However, when you test people, a lot of people have had markers of viral hepatitis, and again this tends to increase with age, so somewhere between 30 and 40 percent of people had markers for either hepatitis B or C or hepatitis B and C. So, basically, history of hepatitis is a very insensitive way of finding out whether people actually had hepatitis B or C previously.

However, if you take this group of people who said, "Yes, I have a history of hepatitis," there is actually one person here, 60 here and 20 here. So if you look at this group of people and say, how did they do at reporting history of hepatitis? So if they say report it, did they actually have a history of hepatitis?

Well, actually they do quite good. Again, there is only one person here so you can discount him, but roughly about 80 percent of people who reported a history of hepatitis actually did have a history of hepatitis, and roughly about 95 percent of people over 40 who reported a history of hepatitis actually had a history of hepatitis. So, basically, if people report a history, it is reasonably believable.

So what conclusions can you draw? At least among cases of acute hepatitis A in the Sentinel Counties, very few people report a history of hepatitis, and this increases with increasing age. Many people with serologic markers of

hepatitis B and C do not report a history of hepatitis. And, finally, most people who report a history of hepatitis do have serologic markers, at least of hepatitis B and C.

And that is my presentation. Thank you very much. I will be happy to entertain questions.

DR. HOLLINGER: Thank you, Ian.

Any questions for Dr. Williams? Yes, Marion?

DR. KOERPER: I am curious, is there a lower age limit? Are you excluding children, for instance, under 18 or under 12?

DR. WILLIAMS: We take all comers, although we rarely see children with acute viral hepatitis, I mean.

VOICE: (Inaudible.)

DR. WILLIAMS: That's right, and again, that's what I mean. Those people would never make it into our study because they have to be acute and symptomatic, so basically they are excluded by the nature of that. But if they are symptomatic, they are in the study, but we have less than 2 percent are actually under 15 or so, but we do see them occasionally.

DR. KOERPER: Right, right, right. And my second question is, what is your definition of chronic hepatitis? You were comparing the hep C's versus the non-A to E, and you said there was a greater incidence of chronic hepatitis.

DR. WILLIAMS: They had to have at least two

follow-ups where the ALTs were more than 2.5 times the upper limit of normal. That is biochemical evidence of chronic hepatitis, and I used a relatively conservative one.

DR. KOERPER: Okay.

DR. HOLLINGER: Mary?

DR. CHAMBERLAND: Do we have, in the Sentinel Counties Study, especially for people who have given a history of hepatitis or maybe routinely, has CDC ever tested for other agents that cause hepatitis? EBV, CMV, whatever?

DR. WILLIAMS: WE don't specifically test for them, although a lot of physicians sometimes will test for it and will note that, but there is not a specific testing form, and that is one of the limitations of especially our non-A through E group. They are not all tested for EBV and CMV, although there is a physician--there is consultation with the physician to help rule those out.

DR. HOLLINGER: Any questions?

[No response.]

DR. HOLLINGER: I guess for purposes of this discussion there would not be a concern with chronic disease anyway, would there not, if they had EBV and CMV?

DR. WILLIAMS: Yes.

DR. HOLLINGER: Thank you, Ian, and there may be other questions a little later.

The next presentation is by Dr. Harvey Alter, and

I think Harvey is going to talk on SEN-V. Is that right, Harvey?

DR. ALTER: Partly, yes. Actually, Robin first asked me to talk on the history of hepatitis question, and I agreed to that. Then he asked me to talk about SEN-V, and I agreed to that. Then he asked me to talk about both, so we compromised and he gave me an hour and a half for my presentation today. So I will be talking about both, and I first want to address the history of hepatitis, and what I want to do is put the history of hepatitis into historical perspective.

Now we have to be aware that history is changing, and therefore that we have to change questions about history. I have to move closer to the mike? Okay. This is going to be hard.

DR. HOLLINGER: It is going in the Federal Register, so we want to get this.

DR. ALTER: So that is really the bottom line of my message. We have to be willing to change these questions at some point, and to do that I wanted to go with the historical perspective.

Now in the 14th century, we asked, "Have you ever, even once in your life, had the bubonic plague?" Now, this was a dynamite question in the 14th century, but by the 15th century you already knew that the yield of this question was

not very good, and by the 16th century the forerunner of the FDA, then called the DAF, which I will explain in a moment, or the "daf", was willing to drop this question, so we haven't asked this question since the 16th century. Now the DAF actually stood for "Don't ask, for God's sake." And so the former FDA was a lot more liberal in adjusting their questions.

Now this is actually to address the question. Robin really has gone over this, and I am going to go through it very rapidly. This is hard to do. Basically, this is the virus. We can maybe clear that slide a little bit. The virus, here we have the virus, and here we have the evidence, how we detect the virus, and then the relevant residual value. And I think we would all agree that for HAV, HEV, there are no carriers, transmission is rare, and the value is nil.

For HBV we have superb tests, almost no evidence of transmission for almost a decade. There is the question whether there are seronegative viremic individuals, but there is no proof as yet that these have ever transmitted disease. So I think we are not making it nil, but near nil for B.

For HCV, we know that only about a quarter of the patients have a clinical history, so the history has limited value there, and we have superb testing now with NAT

testing. The residual risk is less than 1 in 500,000, I think, maybe closer to a million, and I think we have really no residual benefit of a history question for HCV.

So we are down to non-A to E, and we know that the vast majority of these cases, if you take all comers, are subclinical, but the CDC data shows the other end, that there are some people who have clinical disease, and I think Ian's presentation is very valuable in that respect. We don't know much about the severity or the frequency of chronic hepatitis in non-A to E.

And we know that there is some value of existing assays. I will show you from my own data. Anti-HCV and perhaps HBV markers overlap with the non-A, non-B cases, so there is probably a surrogate value of our existing assays in preventing non-A, non-B. So the value of our history question in regards to non-A to E is really the crux of the issue. It is a theoretic value at this point, and I don't know that we will be able to resolve that in this talk or this session.

This is something I used in the workshop last time, trying to project the impact of the question, and I think we know that about .1 percent of people give a history of hepatitis, so if we take 1 million donors and .1 percent give the history, it would be 1,000 donors who would have the history. And using the CDC data, a 3 percent change

that, of these 1,000 donors with a history of hepatitis, that they might have had non-A, B, C. So that would get us down to 30 people.

From our data, and this is very soft data, but we usually estimate about 30 percent of people with non-A to E might become chronic, and in Ian's data it was 22 percent, and I think it is probably closer to the lower. But even if we said 30 percent, a maximum number, we would then have nine potential carriers out of that original 1 million people, or .0009 percent of the 1 million.

The chance that these carriers would be interdicted by some other history question, by other viral testing, I think is around 50 percent, but that is a guess. So there would be 4.5 eligible non-A to E carrier donors. We don't know the transmission rate, but all the other viruses seem to be about 90 percent, so we have four potentially infected recipients.

The risk of overt hepatitis in those four people is, again, 3 percent, so about .12 recipients might have overt hepatitis, and the risk that they would develop chronic hepatitis is about 30 percent, so there might be one recipient who would develop chronic hepatitis out of each million people screened.

And therefore we would exclude 1,000 donors based on the history, to theoretically prevent one case of chronic

hepatitis, and each 1,000 donors means 2,000 donations and 6,000 products. So the impact is quite high, but you can't say that it would never prevent a case. I mean, that is the conundrum that I will get back to at the end. It is hard to say you would never prevent a case by dropping the history question, but the yield is going to be exceedingly small and the loss quite considerable.

So I would propose, really, that we have to bite the bullet and say you can never say never, and not stay here to tweak the question and to liberalize it a little bit, but actually drop the question. At some point we will. I am always jumping ahead.

But I say we drop the question now and ask the question, "Have you had hepatitis, or been closely exposed to somebody with hepatitis in the last year?" And if they say yes, then you defer them for another year, and then you depend on your serologic markers and your NAT testing. It is skipping the issue of non-A to E, I grant you that, and that is the sticking point of this argument. So we are going to get back to the importance of that potential.

So I still feel, though, that we have to be able to move with the times, perhaps even with the New York Times, and that leads us into the SEN-V discussion. So I have been allowed at this time to present some of the SEN-V data, and this is coming primarily from Danieli Primi, who

was the discoverer of this agent, working with a company, DiaSorin.

And what we now know about SEN-V is that it is really a family of viruses, at least we think it is a family of viruses, and these are DNA viruses. It is not clear yet whether they are single-stranded or double-stranded or perhaps, like hepatitis B, both single-stranded and double-stranded. They can't figure that out. But Dr. Primi feels that this is a linear virus. Everything he has tried to do to show that it might be a circular virus has not worked out in that regard. So that would make it different than TTV, which they now feel pretty certain is a circular DNA virus.

It is a small virus. Average length is 3,000 nucleotides. Each of the SEN viruses--and I will show you this in a minute--encodes for three open reading frames, so there is a potential that a protein can be expressed and a serologic system set up, but right now that has not worked out. And the length of the ORF1, ORF2 and ORF3 varies with each of these different agents.

This is not very clear but it just shows you the general structure there. There is an untranslated region like with hepatitis C, there is a long ORF1, a smaller ORF2, and an ORF3, and another untranslated region on the other end. And this is the different variants. Well, I will show you that better here.

So there are now, if you thought bringing SEN was bad, we now have actually multiple SENs. SEN is the name of the patient, the initials of the patient, S-E-N. On the end of each of these is ORF1. This is all ORF1. And then we have SEN-C, SEN-H, SEN-B, SEN-A, SEN-G, so essentially it is A to H. SEN-C and SEN-H are closely related, but the others are quite variant from one another. These differ from each other by 35 to 45 percent. These are very distantly related.

And to put this into perspective, this inner circle is the total range of the variation of hepatitis C, the various strains of hepatitis C, the subtypes of hepatitis C. If you went from the furthest ones apart, it would be encompassed in that circle. But here we have divergence that is markedly greater, and it is even hard to say these are just a single family, but they have strikingly similar characteristics, and that is--so that is the one point to bring across.

Now, we have focused, because of the initial work with our transfusion study, we have focused on SEN-C/H, counting this as sort of one agent, and SEN-D, because those two variants, if you will, or those two members of the family seem to have the closest association with post-transfusion hepatitis. So just go, go down.

This is--I think the only thing I want to point

out here is that this is a dense agent. On seizing choride banding it bands at 1.4 grams per centimeter, meaning it is a heavy agent. This is not typical of an envelope virus. This is not proven to be a non-envelope virus but is probably a non-envelope virus, a dense DNA non-envelope virus.

When the company originally looked at different populations, they found if they looked a blood donors, and these were primarily European blood donors, that the vast majority of blood donors tested negative for this agent, but that some had SEN-B, some had SEN-A. The rates of the two viruses that we are interested in, SEN-CH or SEN-D, were very low in the European donor population, around 1 to 2 percent.

When they looked a interven--I am just going to concentrate now on C, and I will call it C and D for ease of it--when they looked at drug users, they found that 15 to near 30 percent of drug users had one or the other of these agents, suggesting it was a parenterally transmitted virus. And when they looked at polytransfused patients, they also found rates of 10 to 15 percent among thalassemics. So this was consistent with this being a transfusion transmitted and an IV drug use transmitted agent.

One last piece of data from the company that I think is important but as yet unconfirmed, and this was a

study designed to see if this virus replicated in the liver. They used liver tissue from patients who had hepatocellular carcinoma, not looking for a relationship to hepatocellular carcinoma, just that was the liver tissue that they had available.

And what they did is, they extracted the nucleic acid from the liver, they treated it with DNAse in the hopes of destroying any liver DNA, any DNA present. They then activated the DNAse. They then reextracted the nucleic acid, so presumably the only thing that is left is RNA. They then converted this to complementary DNA, and then amplified using specific SEN primers, and entered a detection system by an EIA method.

So essentially they are looking for cDNA, and they found cDNA in these two different patients. They found cDNA in the liver, both in the tumor and around the tumor, and finding the cDNA implied that they were picking up a replicative intermediate of a DNA virus, so they were picking up an RNA that was converted to cDNA. So this suggested that the virus was in the liver and that there were replicative intermediates in the liver, and that is the best piece of evidence that we have that this might actually be a hepatitis virus, but it needs to be confirmed. We need more livers, and we are working with Ed Tabor, in fact, to look at this.

So now I want to go into our own data. Would it help to get this podium out of the way? Is this blocking people's view? No? Okay.

If you look at our patients, this is in our prospective transfusion studies, looking at people, we had a group who were actually transfused and a control group who were not transfused, and among people who were not transfused but were prospectively followed, we found new SEN-V infections, that is, they were negative before transfusion, became positive. This is a six week post-transfusion sample. They became positive after their surgery, 3 percent, but among those who were transfused it was 40.6 percent. This was a highly significant difference, and it suggested that this is a transfusion transmitted agent, although there might also be a nosocomial transmission because 3 percent seemed to acquire the infection in the hospital without getting a transfusion. That is similar to what happened with the TTV work we did.

The relationship to transfusion is shown here. There is a seeming step-wise gradation from no units, to one to two units, to three to four units, to five to six units, but after six units it levels off, for reasons I am not totally clear. We don't know who is susceptible among the donor population, who isn't susceptible, but at least in this range the number of units seems to correlate with

whether or not you get infected with SEN-V.

And here are the prevalences in donors. We have tested 436 voluntary donors, current NIH donors. Eight of them or 1.8 percent were positive. This number keeps coming up. The number I think in both Europe and the U.S. among volunteer donors is somewhere between 1 and 2 percent, possibly higher in Japan.

We have tested now some donors prior to 1990, and we just started doing this. We will get a bigger number. Because that is when these cases occurred, we want to know what the donor population was then. The rate may be a little bit higher, but the numbers are so small, we can't say.

We also--this is the rate of SEN-V in patients before they were transfused, so this is sort of the background population, background prevalence in the population coming to a hospital. So it is a relatively low prevalence agent compared to TTV, for instance, perhaps in the range of what HGV was, but that proved not to be a hepatitis virus.

So the crux of our study is here. When we looked at the cases, and we had 13 cases of transfusion-associated, non-A, non-B hepatitis, one of those patients had preexisting SEN, so we could not determine anything in relation to SEN in that patient. But of the other 12

patients with non-A, B, C, 11 seroconverted, 11 became SEN viremic, and everything I am talking about here is viremia, we don't have an antigen, antibody test. So 92 percent of the acute post-transfusion, non-A to E cases were acutely viremic for the SEN agent.

Among the group who were transfused and identically followed but did not develop hepatitis of any kind, the rate was also high. Thirty-four percent developed a new SEN infection. This difference is highly significant. It is p less than .0001, so there is a relationship to non-A, B, C, but a disturbingly high background among people who don't get hepatitis.

When we looked at cases who had transfusion associated hepatitis C, the rate was 41 percent; again, different from this rate. The rate of those who didn't get hepatitis, got hepatitis C, was the same. And among the non-transfused group I just showed you, only 3 percent had SEN infection. So there is a strong, a very strong--I mean this is dramatic, with TTV it was like 25 percent in all three of these groups--so there is a dramatic incidence in the patients who get non-A, B, C hepatitis, but this high background that confounds the interpretation of this information.

So we tested all the patients who got non-A, B, C, so that's the 92 percent who were positive, but we only

tested 94 people who were not already--we started with 100, but 6 of them had antibody ahead of time. So then we tested 94 of 776 patients who didn't get hepatitis. So we have to make an extrapolation. Thirty-four percent of them were positive, I just showed you.

So I have said that if the sampling is random, then among this 776 we would have expected 34 percent or 264 patients who did not develop hepatitis to be SEN-V infected. I am just trying to equate the numbers. So there would have been a total, if this assumption is correct, there would have been a total of 275 post-transfusion SEN infections, of which only 11, that is 11 out of 12, would have developed non-A, D hepatitis or 4 percent.

So what we are saying, then, is that in probability, we are saying the probability is that the vast majority of people who get infected with SEN do not develop hepatitis. I have another slide about that. I thought it was right there, but we will probably come to it.

This is just to show you some of the cases where I have tried to equate the ALT level shown in blue, the ALT level in blue versus the level of virus in yellow. Now, this is a very crude level. I am giving copy numbers here and the copy numbers are quite low, but we don't, the quantitative assay, we don't really know what it means. But I think it gives you a relative level of virus. Just take

it as that, and not as an absolute copy number.

And what we found, for instance in this case there were some low-level early ALT elevations that we often find in these post-operative cases, but the hepatitis actually, for our definition of hepatitis, the hepatitis actually began here. You see the virus was present at that point, and virus sort of came up with the ALT, and the virus went down as the ALT went down. So this is a nice correlation between viremia and ALT level.

There is another case where again it is sort of these early, low level, but when the actual true hepatitis began, the virus was coming up at the same time. Here there was a different ALT and a different virus, a rise in virus, a rise in ALT, and then again. So this was the best example. I am picking out some nice examples here.

And here is another case where the virus was there first, which is what you see often in hepatitis C, but really actually not there first. Actually the two came, the ALT was going up as the virus was there. In this case the virus came down but there was a lag before the ALT came down. Again, this is something you can see in hepatitis C as well. So these are all consistent with a temporal relationship between viremia and ALT level, but not always a perfect correlation.

And here is one that is seemingly less perfect.

Here the level of virus is low, the level of ALT is high. This was the patient with the highest ALT level, but the virus was there when the ALT went up, and then the viremia seemed to go up and actually be present longer than the ALT. The ALT came down very fast.

So this is a little bit off skew, but I think the important point is that in all these cases, there were no cases where we considered the hepatitis blip of ALT, there were no cases where the ALT went up before the virus was present. In other words, there was always viremia at the time the virus was present.

And here are the cases of this non-A, D hepatitis. Out of these 11 cases, none of them were icteric. The mean peak ALT, because these were all comers, these were not presenting as clinical cases, the peak ALT was 396. If you take out one patient who went up to 1,740, the mean was 262. The range was from here to here, but the median was only 200. So this was a very mild hepatitis. Not a single case was symptomatic. Two cases had what I would call substantial ALT elevations, but as a whole the ALT elevations were quite low.

Now, in judging how many of these went on to chronic hepatitis, the data is soft. These cases were all mild. We have no biopsy data. Looking at ALT elevations, I would say that two of the cases clearly went on to chronic

ALT elevations and two others had sort of intermittent lower level ALT elevations which might have represented chronic hepatitis but there is no way to prove it. I will show you on the next slide, this is kind of interesting, this two versus these two.

And here is some persistence data that we have just come up for. This is combining cases who had SEN-V positive non-A, B, C hepatitis and those who had it in coexistence with HCV. I have included the HCV cases because we followed them longer. We had longer serial samples. But what you see here is that the majority--this is pre-transfusion. They are all negative. This is six weeks post-transfusion. They are all positive.

And this is six months post-transfusion. So by six months post-transfusion, more than half the patients have lost SEN-V, so it tends to be a predominantly transient agent. Here is a case, we don't know because we didn't get follow-up, but here is cases that lost it some time after one year, and these cases here lost it after four years, but cases down here that are persistently viremic after 12 years.

So this definitely is an agent that can be persistent. It may be associated with chronic hepatitis, at least in two cases. And interestingly, in the two cases where the ALT elevations were substantial, into the chronic

phase, these two cases had prolonged viremia that went along with the ALT elevations.

I will run through this. It was just to show that the virus had no apparent impact on hepatitis C. In the cases of hepatitis C that were SEN positive or SEN negative, the ALTs were the same, and the rate of chronicity was the same. So we have no evidence that this makes hepatitis C worse.

We have now looked at some cases with acute liver failure, using the fulminate hepatitis repository of Will Lee, and found no association of this agent with acute liver failure. Among 17 cases of non-A, B, C acute liver failure, none of them were SEN positive. There were nine positives in those who had acute liver failure of other etiologies.

We are looking at a lot of groups with chronic non-A to E hepatitis. I am only showing you one slide from Japan, where the rate of SEN in the chronic hepatitis patients, chronic non-A to E, was 25 percent, but the background rate is higher in Japan and it is more difficult to evaluate. So I don't really have any generalizable data as yet on the frequency of this agent in patients with chronic non-A to E hepatitis outside of the transfusion setting.

So what do we conclude? Well, SEN-V is a novel agent. It is not in the gene bank before. It is small, it

is linear, it is non-enveloped. It is clearly transmitted by blood transfusion, possibly spread by other nosocomial routes. It is found in relatively low prevalence in Western nations but in seemingly higher prevalence in Japan, just as the TT virus is.

The incidence is significantly higher, 92 percent, in patients who develop transfusion associated non-A, B, C than in those who don't develop hepatitis, where it is 34 percent, and that is .001. I think there should be another zero in there. And is much higher than those who aren't transfused, as I have shown you. In patients who develop hepatitis, there is an apparent temporal association of varying degrees of validity between the appearance of the virus and the appearance of ALT elevations.

But here is the key. Because the hepatitis population is small and the non-hepatitis population is large, it is projected that less than 5 percent of those who are SEN-V infected actually develop hepatitis. And that may not be surprising because the virus seems to be present at a very low level, and it is possible that there is a threshold for causing hepatitis.

So how do we explain the absence of hepatitis in most cases of SEN-V infection? Well, one explanation is that this is not a hepatitis virus; that despite the statistical associations, this is just a fluke and it really

has nothing to do with the hepatitis we are observing. That is certainly a viable option.

The second possibility is that the development of hepatitis may reflect either the titer of the virus or the particular virulence of the infecting strain, and implicit in this assumption would be the fact that most SEN agents are low titer and/or not virulent.

And, lastly, that there is some host susceptibility factor that determines clinical outcome of any given SEN infection, and this, there are parallels to this such as CMV or EBV. How often do people who get EBV actually get infectious mono? It is a minority of people.

This is just about the clinical part. I won't go through this again, but I think to prove this is a virus, in addition to developing more epidemiologic data, we really need more data that this goes to the liver and replicates in the liver, that you can detect the virus and replicate intermediates in the liver.

We have some preliminary evidence for that. If this were confirmed, I would have a heightened level of appreciation for this virus as a hepatitis agent. If we really can't find it in the liver, then I am not so convinced about the other data I have shown you. But thus far, it makes a picture that could hold up as a hepatitis virus.

So I would conclude one of two things: SEN-V is definitely an etiologic agent of non-A, B, C hepatitis, or secondly that SEN-V is definitely not an etiologic agent of viral hepatitis, and feel very strongly that the probability is, this conclusion will hold up. And I have here at the bottom that p equals "please be pathogenic."

But I just want to end, I have often, for most of the audience, you have seen my slide of the storing of British warheads with the top at the bottom, and labeling the top at the bottom, a very confusing picture. Well, I want to bring you a new quote from a very famous individual that is actually in relationship to this particular issue.

And the quote is: "We have a dilemma because we can't study that without removing it, and then that gets you into a circular logic because you want to be sure you can remove it before you remove it, but you are not sure you can until you remove it."

Now, this sounds confounding, and this issue is confounding, and I would like to bring you the author of this quote, who is one Jay Epstein. And in reality, as always is the case, Jay is right, because what you want to do, just getting back to the donor history question, what you really want to do is stop asking the question to see if it makes any difference, but you can't stop asking the question until you have the data that says you can stop

asking the question.

And that is the circular argument that we are in in the hepatitis history question. But I would lean towards whether or not SEN-V is real or not real, that we may be at the time where actually the value of the question is so minuscule. You will never be able to say it won't prevent a case, but we have so many other measures in place, and we are losing precious donors, that I think it takes a little guts, but the guts is just to change the question to say, "Have you recently had a history of hepatitis?" and then depend on all your other screening measures to prevent transmission.

We know we are down to zero transmission, virtual zero of not only C and B but also non-A, non-B. It has disappeared, along with C. So get your courage up. Okay, that is my presentation.

DR. HOLLINGER: Thank you, Harvey.

Yes, Dr. Tuazon?

DR. TUAZON: Do we have any information on the histopathology of these patients with SEN-V?

DR. ALTER: No, we don't, because they are all so mild that we never did biopsies. Now, you know, we have biopsies on those who were SEN-V and HCV infected together, but there is no evidence that it makes HCV worse, and you assume that the damage is due to the C.

DR. SCHMIDT: Harvey, Paul Schmidt. I presume that if you followed the rules, the donors with viremia had no history of hepatitis, but how about these patients? Would they have given a history of hepatitis?

DR. ALTER: No, no, no, no. Not a single one, no. And I knew you were Paul Schmidt. I used to work for you.

DR. McCURDY: Harvey, can I assume that you have a high degree of confidence with these observations?

DR. ALTER: Yes, I have a high degree of confidence in what we have done. I don't have a high degree of confidence in the meaning of the data, but I think I have moved from being real down on this to being kind of level on it, and I am--you know, when 11 of 12 cases are positive, and when the temporal relationships are there, if the liver, if further evidence shows it replicates in the liver, then I would lean towards accepting it. And I don't know whether it is the only agent, or there could be another agent that is there at the same time.

DR. HOLLINGER: But, Harvey, if you had asked these--you said if you had asked these 11 or 12 whether they had ever had a history of hepatitis, they would have answered--

DR. ALTER: They would have answered no, because these were mild. Even the one patient who had the 1,740, I actually went to his house to draw his blood at that time,

and he was bouncing around. He didn't feel sick at all. So he would never have known he had hepatitis.

DR. HOLLINGER: Yes, Marion?

DR. KOERPER: It sounds like not only was it clinically a mild hepatitis, but that everybody got over it.

DR. ALTER: No, everybody didn't necessarily get over it. There were two who had chronic ALT elevations and persistent viremia, and two who had--

DR. KOERPER: Oh, they had persistent viremia?

DR. ALTER: Yes.

DR. KOERPER: Okay.

DR. ALTER: And two who had up and down ALTs without persistent viremia, that may be--you know, it is so hard when the ALT values are low, you never know what they are due to.

DR. KOERPER: And what was the follow-up period?

DR. ALTER: Well for some of them, out to 12 years. But mostly--of those 11 cases, however, only 2 of them were followed out a long time.

DR. HOLLINGER: And it is true, I believe, that some--that the test has actually been improved somewhat, so it is much more sensitive now and may be detected, so some of those that may not have had persistent viremia may now have persistent viremia. Is that a correct statement?

DR. ALTER: The sensitivity of the test hasn't

changed much. What has been termed the robustness of the test has changed, in that the answer is more believable. I will say that on these you can--because I think what is happening here is, you usually, at a very low level of virus, under any given assay, you may or may not pick it up. So all of these data, particularly on the cases, have been repeated over and over again, and they are not just based on PCR, they are based on cloning and sequencing. So each of those 11 cases are proven to have the virus. That there is no doubt about. We didn't clone and sequence all the controls.

DR. HOLLINGER: Yes, Dr. Stroncek?

DR. STRONCEK: Harvey, what about the viremic patients, the 34 percent that didn't get elevated ALTs? Did their viremic levels just spike, too, or did they go up and stay up, or did they--

DR. ALTER: Yes, that is a very good question.

DR. STRONCEK: --or was it an error? You know, so did they have one up? And did you follow ALTs for some time periods to make sure you didn't miss it?

DR. ALTER: Well, we only have the ALTs where we have the ALTs, so the way this study was designed, we had them at least every 2 weeks for the first 12 months and then once a month after. But that is a very good question, and we haven't done that yet. And one thing, I would wait now

until we have the quantitation better, but that is definitely worth doing, Dave. It is a good question.

DR. STRONCEK: And did you say--what did you say about the donors?

DR. ALTER: The donors, we haven't done the donors. We are having trouble finding the donor samples.

Jay?

DR. EPSTEIN: Harvey, I just want to make clear that I stand by my previous statement.

DR. ALTER: I know, that's right. I was using it as a joke, but you are absolutely right

DR. EPSTEIN: I do have a question for you, though. Ian Williams' data suggested that for community acquired acute non-A through E hepatitis there was not a strong association with a history of IV drug use, which would indirectly suggest that you are not dealing with a transfusion transmissible agent. And I just wondered if, in putting together your data which clearly show a blood transmissible agent, that there is then the implication that there is yet some other cause of non-A through E hepatitis.

DR. ALTER: Exactly. There is no way to say that SEN, if it is an agent, is the only agent of non-A through E. And in fact, if we look at these failures, it doesn't seem to be there, and these other chronic cases, they are clearly not positive. So this could--at best, I think, is

only a piece of the non-A to E, but sort of a striking piece within our small population. And I think a very important thing is, what about the CDC cases?

DR. WILLIAMS: We are actually in the process of testing that non-A through E group to see how many actually have SEN-V, and we are sort of in the midst of testing so I can't tell you what the answer is. But in about a month there is going to be, at the International Viral Hepatitis Meetings, there is supposed to be a whole session where this whole topic is going to be addressed for, I guess, a whole afternoon, and hopefully our data will be ready for release then.

But we should be able to answer that question. But I think the early look is that they are not--that not all of these are SEN-V positive, that there probably is another route, would be my guess, at least preliminarily.

DR. HOLLINGER: Yes, Dr. Koerper, and then--

DR. KOERPER: Maybe I missed something here. Do you have both an antibody test and a viral test?

DR. ALTER: No, just a viral test right now.

DR. KOERPER: So when you said that 92 percent of those who showed TAH, they were viremic--

DR. ALTER: Viremic.

DR. KOERPER: --and 34 percent of the transfused who did not have chemical hepatitis were also viremic--

DR. ALTER: Right.

DR. KOERPER: --then are you saying that all of those individuals except for two cleared the virus?

DR. ALTER: The problem--let's see--we haven't looked at that group, the group who didn't get hepatitis, for long term. See, because we picked out the group--we were just trying to address the issue of persistence of the agent, and we took the people who had the transfusion-associated non-A, B, C and the people who had transfusion-associated SEN plus HCV, and it is in the HCV group that we had the long-term samples because we were following HCV, so we could go out 10, 12 years.

We didn't look at the other group. We could do that. I don't think--I think that answer is probably pretty--we have answered the two parts of it, that a lot of people clear it and some people have persistence. If you want to look at what is the relative portion of those who persist, more numbers would help. But I think we really answered the question. This is, can be a persistent virus, and it generally clears within six months to a year, two years, three years.

DR. KOERPER: So that is based on those that had the combined HCV--

DR. ALTER: That is based on both those who had--had SEN alone and had hepatitis, or those who had SEN plus

HCV and had hepatitis, so it was based on only hepatitis cases.

DR. KOERPER: So that was 31 cases?

DR. ALTER: Yes, something like that.

DR. HOLLINGER: And, Harvey how did you decide, on the ones who did not have any ALT elevations, what samples to test?

DR. ALTER: We took the six-week sample from everybody. Now--

DR. HOLLINGER: Isn't it a little unusual that every six week sample is positive? I mean, I agree it is positive, but isn't that funny?

DR. ALTER: Well, I am assuming that it is probably positive earlier. Well, I was basing it on hepatitis C, that most people would already be viremic by six weeks and they would hang onto it for a while. We really should do another point, yes.

DR. HOLLINGER: Any other questions before we move on. Oh, yes, Dr. Katz.

DR. KATZ: The question we are focusing on here is the predictive value of a history of hepatitis, and I just wanted to be sure that I am hearing correctly that you screen your donors for a history of hepatitis and none of them gave that history.

DR. ALTER: We do whatever the FDA tells us to do.

DR. HOLLINGER: Let's--oh, yes, Dr. Stroncek?

DR. STRONCEK: I am not sure how relevant, but Jay mentioned that the CDC might have data to suggest there is some non-blood transmitted, non-A, non-B hepatitis other than SEN-V. Well, we don't really care, if it is not transmitted via blood, because the blood transfusions won't obviously transmit it, so we don't have to ask about it.

DR. ALTER: If I may say, I think SEN is sort of a red herring in the issue we are discussing here, because if it turns out to be a real virus, we will have a test, but this still will be something else that you would have to worry about. So the issue is really, how worried are you about non-A, B, C? What is the likelihood that these people who transmit non-A, B, C will give a history of hepatitis? And that is where I think it is probably close to none, and I think you have to base your decision on that and not whether SEN is relevant or not.

DR. HOLLINGER: Dr. Nishioka?

DR. NISHIOKA: However, you showed that it is (inaudible) a genotype of the SEN virus, and any particular type associated with that (inaudible)--

DR. ALTER: Any what?

DR. NISHIOKA: Any particular type among (inaudible).

DR. ALTER: Yes. These are actually beyond

subtypes. These are really so divergent that they are different members of perhaps the same family. And the one--we just focused on two of them because those were the two that appeared to correlate with hepatitis in our patients, so we have arbitrarily picked those two. Some of the other ones are much more prevalent and therefore wouldn't show the distinctions.

DR. HOLLINGER: It is like the difference between HGBC and a FLA-B variety group and hepatitis C. It is about that big, 45 percent, 35 percent, up to others, so it is very wide, very major.

Oh, yes, John?

DR. BOYLE: Just one question, and that is, the discussion has been really in terms of whole blood transfusion. SEN-V is described as a non-envelope virus. Does it represent, or can you say anything about what it represents for plasma products?

DR. ALTER: Well, I can't specifically. I would--I mean, there would be no reason not to think that it is in plasma as well as whole blood, and theoretically it would not be inactivated by a solvent detergent but would be inactivated by a nucleic acid inactivating agent.

DR. HOLLINGER: Part of that awaits, I think, finding a good antibody test, I think, John, to be able to pull these other things in, because you are sort of limited,

even like looking at the hemophilia population from say 1988 to the present time, versus before, to see if there is a difference; unless you assume that there is a viremia that persists in many of these individuals, which, as Harvey had pointed out, may exist.

DR. ALTER: The company is working hard to develop an antibody assay, which would give you then more complete epidemiology.

DR. HOLLINGER: Okay. Thank you.

DR. BOYLE: Thank you.

DR. HOLLINGER: All right. Now we are going to the open public hearing, and there is only one who has asked to respond at this time, I think Dr. Katz for the AABB. Louis, are you? Yes.

DR. KATZ: We have the distinct advantage of writing these statements without hearing the new data, but everything I have heard today I think supports the statement that AABB is endorsing.

We support eliminating the requirement to permanently defer potential volunteer donors with a history of viral hepatitis after the age of 11 years. Our rationale is based on accumulated lines of evidence suggesting that this action will not decrease recipient safety. Further, it will reduce the unneeded loss of over 10,000 donors yearly at a time when the demand for blood components is poised to

outstrip the supply.

We have reached a point where we believe donor historical screening should focus on current rather than historically remote risks, and when simplification of donor historical screening can allow us to focus on material threats to the blood supply and donor safety in a more straightforward fashion.

In the 1960s, with paid donors of unscreened blood, hepatitis was a common outcome of transfusion. Since 1990, using sensitive assays for HBV, with the identification of HCV and implementation of successively more sensitive and specific HCV screening tests, post-transfusion hepatitis has become so rare that prospective studies have had to be replaced with mathematical modeling to estimate its frequency.

After the implementation of HCV RNA screening and minipools under IND, credible estimates suggest a risk for this virus as in the range of 1 in 1 million. The use of current hepatitis B surface antigen screening for HBV infections is far more sensitive than a history of hepatitis for this virus, given the high rate of unsymptomatic and unrecognized infection in that subset destined to become chronic carriers.

Current rates of post-transfusion hepatitis are exceedingly low. Ongoing prospective surveillance for

clinically significant post-transfusion hepatitis at the NIH Clinical Center, Harvey's cohorts, in the interval after effective anti-HCV screening was implemented, is unable to demonstrate a persistent problem.

In the U.K., donors with a history of jaundice are permitted to donate, provided they are hepatitis B surface antigen negative and more than one year has elapsed since acute hepatitis B. In addition, since 1997, donors who provide a history of hepatitis B in the U.K. are tested for anti-core. If the anti-core is negative, they are qualified donors. If the anti-core is positive, an anti-HBs is done, and if protective levels are present, they are qualified to donate blood.

Recently published data from the U.K.--reference has been provided to the committee, I believe--reported the prospective evaluation of 5,579 recipients of almost 22,000 units of blood for post-transfusion viral infection. No infection attributable to transfusion was found in this ongoing prospective cohort.

With regard to the putative non-A through E agents of viral hepatitis, the evidence that clinically recognized hepatitis would allow deferral of these donors is lacking. That is, the history of hepatitis is an insensitive test that will miss the majority of these individuals who had no clinically consistent illness, and are characterized only by

abnormal transaminase levels, if that.

It is estimated that the proportion of clinically apparent non-A through E cases is very low, based on studies both at NIH and at CDC. Also, there is as yet no convincing evidence of clinically significant chronic sequelae. Data from a number of sources have documented the non-specificity of the history of hepatitis which defers donors with prior HAV, or donors who have been told by their physicians that they had hepatitis associated with CMV or Epstein-Barr Virus infections. These donors represent no additional threat to blood recipients.

In summary, the AABB recommends elimination of the requirement to exclude donors with a history of hepatitis as an insensitive and non-specific donor screening tool. Failing this, adoption of a system modeled after that in the U.K. might allow blood collection facilities the option to salvage many thousands of safe donors yearly. Thank you.

DR. HOLLINGER: Anybody? Yes, Dr. Bianco?

DR. BIANCO: I would like quickly, representing America's Blood Centers, to support both the proposal from AABB and the proposal that Dr. Harvey Alter made, and I would request that the committee think that we should not be acting out of fear and keeping things as they are when we have the best opportunity for change we ever had.

The entire Workshop on History of Hepatitis was

almost unanimous recommending that we drop the question because there was no value that could be recognized to the question. And again today we heard a lot of information saying that this question does not contribute to blood safety. Let's let our donors focus on important questions--they are the questions about risk behavior and the questions about drug use--and think about what they did last week, not what they did many years ago or what happened when they were 11 and a half. Thank you.

DR. HOLLINGER: Thank you. Is there anyone else from the public sector that wishes to make a statement or comment? Yes, please, and state your name, affiliation.

MR. HEALEY: Hi. My name is Chris Healey, and I am the Director of Government Affairs for ABRA, the source plasma collection trade association. I just want to say that we support elimination of the question, as well. There has been a lot of science discussed today that makes it pretty clear, at least to us, that the question doesn't add anything in terms of public health.

What hasn't been touched on is the donor screening issue and the donor history questionnaire. I think Celso started to go down that road. But we think that is an important issue that needs to be taken into account as you deliberate on this.

Our donors are overburdened with an overly lengthy

and confusing and complex questionnaire that is often a turn-off to them, and we see fewer and fewer donors coming in the door. We can attribute some of that to the questionnaire. We think the history of hepatitis is sort of a win-win in this case, because not only will it give us an opportunity to streamline the questionnaire a little bit, it won't do anything in terms of negatively impacting the public health. Thanks.

DR. HOLLINGER: Thank you. Anyone else?

[No response.]

DR. HOLLINGER: If not, I am going to close this portion of the session to the public for comments, and we are going to open this up for committee discussion.

I will tell you a couple of things I might take the liberty to do, if I may. Robin has had, on the back page of the information you have, has four different options. I would prefer, I would like to go down through these options in terms of taking a vote on 1 through 4, because I think it follows a little bit longer.

And here is how I would like to do it. I would like to get a vote on whether we should keep the exclusion the way it is. And then, depending on what that vote comes out, then I would like to vote on entirely eliminating the exclusion. And then I would like to come and talk about modifying the exclusion by excluding donors with a history

of hepatitis that occurred during a limited period of time, that is, during the past year. And then finally the question which is before us, which is modifying the exclusion by accepting donors whose previous viral hepatitis could be documented.

Is there any objection on the committee to going through these four, voting on each one of them specifically, and seeing where we are? Anybody have any objections to that?

[No response.]

DR. HOLLINGER: Okay, so I would be glad to open this up for discussion right now, or we can just sort of start to vote and go with that one.

Yes, John? Please.

DR. BOYLE: Just one thing, and it is the year exclusion. If it really is a year that you are concerned about, if you do surveys and you ask questions about recency, people telescope. They telescope bad things further away and they telescope good things closer to. So if you really are aiming to make it a year, then you better ask about two years, because otherwise you are going to get people within a year. That is the way people answer questions.

DR. HOLLINGER: Yes. I think their feeling is that six months is probably what they want, and that is why

they ask a year. But your point about telescoping is exactly right, and I think that was the reason for it.

Yes? Toby.

DR. SIMON: I would just like to make a couple of comments. I did also participate in the workshop, and I wanted to reiterate what other people have said, that from the expertise in the field there was, if not unanimity, there was certainly a strong sense that this question does not add to safety, and it is a step that we could take to add more donors and to simplify the screening procedure without impacting the safety of the product.

But I think another point that I don't think has come up, and at the workshop Dr. Bianco had presented his data from the survey and there was this estimate that we are losing 13,000 donors a year, I believe, and that number has kind of now managed to make its way through a number of presentations, I would just like to make the point that that is then every year.

So presumably the people we are excluding from hepatitis this year are not the same ones we excluded last year and the year before, so just from people who have excluded themselves or who have been excluded in the interview from asking that question, we are probably well over 100,000 blood donors in the last 10 or so years, and maybe an almost similar number of plasma donors. So the

gain could be quite considerable.

And I would also like to point out that both blood and plasma centers for many years in their recruitment materials have always had that little item, that if you have had hepatitis, or more recently hepatitis since age 11, you cannot donate. So this information has been given out to people before they come in, so it is likely that the number of people excluded by the question underestimates what the gain would be in donors if we put out the information that you could donate with a history of hepatitis, depending on how you wanted to handle that. So I think the gain in donors here could be very, very significant.

DR. HOLLINGER: Thank you, Dr. Simon.

Yes, Dr. Macik?

DR. MACIK: Yes. I am not a blood banker, and I am always amused by what I find out, because I until today didn't know about this 11-year-old, and so I have been donating blood for years, having had hepatitis when I was 15. I don't know why, I just turned yellow. So there is, I think--and I am a doctor. So there must be a lot of people we don't catch that have been donating for a long time, so you know, these questionnaires become so long that you don't really think about what goes on. Maybe I haven't donated since they have added age 11 in, to think back that far.

DR. HOLLINGER: You are still yellow.

[Laughter.]

DR. HOLLINGER: Go ahead, Dr. Chamberland.

DR. CHAMBERLAND: I just had a question that pertains to option four on the list, which I guess is the option that FDA actually presented preferentially before us, in the--when Robin went through the pros and cons, acknowledged that it would be perhaps difficult for people to produce documentation as to the type of hepatitis they had. And I was just curious if anybody has ever attempted to do that, if any of the blood banks have attempted to probe further and find documentation, and what can you tell us about how successful people are in producing the documentation, and what is it that you find with it?

DR. KATZ: At the risk of speaking for my colleagues, this comes up particularly in reference to the donor that comes in, "Well, I think I was told it was when I had mono," which are by the way unacceptable donors without an exemption from the FDA, anyway. And less than half of them in fact can, if the doc is still alive, dredge up the records and bring us the documentation of what laboratory studies, looking strictly at laboratory documentation. It is very unusual. Not zero, but--

DR. CHAMBERLAND: So what we would be asking for, for example, in that history of the infectious mono, you would be--the documentation that you would be seeking is not

only serology for EBV but also elevated ALTs?

DR. KATZ: Well, I won't speak for the agency, but I would suspect that they would be interested in seroconversion or IgM--

DR. CHAMBERLAND: Right.

DR. KATZ: --or something of that nature that would have identified the acute episode as in fact related to that agent.

DR. CHAMBERLAND: Okay.

DR. HOLLINGER: Yes, Dr. Schmidt?

DR. SCHMIDT: I think you might have to wait with that last one until somebody buries that little chip in your head with your medical history, but then be sure nobody sells counterfeit chips, so it is difficult.

DR. SIMON: Just as another example, with the dura mater question for CJD risk, we get a lot of people, "Well, I had neurosurgery, but I don't know whether I had it." Often it is with childhood, and it has been very frustrating to try to get the information from some source, whether they did or did not have a dura mater graft. So I think it would be extremely difficult to get documentation.

DR. HOLLINGER: Okay. What I would like to do is --yes, Marion?

DR. KOERPER: It seems to me that this kind of question was appropriate back in the days when we didn't

have good serologies, but now the serologies for hepatitis itself are so good. Probably a lot of people like Dr. Macik had mono, but probably nobody even did serologies back then to see if she had mono. And so I think we have really good serologies for hepatitis, for the kind of hepatitis that we are worried about, and I think that is much more important, that the testing is being done, rather than making people dredge up old medical records from 20 years ago.

DR. HOLLINGER: Thank you. Yes, Robin? Please.

DR. BISWAS: I just wanted to say that in regard to the fourth option, where we would be sort of looking for documentation, I mean you remember that when people go say to a restaurant or they go to a picnic, or they eat a lot of strawberries or something and then turn yellow, many of those people are going to go to their physicians, and if they are good, sharp physicians, you know, they would be doing an HAV IgM. So what we are saying is that, yes, getting the documentation might be difficult, but in some cases, in a few cases one might be able to eventually reenter these people.

The other thing I would just like, you know, to say before you, you know, before you vote on it, is that Harvey has shown us a lot of very, very interesting data, but remember that the data gathering is still carrying on, and in fact Ian Williams has not yet tested all his--is that

what I understood?--has not tested all his non-A through E's for SEN-V, and that data will be presented, hopefully, at the Atlanta meeting next month. So I just wanted to remind you that there is still data gathering going on, and that is what I wanted to say.

DR. BIANCO: If the chairman allows me, I would like to ask Dr. Biswas, how do you think this data will contribute to the value of medical history?

DR. BISWAS: Could you--Celso, what exactly do you mean?

DR. BIANCO: I mean this subject that we are trying to discuss. I think that all the evidence that I heard from Harvey and the other presenters is that these people, there is no medical history, and the people that are being studied by the CDC, they all have a medical history. And if they have SEN-V or not, 2 percent of them have, but what is the relationship with the question about hepatitis and medical history? The only way we are going to eliminate these people is if we find that this is important and we have a test and we do that.

DR. BIANCO: Well, I think that maybe, you know, Ian can answer that. I mean, one thing that I heard him say is that 22 percent of these, of the acute non-A through E cases become chronic.

DR. WILLIAMS: I think the point is, is that we do

see people who really are acute, acute non-A through E hepatitis, and we know a small portion of them, around 4 to 5 percent of these non-A through E's, are transfusion-related, so there is probably an agent out there that may be a blood-borne pathogen. There may be multiple agents left in that small part of the 3 percent. So the question is, are there other agents out there? And we are looking through our group of non-A through E's to see, maybe some of these are SEN-V. I don't know. That is all I am saying.

DR. BIANCO: Well, we defer individuals that received a transfusion for a year from donating blood.

DR. WILLIAMS: The people in our study are people who got acute hepatitis and they had a blood donation within the six weeks to six months prior to their onset of illness.

DR. HOLLINGER: What percentage also were chronic? I want to be sure--

DR. WILLIAMS: Of the whole non-A through E group, about 22 percent. About 22 percent went on to develop biochemical evidence of chronic hepatitis.

DR. HOLLINGER: That persisted for at least--

DR. WILLIAMS: At least two follow-up visits, which--because not everybody comes back--so it would be at least a year, but some people, it is longer than that.

DR. HOLLINGER: And they have been biopsied?

DR. WILLIAMS: No.

DR. HOLLINGER: So why hasn't somebody biopsied them at this stage?

DR. WILLIAMS: We would have to go back and find them and biopsy them. It is not part of our protocol to biopsy people.

DR. HOLLINGER: Mary?

DR. CHAMBERLAND: Ian, I think that is like one of the sticking points in terms of the universe of data that is being examined to address this question, is that 3 percent in the Sentinel Counties, and I think maybe with this nagging question, what is it that these 3 percent have? And I guess I just wanted to ask you to clarify one more time, besides evaluating these individuals for SEN-V, is there anything else that either we at CDC do systematically to further evaluate them, or through a more passive approach of trying to pursue medical records, at least to review the medical records, either prospectively or retrospectively, to just try and get a better answer on what is it?

DR. WILLIAMS: Not beyond their acute phase. I mean, these people are acute symptomatic cases. I mean, really epidemiologically they are different. They tend to be more white; they are not drug, they tend to be less drug users; they are not transfusion related. I mean, they look like a different group when compared with hepatitis C. So epidemiologically they are different than people who have

hepatitis C. That is about what I can tell you.

But we don't do systematic follow-up on these people. That is not the point of the study. So I can't tell you a whole lot about the natural history of non-A through E. We do have a small group of people, about 20 people, that is part of this 1985 or '86 cohort we are following, and those people are as well being tested for SEN-V, but I mean we are only talking about a handful of people, so it is hard to make conclusions based on a couple of people.

DR. HOLLINGER: And I presume all of the other things have been ruled out, Wilson's disease and autoimmune hepatitis, and how about obesity?

DR. WILLIAMS: Yes, we try to. We consult with the physician. We do extensive medical chart reviews. We do everything we can to try to rule out other causes.

DR. HOLLINGER: Marion?

DR. KOERPER: Has anyone looked at a group of patients who have either chronic active hepatitis or cirrhosis, to see if they are SEN positive?

DR. ALTER: We are looking at that now. We have--

DR. HOLLINGER: Harvey, could you use a microphone, please?

DR. ALTER: We are looking at a lot of groups of patients with chronic cryptogenic hepatitis, cirrhosis,

liver cancer, etcetera, and we have already done some. I don't have the data in a clean form to present. There is no dramatic picture that this is the agent of typical cryptogenic hepatitis or cirrhosis. The best data are really in the transfusion study, and the rest are much more equivocal.

DR. WILLIAMS: We actually are looking at a similar group from Harlem, from the previous data that was published in Hepatology last year, and hopefully will have results from that in a month, as well.

DR. HOLLINGER: We have looked at 360 patients with hepatocellular carcinoma in this group, and the odds ratio is about 2.8 so far in early studies, in patients who do not have B or C, in which the odds ratio is running between 19 and 30 that--and the confidence interval is above 1 in this group. So there does appear to be, at least in the early studies, perhaps some relationship with hepatocellular carcinoma.

Anybody else? Any other comments?

[No response.]

DR. HOLLINGER: Okay. If not, let me just start by, and you can ask questions any time you want to, but let me start with at least the first one. First I would like to have the vote on how many of the committee members would vote on keeping the exclusion the way it is. So the

question is, how many would vote for keeping the exclusion. All those in favor of keeping the exclusion, raise your hand.

[No response.]

DR. HOLLINGER: All those opposed?

[A show of hands.]

DR. HOLLINGER: All right. Abstaining?

[No response.]

DR. HOLLINGER: And Dr. Simon and Ms. Knowles?

MS. KNOWLES: I would vote to--with the rest.

DR. SIMON: Also, same.

DR. HOLLINGER: Okay. Yes, please.

DR. SMALLWOOD: The results of voting on keeping the exclusion as it is: There were no "yes" votes and there were 13 "no" votes. The consumer and industry rep both agreed with the "no" votes.

DR. HOLLINGER: The second question I would like to bring up is, how many here would be in favor of entirely eliminating the exclusion for a history of hepatitis? This would be for entirely eliminating the exclusion for a history of hepatitis. All those in favor of entirely eliminating the exclusion for a history of hepatitis, raise your hand.

[No response.]

DR. HOLLINGER: All those opposed?

those in favor of that, raise your hand.

[A show of hands.]

DR. HOLLINGER: All those opposed?

[No response.]

DR. HOLLINGER: Abstaining?

[No response.]

DR. HOLLINGER: Dr. Simon?

DR. SIMON: I vote for it, yes.

DR. HOLLINGER: Ms. Knowles?

MS. KNOWLES: Yes.

DR. SMALLWOOD: Results of voting for modifying the exclusion by excluding donors with a history of clinical hepatitis for a limited time period, e.g., for one year after disappearance of symptoms: There were 13 votes in favor. There were zero "no" votes, no abstentions, and the consumer and industry rep agreed with the "yes" votes, those in favor.

DR. HOLLINGER: Thank you, Linda.

With that in mind, I don't see any reason to vote on the last question, then, at this point. So I think that probably concludes this issue here. We are going to take, let's see--yes, we had better. Can we take about a 15-minute break, and then we are going to come back and start dealing with the nucleic acid, HBV DNA and nucleic acid issue. Thank you.

[Recess.]

DR. SMALLWOOD: May I ask the committee members to please return to your seats? May I have the cooperation of the audience? May I ask that everyone please be seated so that we may continue? Since I now know you like to stay late, we can start the meetings later in the morning.

DR. HOLLINGER: Thank you, Dr. Smallwood. You see Dr. Chambers actually eating her supper here, so she expects to be here until about 9:00 tonight. I hope the rest of you got supper.

Okay. This session, we are going to discuss HBV Nucleic Acid Testing, and Ed Tabor is going to give us the introduction, the background to the issues here, and then we will have several presentations following this. Ed?

DR. TABOR: Throughout the blood and plasma industries in the United States, investigational testing systems under INDs have been put in place during the past two years to test minipools for HCV RNA and HIV RNA by NAT. By the end of 1999, approximately 95 percent or more of blood and plasma collected in the United States was being tested by NAT on minipools for both HCV and HIV.

Although some plasma donations are being tested by HBV NAT in minipools at present, screening by HBV NAT was not implemented at the same time as for HCV and HIV because the benefits of HBV NAT were initially thought to be much

less than those that would result from HCV and HIV NAT screening.

For instance, although the prevalence of window period donations was known to be higher for HBV than for HCV or HIV, 1 in 63,000 donations for HBV compared to 1 in 103,000 for HCV and one in 493,000 for HIV in the classic paper by Schreiber et al., NAT for HBV was expected to detect fewer positive donations. HBV titers are lower during the window period than during subsequent months of infection, whereas HCV and HIV titers in blood are highest in the window period. The lesser sensitivity in general of HBV NAT when compared to HCV NAT and HIV NAT would also contribute to its lesser utility.

Concern had been felt that removal of HBV NAT positive donations might inadvertently reduce the anti-HBs concentration for plasma pools, if it transpired that some resolving infections had HBV DNA in serum as well as anti-HBs, as suggested by some recent publications.

Another point of view, proposed by a panelist at the CBER workshop on NAT implementation held in December 1999, was that setting a more sensitive detection level as a requirement for release of HBsAg test kits might achieve a similar reduction in the number of window period cases as would NAT screening on minipools for HBV DNA. The relative benefits of more sensitive HBsAg immunoassays and minipool

NAT testing cannot be stated precisely without additional studies.

Some currently licensed HBsAg screening tests are already so sensitive that they can detect samples in which the viral load is 1,000 copies per mL. Thus, the only undetected window period cases for units screened with those tests would contain fewer than 1,000 copies per mL. For this reason, NAT minipool testing for HBV would have to be very sensitive to be useful as an adjunct to better HBsAg assays, and even testing a 20-sample minipool would so dilute the positive sample that the testing would be inadequate in many cases.

However, just as the implementation of HCV NAT and HIV NAT, particularly HCV NAT under IND, occurred sooner in the United States than would have occurred otherwise due to the requirements of the European regulatory authorities, there was concern that pressure for HBV NAT screening might occur sooner than would be practical if the Japanese regulatory authorities required it for plasma or for plasma derivatives imported to Japan.

However, during the December 1999 workshop, an official of the Japanese regulatory agency stated that Japan would not require HBV NAT for plasma until U.S. manufacturers were able to do such testing. In contrast, Japan has been requiring HBV NAT testing on minipools for

whole blood since October 1999, and we will be hearing more about that in some of the coming presentations.

Preliminary data from a number of studies suggest that the rate of detection of HBV by NAT screening has been higher than was expected, and these data have caused a reexamination of the possible benefits of HBV NAT screening. This is, in part, the reason for placing this topic on the agenda today. The present session of the March meeting of the Blood Products Advisory Committee was designed to address this issue, and we are grateful to the speakers who will be presenting recent data from the use of HBV NAT screening of minipools. Thank you.

DR. HOLLINGER: Thank you, Ed.

The first presentation, then, is going to be by Dr. Susan Stramer.

Yes, Dr. Simon?

DR. SIMON: One quick question. I gather this is just to inform us and keep us--there is no specific--

DR. HOLLINGER: Yes. There are no questions, to my knowledge, associated with this session. To inform us, but also to provide some questions for them, too, and answers, hopefully.

DR. STRAMER: Thank you, and thank you for the tolerance of the committee, for allowing me to rearrange the schedule a little bit so I can catch a flight.

Thank you. In keeping with Dr. Tabor's introduction, what I am actually going to show, even before you see the results of pooled mini testing for hepatitis B DNA, I am going to show you alternatives to that, and Dr. Tabor alluded to those in some studies with improved sensitivity HBsAg tests. This really will involve three studies, but just to allow you to keep in mind that there are alternatives to HBV DNA pool testing that will achieve equal or better sensitivity.

So my outline includes three presentations, as I said. One will be an evaluation that we did with pooled PCR with National Genetics Institutes tests versus HBsAg using the Abbott Auszyme test. Then the second two studies were actually two different protocols with HBsAg tests. One was the Ortho current test, versus the Genetic Systems newly licensed test that uses a Shaker protocol, and in that way allow much enhanced HBsAg sensitivity. And then the last study I am going to show is from the U.S. clinical studies of the current Abbott test versus PRISM.

Now all of these studies used different panels and each of these tests has different sensitivities, so I hope this isn't too confusing as I go through the 2 by 2 studies.

Firstly, I have shown this slide probably at Blood Product Advisory Committee meetings before, but it shows the results of seroconversion samples, 13, I should say

seroconversion panels or 13 seroconverting donors with 181 samples, and it shows the different stages during HBV seroconversion and HBV marker development in seroconversion.

The first stage here is the stage that we are primarily interested in. This is the stage where DNA is positive for hepatitis B but all other markers, that is, HBsAg, are negative. This next stage, now, represents HBsAg positivity, and you can see the median of these first positive panels is at about 100,000 copies per mL, so quite a high viral load. But in contrast I am going to focus first on these samples, because these are the samples that we are talking about in minipool testing.

Firstly, when we did the study with NGI, we looked at a pool size of 500. At a pool size of 500, this would be a 6,000 copy per mil sensitivity. So if you look at the population, these represent the outlyers or those samples outside 95 percent confidence. We would not detect 95 percent of the population.

If we used a more conservative cutoff of 1,600, which is comparable to what the Red Cross screening program is using, in that if we used a test that had 100 copies per mL sensitivity and a 16-member pool size, we would have 1,600 copy sensitivity cutoff, and then you can see we would detect more samples.

So to go through that in a little bit more detail,

of the 13 panels I showed you, the median viral load in the first samples DNA positive was 600 copies per mL. If you look at all of the samples from these 13 panels in that first category, that is, DNA positive, HBsAg negative, they had a median copy level of 750 copies per mL.

So if you look at what the cutoffs would be, at a 6,000 per mL cutoff for a pool size of 500, only 3 out of 13 individuals would have been detected. The three that would be detected, that would have concentrations above the cutoff of 6,000, have copy numbers of 6,500, 8,000, and 10,000. But again, the other samples of these 32 would not be detected.

If you drop the cutoff now by using a smaller pool size and going to 1,600 copies per mL, you would detect 5 of 13 of these seroconverting individuals. Now eight samples would be positive, so in addition to these three concentrations, we also would pick up samples that had this viral load, 4,400 to 6,000. Of these eight samples, there would be a four-day median window period reduction of a nine-day total.

However, the panels not detected at a 1,600 copy per mil cutoff still included the majority or eight, and involved 24 samples that had a concentration of 100 to 1,500 copies per mL, or a 550 median copy level.

DR. HOLLINGER: Sue, before you go on to that,

just one minute. That should be five, should it not, under the number apparently detected at 1,600, five samples, not eight?

DR. STRAMER: Not detected at 1,600.

DR. HOLLINGER: No, the one above that.

DR. STRAMER: No, these are five donors with eight samples.

DR. HOLLINGER: Okay. Thank you.

DR. STRAMER: Right. I don't mean this to be confusing, but we are still talking about detection of individuals and then how many samples that these individuals contribute to the study. But the main points here are just to look at the viral load that we are dealing with pre-HBsAg, in this particular study with these particular samples.

Now to look at the profiles of some of these representative seroconverters, looking at the two different cutoffs so you can see what would be detected. The pink line here represents HBV DNA. The orange line represents HBsAg. Looking at a period of time this long, these two almost exactly virtually coincide, and actually in this case the first day detected by a pooled test at a cutoff of 1,600 would equal the first HBsAg sample positive, even with current testing.

Looking at another sample, another donor, you see

the same thing. This again is a very long period of time. Here you see a little shoulder of HBV DNA, but as Dr. Tabor mentioned, this is a very low DNA copy level. First samples detected by pooled NAT or HBsAg would in fact be on the same day.

This is a profile in a chronic carrier. Here you actually do see a four-day difference, although you can see it is very short, four days, where DNA is detected before HBsAg.

Moving on to the next study, we looked at increased sensitivity HBsAg tests that have recently been licensed. We use the Ortho test currently, and for this evaluation we looked at Ortho versus the Genetic Systems assay which uses a Shaker mode. We looked at a total of 21 seroconverting individuals. They weren't necessarily the same panels I just showed you, but they were 21 that were commercially available for the study.

Interestingly enough, of these 21 panels, we looked at 184 total samples. There were 57 discordant samples, meaning that they were positive by Genetic Systems but negative by the current Ortho test. Interestingly enough, even using Neat PCR, which in this case had a 400 copy per mL cutoff--we used the Roche test--56 of 57 were PCR positive, so one wasn't even PCR positive that was detected by the Genetic Systems test.

Calculating window period reduction and based on incidence of HBV, what we think we would detect looking at the Ortho test, Genetic Systems, and PCR in a qualitative mode, we would see a 9-day with a range of 2 to 18 day window period reduction just by going to a different test for HBsAg, leaving only 7 days or less than half of the 16-day window period available for single donation testing, even at a 400 per mL copy. And we expect that when single donation testing is available, it will have better sensitivity than 400 copies, but from the results of this study, this is the break-out.

If you look at incidence based on--if you look at detection based on 9.5 per 100,000 incidence, we predict per million donations screened, this would be an additional pick-up of 2.3 per million and then leaving an additional 1.8 for single donation PCR, if it has this kind of cutoff.

Now to show you some of these representative panels, that is what I have in the next couple of slides. The blue bars here represent HBsAg concentration. The orange line represents the Ortho test. Green, Genetic Systems; and pink, DNA quantitative copy level. We only had quantitation for 8 of these 21 panels, and this is a sampling of those 8.

So here you can see that using an EIA cutoff, this is the first positive sample. Using a pooled NAT cutoff of

1,600, which would be a 100 copies per mL sensitive test in a pool size of 16, we would detect DNA at the same time that we detect HBsAg, so no improvement. In this panel we would actually see DNA come up after HBsAg, since this sample is above the cutoff and this sample is above the pooled cutoff by PCR. And then this is the first sample positive by the current test. Lastly, here is another panel that shows equivalence, DNA, Genetic Systems, and the Ortho test.

Of the eight that we could do quantitative analysis on, Genetic Systems picked up HBsAg using quantitation of nanograms per mL, which is the convention used for purified HBsAg, but in these panels detected at .14 to .34 nanograms per mL, and in the Ortho test at greater than 8 nanograms per mL.

Looking at the DNA testing that was done in quantitation with a 400 copy per mL cutoff, the first sample per panel that was detected by Genetic Systems, that was missed by Ortho, had a median copy concentration of 6,500 with a range of 800 to 20,000. Now, 800 is below the cutoff of the 1,600 pool size, so that equates to missing or not detecting two out of the eight panels by pooled PCR that first would be detected by the improved EIA.

Similarly, if you look at another study looking at another group of seroconverters, in this case 25 seroconversion panels analyzed by a five-stage Markoff model

that was developed by Glen Satten, or applied by Glen Satten to these data, here we are looking at seroconverters over time. The yellow line here--excuse me--the orange line here shows you days theoretically of HBsAg EIA positivity by the current test.

If you look at PRISM, which is an improved HBsAg detection automated system, you see a 12.6 day extension into the period of time where anti-core becomes positive, but more importantly, you see an extension of 6.8 days forward into the pre-HBsAg positive window period, and this entire window period here is covered by DNA. So we see 6.8 days on the front end of seroconversion and 12.6 days on the back end.

The green bars show you the DNA concentration performed out of a subset of these 25 panels. The quantitation was only done in this case on seven panels.

Now to look at the copy concentrations of relevance, if we look at the DNA positive samples that were PRISM positive, current test negative, the mean detection in copies per mL from the seven panels was 3,340 copies with a range of 100 to 8,000, and I mentioned the 6.8 day earlier detection. But again you see this recurrent theme that not all would be detected by pooled NAT testing using a cutoff of 1,600 copies per mL.

Looking at the anti-core positive samples, that

is, the back end window, we would see a 12.6 day extension of HBsAg detection, and again very low copy numbers here. But again these are anti-core reactive samples, so these are not necessarily the samples of interest.

If we look at the DNA positive samples that were PRISM negative, the very early seroconversion samples or the very early positive samples, the mean copy level was 240 copies with a range of 120 to 500. Therefore, none of these would be predicted to be detected by NAT testing, that is, in pools.

To apply the last two studies kind of side-by-side to see how they rank against the two HBsAg subtypes, Ad and Ay in the U.S., we have the first study of Ortho and GS and then the U.S. clinical trials, Abbott current and PRISM, and this shows you a comparison of purified nanogram per mL detection, so clearly these two tests have greatly improved sensitivity over the two tests that most blood banks use.

So, in summary of the three studies, and I have one slide for each study, pooled NAT testing in our first study offered little improvement in sensitivity versus the current test that was the comparator. Now in the NGI study, even when we dropped the cutoff to 1,600, we only saw 5 of 13 individuals who were detected by pooled NAT, with a window period reduction of four over the nine-day total.

The second two studies, looking at improved tests

for HBsAg, actually showed a slightly better sensitivity than pooled NAT testing. In the Ortho versus Genetic Systems Shaker assay, we saw all 21 individuals studied had improved detection over the current test, with a 9-day window period reduction over the 16-day total, significant improvements in the concentration detected of HBsAg, and the first discordant samples or the first samples detected had a median of 6,500, with a range going down to 800 to 20,000. But again, two of eight of these would not have been detected by pooled NAT testing, whereas the EIA would have detected all.

And then lastly, in the last comparative study where 25 individuals were looked at, all 25 had improved detection over the current test with a 6.8 day mean window period reduction, but in this study the mean copy level was 3,440 and the range was 100 to 8,000. So, again, not all would have been detected by pooled NAT testing at a cutoff of 1,600.

And lastly, looking at the DNA-only positive samples, none of these would have been detected by pooled NAT testing, again at a 1,600 copy per mL cutoff, because of the very low viral copy number in these early positive samples. So, again, alternates to pooled NAT testing do agree if we move to more sensitive HBsAg tests.

Thank you.

DR. HOLLINGER: Thank you, Sue.

Anyone have questions of Sue? Because she will not be here, probably, later on. Yes, Dr. Simon?

DR. SIMON: I didn't quite understand that last comment. You mean we need a better test than the pooled NAT?

DR. STRAMER: I am saying more benefit could be gained from simply going to HBsAg tests of improved sensitivity versus doing pooled NAT, even in small pool sizes of 16.

DR. SIMON: So some sort of test that is not nucleic acid based, is that?

DR. STRAMER: Right. This is just substituting our current HBsAg test that the whole blood industry and the plasma centers use for another test that has better HBsAg sensitivity, so I am saying we would decrease the viral burden, in your case in plasma pools, greater by just doing HBsAg testing that had improved sensitivity over doing a pooled NAT test.

DR. HOLLINGER: Any other questions of Dr. Stramer? Yes, Dr. Conrad?

DR. CONRAD: I hate to contradict ourselves, but when we went and looked for--there maybe something wrong with those panels, because when we go look forward now, I think from Centeon and from us, you will see that we are

finding a ton of people that are persistently viremic but not coming up positive with the antigen tests. Now, we are using PRISM or Auszyme, and it is contradictory to that, and I don't know if it is because those panels are somehow modifying what we found, because de facto we knew they seroconverted.

And so there is something strange about it, because we are seeing prospectively many more than we ever thought we would, because looking at that, we thought, well, it is not going to yield very much, but somehow it is yielding because we are only doing PCR and then later going to do the other tests. So--

DR. STRAMER: I am not saying we wouldn't detect samples. I showed 5 of 13 that would be detected in a pool size of 16. I can't comment on what "tons" mean, and we have to put some number around "tons".

DR. CONRAD: Tons are 1 in 3,900 donors. I mean, we will show that.

DR. STRAMER: I understand that, and again, we can't compare incidence in perhaps plasma versus whole blood, and again, we have to look at the factor of anti-core testing, because I didn't mention but we also do anti-core. And I believe many of the plasma samples that are positive, we would have picked up with anti-core testing.

DR. CONRAD: We did anti-core testing, and about

half of them were.

DR. STRAMER: Okay. Well, that is half ton, then; half a ton.

DR. HOLLINGER: Any other questions?

[No response.]

DR. HOLLINGER: Thank you, Sue.

The next presenter, then, will be Dr. Nishioka from Japan, to talk about their experience.

DR. NISHIOKA: Thank you, Blaine. It is my great pleasure to be invited here as the only non-U.S. citizen in this important meeting. I would like to present our experience of the NAT screening of hepatitis B, hepatitis C, and HIV simultaneously, using a triple experience. May I have the first slide, please?

Yes. You know the serological marker is a rather indirect measurement of hepatitis B, and the seroconversions are rather late, but the NAT marker is a direct measurement of hepatitis B. We don't worry about a non-viral protein or something, and the NAT conversions are early. Next.

The growth curves of hepatitis B, that is based on a BBI panel. It is (inaudible) it against the date, starting from the extrapolated 10 to the zero copies per mL, it shows exponential growth in the early stage of infection, and if we could shorten that window period, that means the virus load escaped from screening is exponentially reduced.

The doubling time or log time to hepatitis B, based on this, calculated 6.5 days for hepatitis B, and hepatitis C is 1.0 days, and HIV is 1.9 days.

Well, we can change that, not 100 percent, but NAT narrows the window period as well as exponentially reduces the virus load escaped from the screening tests for blood transfusion and plasma sources. We start the NAT screening for the plasma derivatives in 1997, but we have (inaudible) to the window period blood transfusion of HIV last October, and since then we shift our system to the entire blood transfusion, very rapid screening, and to the entire blood transfusion, two days after the blood transfusion.

For that, the NAT screening for blood, the serological prescreening, it is faster, very fast, to avoid the carryover in the NAT test and automatic agglutination test using PK7200. That is very rapid screening out, although the sensitivity is not for--it is very (inaudible), rather lower than EIA, but for the rapid screening and to screen out the high titer of HBV it is very important.

And then shipped to the NAT center by air freight service, and we have an automated pooling system, excluding the seropositives with ALOKA. And the testing reagent is multiplex prepared by Roche Japan and Roche United States. And hepatitis B, hepatitis C, HIV, is at one time extracted in that test. The automatic extraction system using a GT-

12, now we are using GT-X by Roche and a PRISM 7700. Then the reporting system to blood centers through NEC network, and resolution by individual NAT and notify to the donors.

This is in Japanese, but I don't have time to. That day is the blood donation, and during the night time that transportation, and then the screening later went to the NAT center, and then it eliminates--eliminates screening positive sample, and then pooled the next morning, then the NAT is done. That next morning we can get the answer to each blood center, and they are ready for patients, and the total is two days.

This is our transportation system. Now all the donated blood is through air freight or surface and transported to the two NAT centers right now in Tokyo and one in Hokkaido. This is a sample. Then the (inaudible) is excluded from--to prepare the NAT, and then centrifuged, and then the automatic pooling system by aliquot. Only a few people can do hundreds. The human resources, not much required, but the expert can do a hundred very smoothly. And this is GT-X, 7700 PRISM. Then in that day we can get the answer.

And first we start with 500 pool size, then reduce to 50 pool size, and as for the hepatitis B, 96 percent sensitivity is 25 copies per mL, and so we can pool 50 right now going on. And we found 26 of HBV DNA and 9 of HCV DNA,

unfortunately none of HIV, they are all prescreened by (inaudible) assay.

Then we analyze 22 cases, it is a wild type of hepatitis B, and that is 16 is EIA HBs antigen. This is 16, and one is wild type anti-HBs and anti-HBc, the present (inaudible). Then the other four is infection with a pre-core mutant, analyzed, and then all of them gave no reactivity of the (inaudible) by Ortho overnight tests, zero.

Well, just (inaudible), this is current state of the (inaudible) we start our (inaudible) to screen high titer of the HBs antigens, but this (inaudible) we detect by EIA (inaudible), overnight EIA. But this (inaudible) is not detected by Ortho overnight EIA, and (inaudible) of the copies are (inaudible). I would like to emphasize, this is very interesting question, and these are that wild type but the green line is pre-core mutant exists.

Well, you know the hepatitis C copy is rather high than hepatitis B. These are tested, and then we follow up all these positive cases of the donor because it is important to know whether this type is transient infection or chronic infection, and to identify this quality of data is really virus itself, not non-viral protein or non-viral (inaudible), so we start (inaudible) study.

And among the wild type, I would like to

emphasize, 17 of them all seroconverted IgM anti-HBc, and this is anti-HBc, all seroconverted anti-IgM, anti-HBc and anti-HBc, and some of them are anti-HBs seroconverted. And among them so far we followed that, that 10 cases this HBV DNA disappeared during the observation period. And one interesting case is hepatitis DNA present with anti-HBc and anti-HBs present together. That is a very unusual case of the wild type.

And pre-core mutant, we can follow three cases. All are IgM, anti-HBc, no seroconversion, showing some, this is persistent infection, and a very low level of anti-HBc continued.

Well, this is, I put this here in the January meeting in (inaudible) Japan, and you can see this increase in the (inaudible) blood donor of HBs antigen. I just show the slope. It is very similar to what we observed in the BBI panel, lower limits increase, and many going down, the HBV DNA going down, undetectable level, without any elevation of ALT. So we have a two types, going up, or going down, and later we showed a pre-core mutant is going persistent infection like that. And also some of that, I (inaudible) for this, and remember we saw all (inaudible).

This is one of the cases, the EIA negative cases. For that 111 days HBV DNA disappeared, and (inaudible) 160 days it disappeared, so these cases can be--have

specifically the (inaudible) of this patient, of this donor to (inaudible), and this is again show going down, (inaudible), and this is again 43 to 87 days that it will be DNA not observed, and all these cases of anti-HBc responds like this. This is again that same patient after 25 days or 13 days, HBV DNA disappeared.

Well, one of the cases that it wild type, and then after (inaudible) donations the ALT is normal, and the ALT is going up 44 days, 58 days, and then at this time we recommended to hospitalize this patient, and then the donor is (inaudible) now and he is now going--his HBV DNA is going down like this.

Well, this is wild type, but very interestingly here ELISA negative but here anti-HBs, anti-HBc and anti-HBs present together. But for the immunological assay, the antigenicity of this donor was broked but in the presence of anti-HBs or anti-HBc, broked by immunological activity. So it comes out that immunological activity nothing, but the titer of the virus is rather high and continues in that day. Such a case cannot be detected by immunoassay.

Well, another point important is, so for the four cases of pre-core mutants, and then we hold up in this way that virus continues and no anti-IgM core anti (inaudible) at all, so all these three cases (inaudible) the past (inaudible) and may be a later stage, not early stage, and

antibody reactivity is not shown in this station, but the virus continues. It may be an (inaudible) mutant. We confirm that is a mutant, is a pre-core mutant, and such a case continues in the viremic state. And this kind of donor can be detected only by NAT testing, and antibody testing cannot detect. This is very interesting virus (inaudible) here.

Well, I can say the NAT screen detects HBV DNA in persistently infected individuals with extremely low level of HBV antigen and antibody often observed in case of HBV mutant. Next one, please.

So another point of interest is, we have to consider about the health care for the (inaudible) donors, and as I said before, we can notify at very early stage of viral infection, before clinical manifestation. And the follow-up study shows a difference, whether transient or chronic infection, and disclose the virus dynamics in early stage of infections, and further maybe reentry into blood donorship can be when they are in a transient viral type infection.

Finally, I want to say, I already mentioned that hepatitis B post-transient infection is much higher than hepatitis C in the United States, like discussed today also, and also that situation is the same in Japan. But after reconsidering the prevalence of hepatitis B in Japan, it is

much higher than in the United States, but the incidence of the HBV infection I think is not much higher. And so the hepatitis B NAT screen is highly (inaudible) donor, high incentive, much (inaudible) be recommended with, and that, that screen, on the basis of hepatitis B, hepatitis C and hepatitis I together is very time--the time short, a limited time, and cost of testing much lower than in individual test.

And finally I would like to thank Dr. Tabor and (inaudible) for inviting me to this meeting. And I am working as (inaudible) of (inaudible) Japan hepatitis panel for 21 years, and (inaudible) and sometimes very (inaudible) study is make much progress on both sides, and I appreciate in this time future progression, exchange of information. Be beneficial, and I hope some of you interest, please visit our NAT center in Tokyo or in Hokkaido. Thank you very much.

DR. HOLLINGER: Thank you, Dr. Nishioka.

Any questions for Dr. Nishioka? Yes, Dr. Katz?

DR. KATZ: Louis Katz, Mississippi Valley Regional Blood Center. Can you describe the sensitivity of the routine hepatitis B surface antigen assay that you are using in your system?

DR. NISHIOKA: I said 25 copies per mL.

DR. KATZ: That is the NAT. I was more interested

in your surface antigen sensitivity.

DR. NISHIOKA: Oh, I don't have any interest in that immunoassay. That is a shadow, or maybe you might worry about non-viral protein or something like that.

DR. HOLLINGER: Any other questions? Yes, Dr. Tabor?

DR. TABOR: So you have detected four pre-core mutuant HBV infections that would not have been detected without HBV NAT, but two of them would have been detected by anti-core as we--

DR. NISHIOKA: Anti-core, it is a very, very weak. The titer is 2 to the 3, and our original screening, if the anti-HBC is higher than 2 to the 4 or 5th, it is proved. That is within the limit of that negative value of the titer.

DR. TABOR: If I read the graph correctly, these four with pre-core mutant viruses were not very low titer, right? They were--

DR. NISHIOKA: Yes. The one is very high titer.

DR. TABOR: --high titer of HBV DNA.

DR. NISHIOKA: Yes. (Inaudible) reactive (inaudible) of the DNA level can be detected. If it is wild type, it should be screened by immunoassay.

DR. TABOR: So we can hypothesize that at least the high titer ones could very well have been infectious for

a blood recipient.

DR. NISHIOKA: Well, that is proven. We have, you know, in Japan the anti-HBc high titer and the HBs antigen low titer, sometimes called the fulminant type of hepatitis, and by screening by sole high titer anti-HBc, we can exclude that potential fulminant hepatitis B. I don't know whether this is a real pathogenic (inaudible), but I am just reminding, some of the (inaudible) hepatitis B virus were, I don't know were identical to the so-called hepatitis B virus (inaudible), hepatitis B type 2 that was discussed often at some previous (inaudible) by the European (inaudible). May be that we have to analyze why this mutant not show the antigenicity, and this kind of antigenicity deficient strain exists, we should be very careful of that. That what I want to say, and why this kind of virus did not show good immuno (inaudible), I think have a molecule (inaudible) would make it (inaudible).

DR. HOLLINGER: Any other questions? Yes?

DR. MIMMS: Perhaps you answered this. Larry Mimms, Gen-Probe. You had mentioned you are a molecular biologist. Have you sequenced the S gene? What would be, I think, more interesting than anti-core mutants--

DR. NISHIOKA: Yes.

DR. MIMMS: And there was no mutation in the S gene that would have led to lack of reactivity in the

Fujirabio hemagglutinin assay? Is that correct?

DR. NISHIOKA: Yes. We like to make it clear we found at this time, just this year, and analysis yet underway.

DR. MIMMS: So you did find an S gene mutant that was not reactive?

DR. NISHIOKA: Not related in this (inaudible). We have yet another (inaudible).

DR. HOLLINGER: Kusuya, you are still using hemagglutination. Is that correct?

DR. NISHIOKA: Yes. And the hemagglutination test for hepatitis C is much better than EIA, by following-up (inaudible), and the hemagglutination (inaudible) of anti-HBc is more quickly picked up, the IgM anti-HBc. But also the (inaudible) for the HBs antigen, it is about one order below the EIA. That, I showed some of the tests (inaudible), but the rapid screening is very important to be in time for blood transfusion, so (inaudible) this way.

DR. HOLLINGER: Okay. Thank you, Dr. Nishioka.

The next speaker is going to be Michael Busch on infectious HBV window period and its projected reaction by Nucleic Acid Testing.

DR. BUSCH: Thanks, Blaine. I hate to do this to people--

DR. HOLLINGER: We are not going to see that

window period again, Mike?

DR. BUSCH: Similar analysis, but with a different modeling strategy to something Sue presented, but then applying it to full testing. This is a collaboration with the REDS group, Buput Rawal in my group, along with Mary Kuhns at Abbott and several others I will allude to.

This is kind of the general theme. You know, there is infection, and we think there is about a 50-day period, based on transfusion infection, from inoculation to the detection of surface antigen, and then anti-core comes up and usually persists for the lifetime of the individual. And certainly in the U.S., where we screen for anti-core, some of the concern, some of the data you are hearing about from Japan and you will hear about from Europe that are driving them to introduce DNA, relates to the persistence of viremia after the loss of surface antigen.

And that is not an issue here in the States at present because we retain anti-core. Most of the focus here in terms of deciding whether HBV NAT should be brought forward quickly, and we have been focused on this now for a number of years, has been on the front end, and how much can HBV nucleic acid testing close this early window, and what proportion of this window from exposure to this antigen is infectious?

To address that, we have been doing a series of

studies to quantify the kinetics of HBV replication during the early seroconversion phase prior to surface antigen detection; and understanding the ramp-up rate or the doubling time of HBV during this pre-antigenemic phase; and then developing a model to back-project both prior to the ability to detect it and during the early DNA-only phase, what the levels of HBV DNA would be over time and how much NAT could reduce that, either in the pooled or single donation context; and, importantly, trying to understand further, when does infectivity occur relative to the detection of nucleic acids during that early phase of primary infection.

So to study this we have worked on 17 HBV seroconversion panels from BBI, 173 specimens, tested them with surface antigen tests, HBV DNA tests, and then done regression analysis to estimate the HBV DNA level at the conversion point of surface antigen, and then slope and doubling time analysis to derive an estimate for the rate at which virus replication is increasing.

A few representative panels. So here you can see surface antigen coming up, and what you can see is, for typically several bleeds prior to surface antigen, we can detect HBV DNA. This is the cutoff of the surface antigen test. This was the quantitative Roche assay, which has a 400 copy sensitivity, so perhaps with more sensitive assays,

and as I will show you, indeed with more sensitive assays you can detect earlier specimens. But to do the modeling I will talk about, we relied on the data that was quantitative during this early pre-surface antigen phase.

So in this example you can see that we detected HBV DNA with this test perhaps two or three bleeds prior to antigen, about 10 days earlier, and that the slope yielded a doubling time estimate for the virus levels in the plasma of about three days, so the virus is increasing in concentration twofold every three days.

This is an extreme different--another panel that showed many more bleeds prior to surface antigen that had detectable HBV DNA and a very slow ramp-up. In this case the doubling time estimate was 17 days, and we saw several panels like this that had slowly rising HBV DNA levels.

So in developing a model, the way we approached this was to first try to estimate the concentration of HBV DNA at the surface antigen assay cutoff, and that is obtained by doing a regression of the HBsAg signal to cutoff ratio against the concentration of HBV DNA. And you can see that during primary infection there is really a very nice, tight relationship, and it suggests that during primary infection, that all of the circulating virus is probably particles with DNA inside of a capsid with surface antigen.

And that regression analysis allows you to derive

an intercept and confidence bounds around that intercept, which tells you the concentration, the estimated concentration of HBV DNA at the point that the antigen test would become positive. In this particular example the estimate came out at about 2,500 copies of HBV DNA. It varies with the different antigen assays between about 2,500 copies and 12,000 copies. As Sue showed you, there is substantial difference in the sensitivity, and I am talking about U.S. HBsAg EIA assays, not the particle agglutination assay. But the bottom line is that antigen detects HBV DNA once levels achieve in the range of 2,000 to 3,000 copies up to 11,000 or 12,000 copies.

Now, the next parameter was this doubling time parameter which, as I indicated, in most of these seroconverters the doubling time was in the range of three to four days, and the median was four days. However, there were some outlyers that on average ramped up relatively slowly, with doubling times of 10, 11 and 17 days.

Now from those two parameters, the concentration of HBV DNA at surface antigen seroconversion and the doubling time, we can develop a very simple model that estimates the concentrations of HBV DNA at serial time points prior to the detection of surface antigen. Basically, since it is a four-day doubling time, you reduce the concentration of HBV DNA in half every four days. So

now we can model back the levels of HBV DNA prior to the detection of surface antigen.

The next question we asked was when during that theoretical increasing levels of HBV, during that pre-antigen phase, does infectivity begin. And to look at that, Mary Kuhns made available to and in her own lab we characterized 20 replicates at multiple dilutions that were previously pedigreed in chimps. And so we looked at 50 copies, 30, 20, 10, et cetera, and across several different tests.

And it is a little bit complicated, but the bottom line from this analysis, looking at these replicates, was that one chimp infectious dose is believed to represent in the range of about 10 to 20 genome equivalents. So as soon as you have about 10 to 20 genome equivalents in your transfusion, you probably have an infectious dose of virus.

So with that piece of information we can add to this very simple model at what time point during the early development of viremia would infectivity occur, and it probably occurs in the range of 30 to 40 days prior to surface antigen, because you really need very little virus to transmit HBV in an inoculum.

So then what we wanted to do was really look at some real tests, and we have this theoretical model of how much a test with a particular sensitivity could close the

window, and we wanted to look at some actual specimens and then also calculate the sensitivity of pool testing. So this is an actual example of an in-house PCR research assay at Roche--I mean, sorry, at Abbott--where they looked at a series of panels and they calculated how much prior to the detection of antigen could they detect HBV DNA. And in this example it was a mean of 14 days prior to antigen that they could detect it.

This was an assay that has a 20 copy sensitivity. There were several other data sets that were available. The Roche 400 copy assay closed the window by an average of seven days, and I think you will see later some data from Gen-Probe with an assay that also has about 20 copy sensitivity, that also closed it by about two weeks. And suffice it to say that this is very compatible with our model estimates of how much an assay of a specific sensitivity could theoretically close the window, so it makes us confident that this model strategy for estimating concentrations prior to surface antigen is consistent with empiric observed data.

Now those were individual donation testing using those three different NAT assays. Now the problem we have, as Sue alluded to, is once we start to pool, we lose sensitivity. So theoretically, taking an assay that has 20 copy per mL sensitivity--which is kind of where we could get

to best case, with realistic assay inputs, about .5 mL, at least in the whole blood sector today--and if you have an assay that you test undiluted with a 20-copy sensitivity, you would project a window period reduction of 24 days.

And if you then take this out through the incidence projections, you would estimate that with that assay we could detect, in the whole blood sector, about 75 donors per year who would be DNA positive and surface antigen negative. But as soon as you start to dilute that to pools of 20, or certainly pools of 100, the analytic operating sensitivity is diluted out, and you are then operating with a test that only has 400 copy sensitivity, which reduces the window closure to 7 days and diminishes your theoretical yield down to about 20 per year in the whole country in the whole blood sector.

So it is data like this that led us to recommend that HBV DNA testing for window period closure not be introduced in the context of pooled screening because of the relatively low sensitivity of pool testing and the high sensitivity of the antigen assays and the dynamics of the window.

Two other points that I think are important is, one is, you would think like with p24, once we add HIV RNA, we can stop doing p24 antigen. That is not the case with hepatitis B. That is illustrated here.

We took 200 surface antigen positive, anti-core positive donations, and we then looked at them with HBV DNA assays, and whereas in the window phase you have a very nice relationship between HBV DNA and surface antigen, in chronic carriers that completely falls apart. Most chronic carriers have actually very high levels of surface antigen relative to the DNA load. That is well known. Excess antigen is often produced.

But down here you actually see there were about 20 percent of these surface antigen positive donations--these are whole blood donors--who are actually negative for HBV DNA with a 400 copy sensitivity assay. We sent these on to Mary Kuhns at Abbott, who with the research 20 copy tested them, and we were still left with about, I think, about eight or nine surface antigen positive carriers who were negative for DNA.

So what this tells us is, we are probably not going to be able to replace HBV DNA--I am sorry, surface antigen--even once we bring in single donation NAT. We will have to retain the surface antigen test.

The last point is, right now we are screening with anti-core, which protects us on the back end with respect to a problem illustrated here. This is, again, I think Mary Kuhns was involved in this study, and what is known is that some people in very late stage infection, after they have

lost surface antigen, they will then, in the context of persisting anti-core, they will still have HBV DNA detectable in the plasma for periods of months or even years following loss of detectable surface antigen. And even after they lose the HBV DNA in the plasma, they may still have HBV DNA in the liver.

And this is well known to transplanters, as summarized in this paper. The critical point here is, if you take a liver from a person who is surface antigen negative but anti-core positive, and has low level or absent anti-surface, those livers will transmit--this is the example here--they will transmit HBV 70 percent of the time.

So this is again to point out that right now these people are being picked up in the whole blood sector because we are screening with anti-core, but a critical question as we look at adding HBV DNA that we are studying is, will we have to retain the anti-core test or can we get rid of it?

And the last slide just summarizes a study that is ongoing now of the REDS group led by Steve Kleinman, where we have identified over 5,000 donations that are anti-core reactive in our repository, and those have now been tested with a confirmatory anti-core test that has reductant as well as quantitative anti-surface. We identified 1,200 of these anti-core only donations that are corroborated by an alternative anti-core test and have low level anti-surface,

and those are now being tested by HBV PCR to bring forward data that can address the question of can we get rid of anti-core once we add single donation HBV.

So I think the big message here, we are getting a lot of, if you will, noise from Europe saying we should be doing HBV PCR, some data coming from other countries that suggest there is yield. But we have to remember that is generally in countries that are not doing anti-core testing, and many of these countries are using sub-optimal surface antigen tests. So at least our data supports the conclusion that HBV NAT really won't buy us anything significant until we have the capacity to bring in single donation screening.

Thank you.

DR. HOLLINGER: Thank you, Mike.

Questions? Yes, Dr. Fitzpatrick?

DR. FITZPATRICK: Your comments about core, can you make a comment about the U.K. policy, and there are some other countries that have the same policy, that if you are core antibody positive and surface antibody positive and antigen negative, you are a good donor. And the U.K. data showed they have had no transmission using that policy.

DR. BUSCH: Yes, I agree with that. That is also what the Japanese do. The subset of anti-core positive individuals who may harbor infectious HBV, who are surface antigen negative, usually have absent or very low level

anti-HBs. So if you have good levels of anti-HBs, you are almost certainly safe with respect to HBV, and I think the experience from Japan and Britain is that those donors, those units are safe. If you transplant an organ from people like that, it has been pointed out that those livers don't transmit HBV either.

DR. HOLLINGER: And, Mike, on that same issue, you mentioned that there were about eight or nine samples at 20 copies per mL that were HBs antigen negative, anti-HBc positive, I believe. Do you expect those to be transmissible?

DR. BUSCH: No. Those specimens were all HBsAg positive and anti-core positive, but they were negative for HBV DNA.

DR. HOLLINGER: Right, and I guess my question to you is, my assumption is that these may not be infectious, and the fact that you don't find it may actually speak to that. I think those kind of samples need to be tested, at least in some way or other, whether it is a chimpanzee or otherwise.

DR. BUSCH: Yes. I like that thought, yes.

DR. HOLLINGER: Any other questions of Dr. Busch?

[No response.]

DR. HOLLINGER: Thank you very much, Mike.

Okay. We are going to go to the open public

hearings now, and the first one is going to be Charles Watson from Centeon. Dr. Watson?

While we are looking for some material, we will go to the second person, Dr. Andy Conrad. Andy?

DR. CONRAD: What I am going to talk about is a prospective study, and again, it is such an interesting thing, because when we look back at some of these plasma seroconversion panels, it is just bloody different than the data that we are getting and I think you will probably see in these prospective studies. I want to remind you that these are in paid donors, plasma phoresis donors, which may be a slightly different population. Certainly the frequency of donation is going to be dramatically different.

And I guess this is the first thing. When we began doing this, we sort of postulated that what we would see is two sort of pathways down here to the bottom, and I apologize for this complicated slide. But, basically, since we screen, everybody in the plasma industry is screened for antigen, we figured that the only people that we could ever see would be antigen negative, and they could have surface antibody and core antibody, and those would just be the sequelae of people who had resolved their infection, going through the normal course, and that they would be DNA negative, but indeed that is not what we found.

So what you theoretically think that you could get

is, you could get people who are negative for core antibody, negative for S antigen, and because we are screening, the first line of screening of nucleic acids, all of these people would be positive for HBV DNA. The other class of people you could see would be HBC core antibody positive, S antigen negative, and those people could be S antibody positive, thus masking the antigen. You could have the opposite with people antigenic, which would be the acute people with no core antibody, or you could have people that had both.

And we thought, well, this is going to be hard to find, it is going to be rare, but here is what happened. So what we thought this model would tell us is that there should be a rather rapid conversion from S antigen--to S antigen positive in acute cases, and Mike Busch talked about four-day doubling time. But then there was these weirdos that took 17 days or longer, and those confuse us too. It isn't clear from the kinetics why some people do it so differently than others.

And then we also thought that we must be very careful because we had people coming into the system, they could be so viremic that they could contaminate the pools and cause us to be a lot of false positivity, so we had to be very sure that we pulled out the S antigen samples. And some of the confusion that we may be seeing may have

something to do with, at the donor center, with the durability of HBV, and these highly viremic samples may be contaminating at donor centers, and that has been one of the things we have noticed.

So we did two studies. The first study we did was a pilot study where we took samples that had been run for HCV and HIV, re-randomized them and tested them for HBV. This is, again, the same general donor population that we have been looking at for years with the HCV and HIV.

And then we also conducted, under IND for our friends at the FDA, a prospective study to screen a minimum of 300,000 donations from at least 10,000 donors with HBV DNA by PCR. All the donations were to be surface antigen negative, and screened in this case with the Auszyme, the Abbott surface antigen test. And then we were going to use pools at 512, where you would think from, again, Susan and Mike's presentation that there wouldn't be very much yield. The assay that we have has a mean sensitivity of about 3 copies and 95 percent detection at 18.

Again, the three-dimensional matrix, I know you are tired of this, but just so you know, we take 512 samples, we stick them together in a machine that sort of puts them in a cube. If the cube is all negative, everything is negative. If the cube is positive, we look at the row, layer and column and it isolates the single

positive sample.

So all the data I am going to show you was first screened with nucleic acids. First the S antigen positives were pulled out, then screened with nucleic acids. That is just the column stuff. That was a refresher, and here we go. So here is the first donors that begin to see.

These were donors where it was available for quantitation, and those are numbers that are cut off. But we started getting viral loads--maybe if we could shrink that down a little bit, people could get these numbers, because that is sort of relevant. There you go.

We were detecting a great many people with very low viral loads. Now, I am not quite sure how we got them, but we were getting these in the screen, and when we quantitated them, they had 750, 950, all the way up to levels of 46,000, which again is different than the models would have predicted. And I don't know if it is because those panels were old or not stored correctly, but we found people with viremia, nearly 50,000, that aren't antigen positive, and we have now started sending them out to get other antigen tests, but they have been repeatedly antigen negative, all the way down to these low ones like Sue Stramer showed originally.

The second class of people that we saw was the other end of the arrow. These people are all HBC or HB core

antibody positive, HBs antigen negative, and they have a transient viremia, a low level viremia, exactly what Dr. Busch showed. This class of people tend to have lower viral loads, it is transient, but somehow it is being detected and that is what keeps bothering us, because we keep finding them through the pooling.

This is the last slide that I just wanted to mention, because in this person here we actually followed them out. This one was reactive for 18 days, remained positive for PCR, and ephemerally negative and positive, negative for the antigen, positive for both core antibody. And here is a person that stayed negative for 13 days for core antibody and core antigen--I mean and S antigen, but viremic for 13 straight days, no S antigen coming up. And this is probably like the person that you thought, Dr. Busch, that they can go longer than people think. And so it has been very surprising.

Finally, I am going to give just some numbers on what the prevalence has been, and I feel awkward using word "prevalence" because in some ways these people who are core antibody negative, S antigen negative, are sort of incidence cases in a way, in that they are somewhat--you would postulate that they are proximal to the event of infection, but the numbers we got in that pilot study were 11 positive donors out of 43,000, which works out to be around 1 in

4,000, roughly, 1 in 3,900.

And so far in the ongoing study that we talked about, the new study, we have gotten 4 donors, a total of 4 donors out of 24,663, for a prevalence of about 1 in 5,000, statistically the same. PCR positive, S antigen negative, and half of them were anti-core negative and the other half were anti-core positive, so it went for both directions on the arrow. And that is all for those.

So, in conclusion, what we have is, we have the prevalence of HBV infection in our two studies has ranged between 1 in about 3,900 and 1 in about 5,100, with the low end that is obviously probably the same. It would appear that the donors fall into two distinct groups, what we would call the acute group, which is the group that is core negative, and the chronic group, which has HBV S antibody but remain viremic, S and core antibody but remain viremic.

And, lastly, there is data to support the notion that there is a sustained window period. By sustained window period I mean more than what one would figure with the rapid doubling time that was originally postulated for HBV, probably that second class of people that have 15- and 20-day doubling times, not the 4-day doubling time. And it is odd, there is no real clear explanation for the viral mechanism for the slower doubling time in the absence of immune suppression, unless it is an antibody mediated immune

suppression that is absent.

And one of the things that I worry about is that there clearly may be issues with the sensitivity and specificity of current HBV antigen kits, like Sue showed, because we think that it seems odd that this is happening. And that is all.

DR. HOLLINGER: Thank you, Andy.

Any questions for Dr. Conrad? Yes, Dr. Tabor.

DR. TABOR: Do you think, do you know yet whether there are any mutations in these individuals you detected?

DR. CONRAD: Yes, that is such a good question. N, we don't know yet, but 34 we fortunately have up to 850 mLs of plasma on them all, and hopefully we will be sequencing them soon and looking for that.

DR. HOLLINGER: Okay. Now we will go back to Dr. Watson.

DR. WATSON: Thank you for finding these. You would not want to hear this without seeing the slides. It wouldn't work.

I am Chuck Watson. I am from Aventis Behring. We are a new company. We used to be Centeon, so if I say "Centeon" please forgive me. I appreciate the invitation. We have been wanting to talk about our hepatitis B testing for a while. That is what I am going to present. I may slip from NAT to PCR because that is what we work with. I

would also like to recognize Dr. Fielder, our medical director, for helping with the interpretation. Could I have the next slide, please?

I am going to go through just a quick summary of our test system. If you want, later I will go into our pooling scheme, but I left that out to present the data. We now test for five viruses. All of our samples are serology screen negative. We also do all of our tests--all of our samples are tested for ALT. Remember, this is plasma, so we don't do the anti-core test. Our screening system was developed in-house.

Next one will show our start times. We have been doing tests for hepatitis B, C, and HIV-I since April of 1998, and that is what I am going to report on here, for the approximately 21 months. HAV and B19 high titer have been implemented this month. We started pooling for these today --as long as the lab is still open.

Our hepatitis B detection limit is, in our largest pool, is 2.4 times 10 to the 3 international units. Now, when we go to smaller pools and we follow up the large pools, our detection limit is 27 international units per mL.

As part of the IND we have a clinical study where we invite those positive donors to come back and visit us monthly. We started this at once a month for six months but we have extended that to 12 months, where we do both a

serology test and an NAT test for hepatitis B seroconversion, if we have an HBsAg reactive or we have the anti-core positive.

Now, let's look at some of our results. This is strictly based upon what we have done in the United States. This excludes all of our testing in Europe. Okay? Of the 3.25 million samples, we have 42 donors that are positive; 62 units are also positive. Now, our donors can donate twice a week, and it takes time, since we have a large pool size, to do the testing, so these donors have contributed 272 units after that donation.

This turns out to be 19 donors per 100,000 donors. And, Dr. Hollinger, I am glad to finally be able to answer your question from last March. Which is very similar to what Andy Conrad showed from NGI. I think that was about 25 or 26 per 100,000. As far as per donation, we 2 units positive per 100,000 units that we test. Okay? Could I have the next slide, please?

Let's look at the pattern of these positives based upon the NAT result. We basically have the donors falling into three patterns. One is, on a subsequent donation, do they end up with an HBsAg reactive? And you can see that we have 10 donors in that category. The next category is a single NAT positive. Some of those donors don't come back, so there is no subsequent donations. Some of those donors