for such nonresolvable pools.

Then, in our program and Susan's, we actually reverted to a system based on that experience that actually released those products and, very importantly, we have continued to track the subsequent donation status of donors who were implicated in an unresolved pool, and have zero cases where an infected donor was ever identified downstream after being implicated in an unresolved pool.

Were we to have taken the position early on that all those needed to be worked up and you could never shift while under IND to a perhaps more liberal program, we never would have gotten back to the point of feeling comfortable releasing. We never would have gotten the kind of follow up enrolling people that we were able to achieve by simply not deferring those donors and allowing those people to come back and donate again, and track that data.

So, much better than waiting two years and going more conservative, I would argue let's allow the programs to operate as they have, and perhaps enforce a continued prospective tracking of donors who are in those pools to further generate more data to prove that these are false positives.

DR. EPSTEIN: Well, putting the question another way, in the experience of the plasma industry, has there been an instance in which you have had a reactive master pool, negative subpools, and have identified a positive individual unit, and if so, can you give us a numerator and a denominator?

I think the compelling argument that we have heard from Sue Stramer is the numerator was zero and the denominator was large, and so I am asking you the same question. This was Andy's question. Have you had the experience of a positive individual unit in the face of all "negative" deconstruction subpools, X, Y, Z pools?

DR. HELDEBRANT: No, not to my knowledge.

DR. EPSTEIN: Then, what is the basis for thinking that further testing remains necessary? I don't have a problem with the argument that there should be a GMP investigation about why did you have this reactivity. Certainly, we want it to go away, and we are never going to learn that if we don't investigate it.

But the question at hand is are the units safe to release, and your data--you didn't actually give us numbers, though, and I would like to hear the numbers--but your data, then, do agree with Sue's data?

DR. HELDEBRANT: Yes.

DR. EPSTEIN: You found no individual positive

units in the face of negative deconstruction subpools?

DR. HELDEBRANT: Right. We have had negative deconstruction subpools which, when we retest them, come up positive because of very low levels of virus, and we do find the individual positives, which is the strength of doing a GMP type investigation. I agree with you, additional testing is not always required, but I do believe that as you bring on a new test that is highly sophisticated, that has great sensitivity and has a lot of potential error areas in it, you need to apply some extra care to it.

DR. EPSTEIN: So, let me just see if I understood your answer correctly. What you stated is that further retesting of deconstruction subpools--and we haven't talked about under what method or scenario--has yielded reactive results even though the initial test of deconstruction subpool was negative.

DR. HELDEBRANT: Yes.

DR. EPSTEIN: And in that instance, you found individual positives. So, that would speak to the question of a one-time test on--I presume it was an archived deconstruction?

DR. HELDEBRANT: Yes.

DR. EPSTEIN: Was not adequate.

DR. HELDEBRANT: There have been cases where we have had a low level of positivity, and you don't pick it up

the first time, you test it again, you get it, you investigate, and you find it.

DR. EPSTEIN: Well, I think that is a very important piece of information which we had not previously heard.

DR. MITCHELL: To follow up, was that retesting using the same test?

DR. HELDEBRANT: Yes.

DR. MITCHELL: Thank you.

DR. SMITH: Richard Smith from National Genetics Institute.

I would like to just clarify. In the cases where you retest the subpools, is that not only when you have had at least one dimension come up positive?

DR. HELDEBRANT: It is in the case where we cannot account for all of our positive signals.

DR. SMITH: So, you had one dimension on the subpools come up positive?

DR. HELDEBRANT: Yes.

DR. SMITH: Thanks.

DR. STRAMER: What does it mean?

DR. HELDEBRANT: What it means, imagine we are doing the cube and Dr. Smith will show it in just a second, if you have a positive master pool that comes up, you presume there is at least one infected donation in there

somewhere, which means when you go to what we call the primary pool levels in our system, rows, columns, and layers, which are the three dimensions of the cube, you would again expect to find, if everything is working correctly, a positive row, a positive column, and a positive layer.

That intersection would point to the suspect positive unit, which you then confirm and test in our algorithm. When it works that way, everything is perfectly fine.

Now, if you get to the subpool level and you get a positive row, a positive column, and no positive layer, you cannot account for your positive signal, that is a resolution situation that goes to an SOP until we can find out where is that positive signal.

DR. FITZPATRICK: And so you are saying you have done that test, you had a master positive pool, did your X, Y, Z of 24 were all negative, but then when you did your resolution, you found a positive?

DR. HELDEBRANT: Yes, we would get, for example, a positive row, a positive column, and no positive layer, and we would go back, look at the layers again, and let's say for the sake of a hypothetical example, the layers again were all negative, so we then look at all the suspect units that were involved. We would go until we find a reason for

that positive signal.

DR. FITZPATRICK: But have you had the instance where you had a master pool and a negative row, layer, and column, and followed up and found a positive?

DR. HELDEBRANT: No.

DR. FITZPATRICK: Okay.

DR. van der POEL: I have just one small comment from the Netherlands, a small country compared to yours. We have done NAT testing for a while now, since two years. In our country, when you have a primary pool which is positive, we repeat it in duplicate. When we find no single signal, then, it is released, and I think I would agree to a strategy where if you cannot reconfirm on a second occasion, a signal in a similar test, then, you can release the products.

Thank you.

DR. NELSON: Thank you very much.

There are six or seven people who want to testify at the open public hearing. I would ask that if you have some new data or new ideas or something that will help us, we would certainly like to hear it, but if you don't, if you can either be brief or say I agree with the previous or something.

The first one is Dr. Richard Smith from the National Genetics Institute.

Dr. Smith.

Open Public Hearing

DR. SMITH: I just want to thank you for the opportunity to present to the committee. As I said before, my name is Richard Smith. I am representing National Genetics Institute. As you know, we perform the NAT screening for much of the source plasma industry.

Before I present our testing algorithms, let me say that in many cases, plasma pooling and positive pool resolution is the responsibility of the companies for whom we perform the testing services. These algorithms are designed to work optimally within each system and, of course, with utmost safety of the final product in mind.

That being said, I would like to quickly review our testing algorithm, which does go into in depth what we do when we run across a single positive subpool in one dimension and no positives in the other dimensions.

We have adopted a standard algorithm for dealing with that situation that I hope people will find acceptable.

[Slide.]

Our first slide shows a familiar slide, the cube with three-dimensional matrix in which all samples are first combined into 24 separate primary pools. Each sample is represented in one row pool, one column pool, and one layer pool, the tubes containing 64 members each. All primary

pools are then combined into one master pool.

[Slide.]

We test the master pool and in the majority of cases find it negative and release all the samples. When a positive result is observed, all 24 primary or subpools are tested and in the great majority of cases, one row, one column, and one layer are found positive implicating an individual sample, which is then tested to confirm positivity.

Now, addressing the second question before the committee, occasionally, all 24 primary subpools are negative and in these cases, we, again NGI, releases all the samples as not implicated based on the fact that they have been retested now in triplicate, in pools that are 8 times less dilute than the original master pool.

This is, of course, somewhat similar to the EIA paradigm allowing release of samples after duplicate negative tests following initial reactive.

It is important to note that in the case of NAT pooling testing, however, the retest is performed on much more concentrated samples than the initial test.

[Slide.]

In still other cases, individual confirmatory testing fails to explain all the positive subpool results, and in addition, we could have one primary pool come up

positive in which case we wouldn't go to testing all 64 individual samples, but we would go on to what we call resolution pooling.

[Slide.]

In these cases, all members of the initially positive subpool that isn't explained by the individual samples are recombined into a smaller, 3-D matrix, 4 by 4 by 4, in the same way as before, but we go straight to the 12 primary pools for testing. Again, implicated samples are tested to confirm positivity.

Now, the samples again here are being tested in triplicate, this time in pools with only 16 members versus the 64-member pool that they would have been found positive in last. If all the 16-member pools are negative, we release.

If once again, and this is very rare, a positive 16-member pool is not explained when we test the individual samples, we would test all 16 component individual samples of that resolution primary pool.

[Slide.]

So, finally, if a positive is identified after testing those 16 results are reported, the rest are released, and pertaining to the first question before the committee, if all 16 samples are negative, the individual test results would supersede the earlier result and samples

would be released as not implicated.

And that is our algorithm. Thank you.

DR. NELSON: Thank you. Questions? Thank you, Dr. Smith.

The next person is Dr. Craig Halverson from Gen-Probe. Is he here, Dr. Halverson?

The next is Dr. Larry Pietrelli from the Roche Molecular Systems.

MR. PIETRELLI: Thank you. First of all, thanks for the degree. I am not a doctor.

DR. NELSON: That's okay.

MR. PIETRELLI: I would like to thank the committee for the opportunity to speak. I am Larry Pietrelli from Roche Molecular Systems.

[Slide.]

The Roche COBAS AmpliScreen HCV clinical trial was initiated in April of 1999 at 13 clinical sites throughout the U.S. The sites range in size from 40,000 annual donations tested per year to over half a million annual donation tested per year.

[Slide.]

The Roche pooling procedure is to take sets of 24 tubes up to 96 and to load them onto our pipetting rack. The rack is placed onto our pipetter or an archive plate, and intermediate plate are made.

The archive plate is removed, covered, and refrigerated for possible future reference. The intermediate plate is then used to make primary pools or master pools, each containing 24 donations.

The primary pool or master pool is tested. If the result is negative, all 24 units are released. If the result is positive, further testing is required to determine which sample in that primary pool is positive.

To resolve the positive primary pool, the archive plate is retrieved and used to pipette 4 secondary or subpools. Each secondary pool contains donations from 6 donors. These 4 secondary pools are tested and the positive pool is identified. The 18 units associated with the 3 negative secondary pools are released. The archive plate is retrieved again and used to pipette 6 individual samples. These are tested and the positive unit is identified.

The data on the next several slides are preliminary data from the HCV clinical trial. The data are from all 13 clinical sites and represent 6 consecutive months of testing.

[Slide.]

During the 6-month period, over 1.7 million donations were tested. The vast majority of samples were negative at the primary pool level. Approximately 2 percent of the primary pools were positive and required further

resolution testing. In the 6-month period, there were 1,324 donations that were NAT-positive.

[Slide.]

Eighty-six percent of the NAT-positive donations or 1,138 were also positive for EI and RIBA, 11 were positive by NAT and EIA and indeterminate by RIBA; 9 were positive by NAT and EIA and negative by RIBA. 136 donations were positive for NAT and negative by EIA. For 27 donations, the RIBA result was either not tested or unknown, and for 3 donations, the EIA result was either not tested or known.

On the next slide, I will talk about the 136 NAT-positive, EIA-negative donors.

[Slide.]

Of the 136 donors that were NAT-positive and EIA-negative, 7 were enrolled in the follow-up study and seroconverted. These are confirmed window cases. Four were lost to follow up, but were presumed to be window cases because an alternate source drawn on the same day, in this case the fresh frozen plasma, was positive.

Thirty-three were enrolled in the follow-up study, and all follow-up samples were negative for NAT, EIA, and RIBA. The average length of follow up was over 9 months. All but one subject had six months or over 12 months of follow up. Six were enrolled in the follow-up, all follow-

up samples were negative. In addition, these 6 also had alternate source drawn on the same day, negative by NAT. The alternate source could have been the duplicate tube, the archive plate, the EDTA, or the fresh frozen plasma.

Twenty-one had alternate source, test NAT-negative, 65 had no additional information other that that associated with the NAT testing.

[Slide.]

We have been asked to comment on two specific scenarios. The first is when the master pool is positive and the subpools are negative. At the time when the primary pools or master pools are prepared, 3 replicates are made. One pool is used for initial testing, and the other two are for situations such as this.

If the primary pool is positive and all 4 secondary or subpools are negative, Roche algorithm requires testing the two remaining primary tubes. If both of these pools are negative, all 24 units are released. If one or both of the master pools are positive, all 24 donations are tested individually.

A random sampling of primary pools identified, 8,594 pools that met the validity criteria of the test kit and were within the analysis time frame. Eight of these primary pools had 4 subpools that were negative. Seven were negative on testing of the two remaining primary pools. All

units were released. One was positive. Testing of the individual donations to determine one NAT-positive unit.

The EDTA was NAT-positive, but the fresh frozen plasma was NAT-negative. This donor was an error, not enrolled into the follow-up study. A subsequent donation 7 months later was negative by NAT, EIA, and ALT was normal.

[Slide.]

The second scenario is when the master pool is positive, the subpool is positive, and individual donations are negative. The subpool and individual donations are pipetted, these are pipetted in duplicate. If the master pool is positive, the secondary pool is positive, but all 6 individual donations are negative Roche's algorithm requires testing the duplicate individual tubes. If the repeat testing fails to identify the positive donations, the associated blood components are destroyed, and the 6 donors are placed under surveillance.

The random sampling of 8,594 pools, one pool fell into this category. Of the 6 donors associated with this pool, 3 have not returned for donation, 1 returned, but the bleed was unsuccessful, and the last 2 had 4 and 5 subsequent donations. All of these donations were NAT and EI-negative.

[Slide.]

In conclusion, preliminary data supports the low