DEPARTMENT OF HEALTH AND HUMAN SERVICES

ADVISORY COMMITTEE ON BLOOD SAFETY AND AVAILABILITY

Twenty-Sixty Meeting
Volume II

8:04 a.m.

Tuesday, May 17, 2005

Washington, D.C. 20001

P A R T I C I P A N T S

Mark Brecher, M.D., Chairman
Jerry Holmberg, Ph.D., Executive Secretary

Judy Angelbeck, Ph.D.
Celso Bianco, M.D.
Arthur W. Bracey, M.D.
Paul F. Haas, Ph.D.
W. Andrew Heaton, M.D.
Jeanne Linden, M.D.
Karen Shoos Lipton, J.D.
Gargi Pahuja
Susan D. Roseff, M.D.
S. Gerald Sandler, M.D.
Merlyn H. Sayers, M.D., Ph.D.
Mark W. Skinner, Jr.
John W. Walsh
Wing Yen Wong, M.D.

Non-voting Ex Officio Members

Matthew G. Kuehnert, M.D., CDC
Jay S. Epstein, M.D., FDA
Harvey Klein, M.D., NIH
CDR Michael Libby, DOD
James S. Bowman, III, M.D., DHHS, CMS

C O N T E N T S

C O N T E N T S (Continued)

P R O C E E D I N G S

Call to Order

DR. HOLMBERG: Welcome to the second day of the Advisory Committee for Blood Safety and Availability. We will proceed with the roll call.

Roll Call

Dr. Brecher?

DR. BRECHER: Present.

DR. HOLMBERG: Dr. Angelbeck?

DR. ANGELBECK: Present.

DR. HOLMBERG: Dr. Bianco?

DR. BIANCO: Here.

DR. HOLMBERG: Dr. Bracey?

DR. BRACEY: Here.

DR. HOLMBERG: Dr. Haas?

DR. HAAS: Here.

DR. HOLMBERG: Dr. Heaton?

[No response.]

DR. HOLMBERG: Dr. Heaton has not shown up yet. I'm sure he's here.

Dr. Linden has not shown up.

Karen Shoos Lipton?

MS. LIPTON: Here.

DR. HOLMBERG: Pearl Toy is absent.

Gargi Pahuja?

MS. PAHUJA: Here.

DR. HOLMBERG: Dr. Roseff?

DR. ROSEFF: Here.

DR. HOLMBERG: Dr. Sayers?

DR. SAYERS: Yes.

DR. HOLMBERG: Mark Skinner?

MR. SKINNER: Here.

DR. HOLMBERG: John Walsh?

MR. WALSH: Here.

DR. HOLMBERG: Dr. Wong?

DR. WONG: Here.

DR. HOLMBERG: Dr. Kuehnert?

DR. KUEHNERT: Here.

DR. HOLMBERG: Dr. Epstein?

DR. EPSTEIN: Here.

DR. HOLMBERG: Dr. Klein?

DR. KLEIN: Here.

DR. HOLMBERG: Commander Libby?

COMMANDER LIBBY: Here.

DR. HOLMBERG: Dr. Bowman?

[No response.]

DR. HOLMBERG: Dr. Sandler, we didn't get you on the list here?

DR. SANDLER: I'm here.

DR. HOLMBERG: You're here. I see you.

My eyesight is failing me.

Okay. Just some things from yesterday--Dr. Linden?

DR. LINDEN: Dr. Linden is now here.

DR. HOLMBERG: I knew you were coming, so okay.

Just a few things from yesterday. Yesterday morning there was some discussion about the safety of albumin administration. That was posted on the CBER website yesterday, and I did make copies for the committee and pass that out to you so you have copies of what is currently on the Food and Drug Administration's CBER website.

Something else that I dropped off at your table is an e-mail that I received from Michelle Vogel concerning the IVIg cost or the

reimbursement. Evidently there were some new prices that were posted yesterday on the website which were less than this last quarter. So if you will read that e-mail, it emphasizes that there's a lot more coordination that we need to do with CMS.

Okay. With that, Dr. Brecher, would you take over, please?

DR. BRECHER: Okay. Thank you, Jerry.

We're going to continue now with our topic of emerging infectious disease surveillance and impacts on availability. We are going to stay on time and we are going to get out early.

Our first speaker is Michael Busch from Blood Systems Research. He's going to be talking about NHLBI-funded programs.

NHLBI's funded RED II Program: Understanding the RED II Program and its Role in Detecting emerging Threats - Michael P. Busch, M.D., Ph.D., Blood Systems Research Institute

DR. BUSCH: Good morning. I'm pleased to be here in George Nemo's absence. You were actually supposed to get a couple of manuscripts.

I think you got one, which was an older review paper about the NHLBI repositories. It kind of had tables that walked through the historical repositories up through REDS and talked about the product, the manuscripts, and the approaches. And there's a second paper that's in Press and Transfusion detailing the RADAR repository, Steve Kleiman (ph). I don't know--you got that as well, great.

Okay. This is actually a slide that Steve gave me, just the concept that, you know, we only are going to see emerging agents if we keep our eyes open and look beyond the fence. So I think that's a theme that you'll see through this presentation.

I think many of us that are in this field appreciate very much the effort that NHLBI has taken over the last three or four decades to establish donor-recipient repositories to allow us to both not only establish the prevalence of the emerging agents in the donor pool, but most important, through the linked donor-recipient

repositories to actually demonstrate transmission. And this becomes obviously one of the critical steps in assessing whether a screening strategy is justified for a new agent. And through these linked repositories, we're really able to prove transmission through genetic analysis and demonstrating sequence homology between the virus and the donor and recipients. And many of these studies have also included non-transfused control patients that allow you to really assess background rates of infection.

Not only can these demonstrate transmission, but they can establish the rate of transmission of these agents and also, you know, assess--going back to historical repositories which have existed now for 20, 30 years, as I'll show, be able to establish the prevalence over time of infectious agents so we can look back to repositories of donor samples or donor-recipient from 20, 30 years ago and understand whether this is really a new agent coming into the population or has this been around for decades. And, of course,

if it has been around and transmitted for decades, for example, the GBV or TTV viruses, it's probably not that clinically important if we haven't seen any consequent disease in the donors and recipients. So an important value of having a series of these kinds of repositories.

Another challenge, though, is, you know, because these repositories, as you'll see a little bit later in the RADAR example, are of limited scope, to demonstrate lack of transmission when we're looking now at wanting to reassure the public about risks in the one in a million range, I mean, these studies are not really powered to demonstrate that kind of zero risk.

This is just a summary table of the historical large repositories, and I'm not going to be able to walk through these again. They're all reviewed in that review in Vox Sanguinis. You know, just to mention the transfusion transmitted viruses study really focused on hepatitis. Harvey Alter's NIH clinical center studies are continuing now into what he calls the TRIPS study, which is an

ongoing prospective study with very frequent bleeds from recipients to be able to look for what he calls molecular conversions to a variety of known and emerging agents.

Transfusion Safety Study was focused on HIV, but has been used extensively also for HTLV-related studies.

The FACTS study, which was the Hopkins Houston cardiac surgery cohort, most recently, actually in the last issue of Transfusion, a paper on studying HHV-8 transmission in that FACTS. That was a recipient population where there were no donor samples, so it leaves you with a little bit of uncertainty. Even though they demonstrate seroconversions, the inability to go back to linked donor samples and prove a relationship between recipient seroconversion and receipt of a positive unit is a limitation of that kind of study.

Then the original REDS repositories, which were donor repositories, used two study HVB, CMV, et cetera. The donor repository is a little bit more detailed here from the REDS program. About

half a million donor sera in the first repository and then a second, what we call the GLPR--general leukocyte plasma repository--that for the first time was a large-scale repository that included cellular preparations to be able to look for cell-associated pathogens in the donor samples. These were linked to the donors, but we did not enroll corresponding recipients of these units. So any kind of transmission question would have to be addressed through lookback studies, tracing the recipients, you know, years later after they received these units. But this repository was used for a variety of studies. In fact, the key findings here included looking for the rate of NAT--for DNA for HBV in various hepatitis reactive samples, a T. cruzi study that looked at the prevalence of seroprevalence and the correlates of prevalence around the country in the REDS sites, and then actually zero transmissions observed through lookback studies. A fairly large HHV-8 seroprevalence and viremia study, several studies on CMS viremia, and studies of collaboration that

looked at TTV seroprevalence in donors. And these were using the serum repository from REDS.

Then we have the RADAR repository, and that's the newest large NHLBI-funded donor-recipient repository, a true linked donor recipient repository, established at the five REDS centers and then two additional sites that were supported by CDC--the Pittsburgh ITM and the Tampa Blood Center. And this enrolled recipients and captured corresponding samples from large numbers of donors that were likely to be transfused to these recipients, throws away both cell and serum samples or plasma samples from the donations as well as from the pre- or peri-transfusion samples from the recipients, and then followed these recipients to capture a 6- to 12-month, generally closer to 6-month follow-up specimen.

These units, this study really targeted surgical patients, and most of the units that went into these patients that were part of the study repository were red cell-related products. And there was a large group of both recipients who

didn't get transfused as well as donors whose units did not go into the enrolled recipients.

Now, this study was designed and powered, and, again, in the paper you see extensive detail about the whole objectives and power calculations. But it was really designed with the intent to disprove transmission of some agent that had a moderate prevalence in the donor pool. So it was really trying to establish with, you know, a 95-percent certainty that an agent with a moderate prevalence had a less-than-25-percent rate of transmission. If transmission was observed at some modest rate, then one could calculate infectivity relative to the prevalence in the donors.

The assumption in designing the powering study was that the agent of concern would have a prevalence in the range of established transfusion-transmitted pathogens that we deal with, so in the range of 0.05 to 1 percent prevalence of marker reactivity in the donor population; and that it would have a transmission rate like those agents in the range of 25 to 75

percent. So the study calculations and the power in terms of number of enrolled recipients and donor unit exposures was designed around sort of established pathogens that we know and care about and screen for.

So the study enrolled about 3,500 recipients who fully enrolled, meaning that we had a follow-up sample that they had an evaluable transmission investigation that was possible. There were also additionally 1,400 recipients who enrolled, gave us the baseline sample, but didn't give us the follow-up sample. And these become valuable as we assess background prevalence in the recipient population and pilot assays before we go to the especially evaluable linked donor-recipient samples. Our studies are always designed to assess both prevalence in the donor and pre-transfusion prevalence in the recipients by piloting the assays on the less critical specimens. So that we save these 3,500 fully enrolled recipients and the corresponding donor units for future use where we know we have tests that will be sufficient and we

know we have a background prevalence, et cetera, that will be informative.

So, again, these include the donor samples, which is 127,000, including, you know, a large number--I don't remember the exact number--of units that actually went into these 3,500 recipients. There were also a number that went into recipients who enrolled but didn't give us follow-up. And then there's also a large number that came from donors who went into the repository, but the units didn't go into these patients, because we had to set up special inventory to support the RADAR patient needs, but not all of those units--only about 25 percent overall, I think, went into the actual enrolled recipients.

So, again, the fully enrolled recipients got a mean of about four units. They had a variety of blood exposures, but the majority you can see were red cell exposures with about an equal split, actually--close--of non-leukoreduced and leukoreduced units. Coincidentally the study spanned the implementation of leukoreduction. So

as we're looking at agents like right now HHV-8 or things like T. cruzi that are cell-associated and where leukoreduction could have an impact on transmission, the study fortuitously is built to be able to ask the relative transmission of leukoreduced/non-leukoreduced components.

Now, moving on just briefly to talk about REDS I and what it was intended to do and what it did, as you'll see, it's a similar mission to REDS II, which was just launched. REDS I was designed and intended to really facilitate investigations of transfusion-transmitted infections. Really on the heels of HIV it was first launched, now about 15 years ago. Really a resource to NHLBI and I think to the transfusion medicine industry to rapidly address critical issues of both safety but also progressively over the course of the study we did a number of projects related to availability--donor motivations, deferral policies, et cetera. In fact, the study has just hit its 100th publication, actually, just right about now.

In terms of data presentation at meetings,

policy decisionmaking, again, I think very visible with about 200 presentations at scientific and policy setting meetings. And really the concept of really being very responsive as data was needed, both in terms of generating new data and analyzing existing data to address your policy concerns.

Now, there were a variety of studies, epidemiologic modeling type studies and survey type research, and then there were the laboratory studies. I'm not going to go into this in any detail, you know, as we kind of move into the emerging viruses in REDS II; just to highlight how within REDS I there were large numbers of studies related to HIV assessing test performance, characterizing biodynamics, modeling out and projecting yield of NAT, things of this nature, trying to understand the significance of indeterminate serology. Hepatitis viruses, HTLV, again, a lot of different studies. I'm not going to walk through them.

And then with respect to emerging studies, just as examples of rapid response and approaches

to take to investigating emerging agents, within REDS I we fairly early on had the concern about what was called idiopathic CD lymphocytopenia, an AIDS-like immunosuppression in the absence of HIV. And we actually launched studies that evaluated the role of CD4 screening tests, rapid tests potentially useful in donor screening. This eventually died out and was attributed to either AIDS variants or normal range of immunologic parameters.

T. cruzi, again, we did a prevalence and transmission study there. We worked with (?)-aney to look at some of the variant T. lymphotropic viruses. Actually these variant primate lymphotropic viruses have recently been discovered in humans. There are two new humans HTLVs that turn out to correspond to the very viruses in primates that we had studied in this particular investigation.

We did a large evaluation of HHV-8 serologic tests, and as you'll hear in a minute, we're actually now working on a prospective

transmission study related to HHV-8. And then a lot of studies related to West Nile. That is obviously, from all of us, I think, the paradigm of a recent emerging infection that really came down big time on the U.S. blood supply.

Just a couple of slides to illustrate the kind of projects we did. Again, you are all aware of how that virus spread across the country, and we're expecting actually a fairly significant epidemic on the West Coast this year. A lot of our work actually in the last Transfusion, those of you who have seen it, has this cover, and I think three papers from the REDS group addressing the issues of the sensitivity of various tests on detecting low-level viremia, somewhat the concern, the persisting concern over this tail end, very low-level viremia post-seroconversion, and then a large collaboration with America's Blood Centers where we've compiled the data from all the ABC centers and looked at the rate of detection of viremic donations through the 2003 season.

Now, we actually collaborated with Sue

Stramer of the Red Cross to compile a national yield of West Nile through the 2003 year, both detection by mini-pool NAT and then as shown in this sort of purple color, the detection by targeted individual donation NAT detecting the very low-level viremia that had been missed by mini-pool NAT through the course of the season. And this paper is, I believe, going to be coming out in Annals very soon that compiles the national yield.

But well beyond just contributing a compilation of national yield, what we were able to do in this study was to translate this national yield of donor screening into information on the infection rate in the population as a whole and the proportion of infections that evolve into neuroinvasive disease. And the way we did that was to calculate through the relationship between viremic donations and seroconversion in the donor pool. We did a large study in North Dakota where we tested thousands of donations for IgM and IgG antibody and could determine that in this region, where they had a peak NAT yield of a little over 1

percent, about 5 percent of the donor pool became infected through the course of that very brief epidemic.

So knowing that relationship, we were able to calculate the length of the mini-pool NAT window period, which is 6.9 days. And then we could take the data from every state in the country, NAT yield by month within every state in the country, and from that we could transform the NAT yield data into infection rates in the total population. And you can see this is a map based on this density plot of proportion of infections per state, and from this we could calculate that in different states we figured out exactly what percentage of people became infected and calculated that in that year 735,000 people acquired West Nile infection.

Then we could correlate the proportion of people within each state who developed West Nile infection from our donor data with the number of cases or the rate of cases that were reported of neuroinvasive disease and figure out what the relationship between infections and neuroinvasive

disease was.

So I kind of walk through this to sort of illustrate and emphasize that this donor data is not just relevant to blood donors. As we look at emerging infections entering the population, the donor data is an incredible resource and these repositories an incredible resource to address broader public health questions of both infection prevalence and transmission, but also disease penetrance.

So moving on to REDS II, REDS II is a new program just launched--formally awarded last October, and it involves--oh, shoot, this is an older version of the talk. I don't know how we ended up with this. So it's NHLBI-funded; Westat is the coordinating center. Actually, my group in San Francisco is the central laboratory, and there are six centers that are listed here in the U.S. And the objectives, like REDS I, is to conduct epi and lab and survey research on the volunteer donors.

Now, the focus is safety and availability,

as in REDS I, and the initial focus was the U.S. blood supply. But I think importantly, as you'll hear in a minute, they--this seems to be on auto-forward, sorry. They've added international sites to the REDS II program, are in the process of doing so.

So as we entered this study, each of the competing sites, the lab and the coordinating center, all had to bring forward ideas to be successful in competing. And then we met, of course, we worked through those ideas, and we have six major working groups or working areas of activity. There's a large infectious disease focus, of course. There's a major project related to TRALI. Studies on the impact of regular donation on iron and hemoglobin levels, studies related to recruitment and retention of donors, studies focused on understanding what happens to our deferred donors and can we get them back, and are the deferrals that we have today really useful; and then large studies linked to the donor database and survey-related projects.

On the international front, very exciting, I think, for many of us is that NHLBI has had now the ability, resources to fund two to three

international programs in developing countries to join the REDS group. And this RFP is just out now with the proposals due June 14th. They're hoping to get three--potentially as few as two, but hopefully three awards that will require a U.S. collaborating center with a strong historical relationship with a developing country or developing region. And each of these developing centers, developing country blood center programs must have at least three linked centers and a minimum of a quarter of a million donations represented by those three centers. It's predicted to be a four-year program, although we're always optimistic that REDS will continue forever.

The objectives of the international program are to really do the same kind of epi, lab, and survey research that REDS has pioneered but in the setting of regions that have developing overall infrastructure, but especially blood programs, and

also have a significant HIV/AIDS epidemic. So the areas that are of particular focus are enumerated in the RFP and I know applications are being developed include South Africa, Asia--such as China--South America. And the goal is to help those programs both assess their safety and blood availability issues, but also to collaborate in ways that allow us in the U.S. to have a little bit more proactive global surveillance for emerging infection problems.

So some bread and butter stuff that we do in REDS will be done in these programs, monitoring prevalence and incidence of major bloodborne viruses, and most important, being ready for newly discovered or emerging agents that may pose a threat in those countries or here to blood safety and doing the studies to assess prevalence in the donor pool, transfusion transmission, impact of current or potential screening methodologies both in terms of safety impact and consequences in terms of deferred donors as we look screening strategies for new agents.

You know, studying the donors in terms of risk factors, so bringing back HIV-infected donors. In many of these regions of the world, the HIV

epidemic is heterosexual, and screening donors is very difficult. As you may have read, in South Africa, for example, there is a major political uproar over their approach to maximize safety through targeted recruitment based on really predominantly race, ethnicity, geographic issues. That has really come under fire, and they're going to have to disband that. They're going to be adding individual donation NAT in South Africa, which actually presents a huge opportunity to capture those acute viremic, infected donors. They're projecting 50 to 100 a year people picked up by ID NAT with Clade C infection. So that presents actually an opportunity for us to work with them to study those kinds of donors over time and understand the issues.

Then address the blood safety issues, so there's a large donation and donor-linked database that's required. So these sites must have moderate

capacity. So these are not, you know, kind of very small, rural blood centers we're talking about. They're major urban centers with fairly good computer capacity and some history of research. And they'll serve--the goal is that these centers in these developing regions will serve as centers for excellence to train scientists and clinicians in blood transfusion research and practice over the decades to come.

Okay. So quickly moving on to the REDS II planned projects in just a very brief overview, we have programs within REDS II move through a development cycle and have to go through review internally and then through NHLBI. So we have projects that are really through the proposal and into the protocol development phase related to HHV-8 and parvo B19, and also a project that I think will probably play out also in the international sites and I'll describe in a little bit of detail related to molecular surveillance of incident cases detected in donors. And I'll talk about that a little bit more.

We also have projects that are in development related to tick-borne pathogens, a concept submitted on the effect of leukoreduction

on transmission of cell-associated herpes viruses, and then a generic program, which is really kind of a rapid response and being proactive and being ready to investigate emerging agents in the blood supply, I'll talk about a little bit. And then NHLBI brings some issues to us. They've brought recently the simian foamy virus prevalence/transmission question which has been fomenting for the last couple years. We're interested in studying whether we can drop HBsAg once particularly ID NAT for HBV is introduced in the study there.

Another big issue is as vaccines come into the broader use for things like particularly HIV and West Nile, we'll have huge populations of donors who are seropositive as a result of vaccination, and being thoughtful about how that's going to impact our screening strategies. For example, as HIV vaccines begin to be more widely

used, optimistically, the population will be seropositive due to vaccination. And unlike hep B, we won't have simple tests that can discriminate seropositivity-related and natural infection from vaccine exposure. And will NAT solve the problem? Probably not. So being involved in studies to potentially develop more appropriate serologic screening tests for donors in the setting of prior vaccination.

HHV-8, again, the last Transfusion has the paper from the FACTS program and an editorial by Roger Dodd. This is an agent of concern. The prevalence in the donor pool is in the range of 2 to 3 percent antibody prevalence. And now there's three studies--actually one from the U.S. and two from Africa, this one that's published and another that's just about to be submitted--that really pretty unequivocally show that that virus can be transmitted by blood transfusion, particularly in high endemic areas in non-leukoreduced units.

On the other side of the coin, transmission isn't super common. There have been

some limited lookback studies that have shown absence of transmission of recipients. This was a TSS study. But, also, we don't have a lot of transfused patients who are developing KS, so this is not a crisis in any sense but a study to investigate formal transmission is warranted and is under consideration by the REDS program using the RADAR repository.

B19 is another one where we've had a lot of discussion with FDA, and currently blood programs are actually implementing what we call in-process testing for B19 DNA. This virus is very resistant to inactivation and has been transmitted by some derivatives and, you know, can cause significant disease. So we're concerned that right now there isn't--we may be screening plasma that goes to derivative manufacturers for high-level B19, but we're concerned with the sort of inequity that we're actually allowing that blood to be issued in real time to individual transfusion recipients. And so the study is actually designed to determine the rate of very low level viremia in

the RADAR repository, the distribution of low viremia versus high, and the sero status, and then look at the transmission to individual recipients of B19 from these different kinds of components. So the relationship of viral titre and sero status to infectivity is the focus of the RADAR B19 study.

I just want to present a few minutes on molecular variants. We need to assure that the screening and diagnostic tests that we use are detecting critical circulating strains. A lot of these assays in this country, you know, were actually developed five, ten years ago, often using prototype strains. All of the HCV antigens are still based on the original Chiron isolate. Most of the HIV-1 antibody tests in the U.S. are based on the origin Clade B U.S. virus. And we know from studies in other countries that these tests are not always optimal for detecting variant or--often in many cases these other strains are actually much more prevalent around the world than the U.S. strain that was the original prototype.

So we think that studies to monitor the

rates of particularly transmitting strains, which were defined as the newly infected donors who we can detect through testing strategies that have incident infections, are justified. And just understanding the divergence of the infection trains in the donor pool in turn gives us insights into the transmitting strains in the general population because the donor pool really represents a low risk but a representation of the larger population.

Also, just in terms of broader public health implications of understanding the kinds of strains that are being transmitted in the general population relate to questions about pathogenesis of different strains, relative likelihood that HCV variants would actually cause disease, or vaccine resistance. So if there's a lot of transmission of HBV vaccine escape mutants that we detect in the donor pool, that would, you know, put forward the need to enhance the vaccine with respect to immunizing for these variants.

So, again, our focus in thinking about how

to do this kind of what we call targeted molecular surveillance was to try to focus on donors who we detect as having acute infections. And we detect these actually by various strategies. Obviously NAT yield gives us the acutely infected donor because they're by definition viremic and seronegative, so they'd either be incident infections or what we call long-term immunosilent carriers. But we can also study discordancy between antibody and NAT results to detect testing errors and viral variants that might be missed by NAT assays.

So the approach that we've taken is to design a screening strategy, and this would be a collaboration not only within the REDS group but with the Red Cross and blood systems to capture the critical donations that through routine screening have characteristics of incident infections or possible variant infections. And, therefore, we're sort of focusing on the circulating strains of virus in the general population. And then the focus is to actually sequence the virus in these

particular donors.

And just a couple of slides on HIV and HCV, just to again emphasize with HIV the green here is the Clade B variant, and you can see in the U.S. and Canada and Australia that's by far the predominant, but in Africa it's a rare variant. And we're seeing progressively in many regions of the world expansion of non-B variants.

Just to illustrate how these historical repositories allow you to look at these questions, this table here is compiled data over time from studies that we've done with TSS- and CDC-funded repositories to look over time at samples from HIV-infected originally recipients and hemophiliacs and then progressively donors, and quantifying the rate of non-B infections minor variants in this country accruing over time.

An approach that we've used now in a number of studies uses the detuned or less sensitive assay. You apply this assay to HIV seropositive samples and can detect recently infected seroconverters. It's a strategy that has

been developed with CDC and is widely used now around the world to measure incidence. And the idea here is to take large numbers of donor samples from the U.S. or our collaborating international sites that are found to be seropositive and identify through testing within that group the recently infected subset, and then actually apply a full sequencing of, for example, the polymeration RT genes to understand not only the transmission of variants but also the transmission of drug-resistant virus. And this is an example of a recent study in Press and Transfusion that looks at subtypes and drug resistance in blood donors from Brazil who were identified as recently infected using what's called the STAHRS or detuned testing strategy. And you can just see here--I'm not going to go into any detail, but we can focus and compare the variant distribution in the recently infected donors, those who had evidence of recent seroconversion, serologic profiles, versus those who had longstanding infections. And we can figure out what proportions of both categories have

various subtypes or recombinant strain viruses and look at the trends in subtypes over time. And then this study also sequenced, again, the RT and polymerase gene to understand the frequency that drug-resistant virus, antiviral drug-resistant virus was actually being transmitted in the population, because, again, these are people who were not known to be infected, they gave blood, and if they're harboring a drug-resistant virus, that means they got it from someone who was treated. So the donor pool becomes a resource to monitor transmission of variants in the general population.

Hep C, you know, a similar global diversity of the genotypes there. Again, just comparing historically the TTVS donors. This is from the '70s. The recipients were 90 percent genotype 1B, a pretty dramatic shift through the '70s and '80s where early studies of NAT-positive plasma donors found 80 percent genotype 1a in the plasma donors. And then now work from Sue Stramer is showing the distribution of genotypes in the NAT-positive donors. So we have these NAT yield

donors who we can actually characterize the genotype, and now we're actually monitoring the HCV genotype in newly infected blood donors by targeting the studies to the NAT yield cases. And this is, again, data from Sue Stramer where 158 NAT yield donors detected over the last three years--or the first three years of NAT screening have been genotyped, and it allows us to, you know, really monitor that issue.

This is work from Eric Delwart, who will speak to you in a few minutes, but as an emerging agent hits us, we can immediately attack it through monitoring the molecular variants over time. So this is a phylogenetic tree of a few hundred West Nile variants, both donor samples--150 or so donor samples--and then prototype strains. Until we had donor screening, nobody had sequenced data from humans because all of the sequenced data that was available required viremic samples, and that was coming from birds and mosquitos. When humans get sick, they've cleared the virus to undetectable levels. So it's only with donor screening that for

the first time we can really monitor and classify the strain spreading, and then this is just sort of bar graph of the evolution of these variants. These are minor strain variants. They probably don't have any clinical significance, but they allow us--they're signature sequences that allow us to track the virus, and indeed we're seeing founder effects, as they're called, meaning that as the virus has moved across the country, it's clear that individual strains of West Nile really moved into new geographic regions and then expanded into predominant variants within that region. So it's kind of classic molecular epidemiology.

So, again, I think the concepts of the approach of the molecular surveillance study, the primary objective is to monitor genetically divergent variants. Actually, the focus is on HCV, HBV, and HIV. The West Nile piece we're probably not going to pursue as part of the REDS II program. And we'll be capturing donations again from both REDS programs and collaborating Red Cross and BSI. The central laboratory will sequence the

informative regions and correlate those with data coming from pathogenesis and high-risk population studies. And then also an approach that--we can actually use the correlation between NAT and serology that kicks out these incident infections to monitor also for testing errors and immunosilent infections. And this is just a schematic of sort of how the study will capture what the characteristics are of these acute or incident infections, and then how the samples will be further pedigreed and then characterized genetically for variants.

A final slide just to mention sort of an approach that we're framing out within REDS II but a collaboration we see with larger public health sector programs to respond to emerging infections. Really, I think the U.S. clearly is the engine for discovery of variants, new agents, and everybody's new agent, they'd love it to be a transfusion pathogen. That's where the money is. So you don't have to worry that we kind of don't have an effort to find these viruses and figure out if they're

transfusion pathogens. That's really going on.

The challenge is for us in the blood industry to be ready to assess whether these are important for us. And towards that end, really understanding the virology or the parasitology and having these repositories and having the collaborations to quickly develop the assays, the high-throughput NAT or serologic test to assess their prevalence in the donor pool and look at the transmission question.

One big problem I see--and, again, NAT systems are really easy. It's easy to build a TMA test or a PCR test and apply that to large numbers of donor samples. But the problem is serologic tests which often you're much more powerful at looking at transmission and prevalence with antibody-based assays than NAT test. If you have a virus like West Nile, the viremia is very transient. And a serologic test is much more effective at detecting transmission and prevalence. But the antibody test, we don't have nearly the resources or the commitment to build quick antibody

tests.

So one important point for this committee is anything that can be done to enhance the development of serologic tests to respond to emerging pathogens would be good.

The other problem that I am concerned about is that all of the NAT platforms and focus has been on cell-free pathogens. The plasma extraction methods, the high-throughput screening is all focused on HIV and Hep C. And we were lucky with West Nile virus that we had a plasma virus. If this was a cell-associated virus or some new parasite spreading in the donor pool, we could not have brought up NAT. Those systems, there is no even significant development work going on to develop whole blood or cell-derived nucleic acid amplification technology. So another big concern that, again, we're trying to work on and push the companies to collaborate on developing rapid high-throughput automated extraction for nucleic acids.

Another idea that's really taking off, in

fact, at a global level is the ability to exploit the development of these pools. We're building pools every day from literally thousands of donors. Then they're going in the trash can. And, you know, whether we shouldn't be taking advantage of having established these donor pools and saving some of them or at least having a virtual repository where whenever something becomes a concern, sites can hold those pools so that we can go back and do a quick prevalence study. So this idea of a global virtual repository.

And then finally is just having the knowledge and the collaborations to rapidly respond as these agents come up and using the historical repositories as well as possibly prospective studies to quickly respond to emerging challenges.

DR. BRECHER: Thank you, Mike. Very impressive.

Questions? Art.

DR. BRACEY: In terms of the RADAR recipient group, is there two-way reporting? For example, if there are morbidities that develop

within the recipients that are beyond the usual hepatitis-related morbidities, is there any way to report that back so that the look can begin?

DR. BUSCH: The recipients did have a symptom interview conducted at the time of the post-transfusion visit, sample. I'm not aware that any recipients--or that anyone attended, to be frank, to any recipient reports of what might have been post-transfusion illness. I think that data was captured, but I don't think that--and certainly those recipients, although this is a linked repository and they consented to potentially recontacting them in the future to further investigate findings, there isn't any ongoing active communication, nor do I believe there was, you know, kind of a purposeful "If you have a problem, give us a call back" message given to them. So it's an interesting--

DR. BRACEY: It just seems that, again, in terms of surveillance, if you're looking and you ask the question after you, in essence, get the report back from the public health entities,

another way to look would be basically to look for these morbidities in those populations.

DR. BUSCH: Yes, that's a good point.

DR. BRECHER: Merlyn?

DR. SAYERS: Thanks. Mike, what happened to those discussions not all that long ago about the stability, particularly antibody stability, in samples that have gone through a number of freeze/thaw cycles?

DR. BUSCH: Well, you know, antibodies are generally very stable to freeze/thaw. The nucleic acids and certainly infectivity are dramatically impacted, or more dramatically. I mean, anytime a company, you know, brings forward a test to FDA and anytime any of us develop pilot assays, we always do some level of stability analysis, both, you know, refrigerator/freezer and freeze/thaw cycle work.

In general, I think, you know, there's--you know, everything is dependent on the starting titre. If you barely have any detectable analyte to begin with, a few freeze/thaws or

storage issues can reduce detectability. If you're dealing with the typical high viremias or high-antibody titers, you know, the impact of freeze/thaws on most of these parameters is very small. And when we build these repositories from the get-go, we prepare sub-aliquot. So we're always thinking about maintaining, you know, pristine aliquots from the beginning, and as soon as a sample in the main repository is accessed, it's immediately sub-aliquotted, typically in the 250 microliter sub-aliquots so that they've only gone through one thaw/freeze cycle and then, you know, for the initial testing, and then as others need it in the future, they can access a fresh, you know, minimally frozen/thawed aliquot.

DR. HEATON: Mike, since many of the pathogens we're dealing with have started in other countries and, in effect, been imported to the U.S., how many of your non-U.S. REDS II centers do you envisage there will be?

DR. BUSCH: They're anticipating funding two to three.

DR. HEATON: And they each must meet the defined criteria of having repository capacity and--

DR. BUSCH: Yes, there are very specific criteria, both, you know, specimen--collection capacity, computer data capacity; importantly, also, the ability to send samples to the U.S. for further characterization and repository storage.

DR. BRECHER: Celso?

DR. BIANCO: Mike, is there a connection between REDS II and our policymaking bodies--FDA, CDC--in the sense that before the studies are completed, a lot of the data that is generated can help policymaking on a more scientific basis?

DR. BUSCH: There's a mantra in REDS, for me personally as well: If George Nemo says, "Jump," we say, "How high?" So NHLBI is in charge, and they are always listening and engaged in the discussions here, and we've never gotten anything but a green light to do analyses that can inform your guys' questions and discussion. So I think there is really a strong, controlling force that

keeps us in line, plus, you know, most of us involved are really part of the industry and I think understand and are very interested in the issue.

DR. BRECHER: Okay. Jerry?

DR. HOLMBERG: I appreciate that question from Celso about your response and your capability of responding to the various policies. What is the duration of REDS II?

DR. BUSCH: Well, we're funded for five years, so that would have been October '05 through '09. But, you know, the first REDS II program went through two non-competitive renewals, which is, you know, unusual and certainly as much as you could ever hope for. I think we'd be optimistic that there would likely be at least one renewal phase for the new REDS program.

DR. HOLMBERG: And you mentioned also that when Dr. Nemo says, "Jump," you say, "How high?" So that implies that there's a degree of flexibility to modify REDS II and be responsive to what the Government is saying--

DR. BUSCH: Absolutely. Right, and again, I think if you look back on examples, we've always put enormous focus and effort into new challenges,

and they hold back funding as well. So there's kind of core funding that's initially put into the program at each of the sites in the laboratory. And then as we develop and get approved specific protocols, there's additional funds that are allocated to those protocols. But then there's also additional reserve funds that can be, you know, just issued to support an immediate challenge.

DR. HOLMBERG: And so is it safe to assume that some of the response that you've heard from not only the government but also from the private sector in regards to TRALI has been a motivation for you to put this as part of your study?

DR. BUSCH: Yeah, for sure. Yes.

DR. BRECHER: Thank you, Mike.

We're now going to move on to some other topics, particularly prions. The first speaker is Dr. Jerry Ortolano from Pall.

Orphan Test Development

Prion Filtration

DR. ORTOLANO: Thank you. Well, I guess from the expressions on your faces, you're all foaming over that simian foamy virus or fomenting

over that simian foamy virus comment. I'll change gears right now and talk to you a little bit about the update for our prion reduction filtration technology.

This technology really embraces a slightly different approach. The separations technology is based upon physical/chemical separations considerations. It doesn't really utilize an antibody or monoclonal antibody. It doesn't have a specific ligand to the abnormal protein. But with this proprietary surface modification, we're able to remove not only normal prions but pathogenic prions as well.

As I mentioned, it reduces all prions tested, both cell- and non-cell-associated because embodied in the technology is some degree of leukocyte reduction, although the technology was

characterized originally for a previously leukoreduced blood product.

The surface modification does not impact red cell stability following storage to outdate as determined by indices of both hemo- and biocompatibility and in vivo survival, which are recent data that will be presented shortly, but not at this meeting.

Filtration is a commonly used process, and as a consequence of that, we feel as though this technology will be readily integrated into the blood processing arena.

I just want to share with you information about the rapid growth of prion research in general. Here you see this curve with a linear regression demonstrating the number of articles that have been added to Medline over the course of time. And you can see it's kind of linear. In contrast, if we look at the number of articles related to prion or TSC, it's dramatically increasing at an exponential rate.

There is reason to point that out because

our understanding of being able to develop a prion removal filtration technology really relies upon some basic facts, and some of those facts are actually missing in prion research. So they all relate to the model. Is the model an applicable one for the disease that we're trying to attenuate with respect to transfusion-transmitted vCJD? And the answers to those questions I think are still open.

Basically this is what we're dealing with. We're dealing with humans ingesting beef from infected cattle and contracting vCJD. And then there's a long latency that leaves some of the blood donors infected with prion, and they are asymptomatic, and those levels of prion are estimated to be as low as 10 infectious units per mL. So those are the things that we think are reasonably well known.

Also, unless we hear some revelations today at this meeting, we cannot yet screen for prions in blood, nor is pathogen inactivation particularly effective to date. So with respect to

the testing needs, we really have to simulate the native infection. That's what we'd like to try to do to see whether or not technology would be effective, and then demonstrate prion removal.

Okay. I'm going to try to go back a second. While Renee comes and gives me a quick hand, the next slide is going to talk to you about the technological approach that we employ. Basically the considerations are as follows: How do we get pathogenic prion into the blood?

Well, in the clinical scenario of transfusion-transmitted vCJD--I'm sorry. Is that one? Okay, great. In the setting of transfusion-transmitted vCJD, what occurs is that blood is contaminated with prions probably from the central nervous system or through the immune system. So it's a little bit different in testing. We can test by taking scrapie-infected hamster brain homogenate, for example, and just add it to blood. But the nature of that level of contamination is different. First of all, pathogenic prion concentration is extremely high,

and it has to be because some of the testing methodology that we employ is not very sensitive, like Western blot, for example.

So I'll talk to you about exogenous relating to spiking and endogenous relating to contamination of blood that occurs through the central nervous system or via the immune system and as a consequence of intracerebral administration of pathogenic prion and then waiting until the animals display infection or disease.

So these are three approaches that we employ. We can spike pathogenic prion into blood, and then we can pass the blood through a filter and take the pre-filtration sample, compare it with the post-filtration sample, and analyze that on a Western blot approach. And for those of you who may not be familiar, basically the Western blot is just a gel electrophoresis. Previously the samples were treated with proteinase K and proteinase K digests normal protein but not pathogenic protein. And then you can separate out by the electrophoretic technique the pathogenic prions

specifically. You can transfer it on to a membrane, react that membrane with antibody directed against the pathogenic prion, and then use various techniques to uncover or illuminate the pathogenic prion.

The Western blot technique originally was relatively insensitive. It was kind of a yes or no or roughly. I could get an idea of what the order of the magnitude of reduction of prion is by just looking at the gel. But more recently, Wadsworth and others, utilizing a more sophisticated precipitation technique and coupled with the use of densitometry, were able to now quantify pathogenic prion removal.

We still suffer from some of the same limitations, and that is, you have to have a reasonably high concentration to be able to measure it. So if you're going to spike blood, you have to spike with a very high concentration, and those numbers are comparable, for example, to 10

infectious units per dose--that is a 280 mL of packed red cells as an example--contrasted with

what I mentioned before, perhaps circulating concentration of pathogenic prion on the order of two infectious units per mL. So you can see they're orders of magnitude higher.

The other approach is to use an exogenous bioassay. So, again, exogenous refers to the fact that we're spiking the blood with pathogenic prion. Again, the model is scrapie-infected hamster brain homogenate. And then we either filter or not, and we take the filtrate and the pre-filtration sample and serially dilute them. We take each serial dilution and then inject that into animals, intracerebrally again, and we wait and look for disease. And in this way, we can contrast at what dose does the disease manifest the soonest time possible, or at what dose do we find the disease no longer manifests at a prescribed interval of time of observation?

And so comparing pre-filtration with post-filtration samples in this way, we can get an idea of the magnitude of prion reduction as it relates to the clinical symptom of disease.

The best model, however, is this endogenous infectivity model, and here we're trying to simulate what happens in the clinical scenario

with transfusion-transmitted vCJD. So we infect animals. We wait for the symptoms to express themselves. We take the blood from very many animals, and we pool them and then filter, or not, an aliquot. And then we inject those into animals and, again, now look for disease.

So you can see that the number of animals required to do these experiments, particularly with respect to the bioassay or endogenous infectivity assay, are considerable. They do not lend themselves to screening for pathogenic prion efficiency with respect to various types of media available. So you have to go back to the Western blot to be able to accomplish that. Only when you finally get a prototype that you think you want to use, you can then further characterize with the exogenous bioassay or the endogenous infectivity model.

I realize that was kind of a long-winded

explanation, but I think now it gives you a better idea what we're up against.

Here's an example, the exogenous spiking study with a Western blot. Here we've infected the blood with scrapie-infected hamster brain homogenates spiked into the human blood, pass it through a filter, and determine the log removal using the Western blot. This is the consequence of leukocyte reduction filtration technology alone, and this was published by Gregori in the Lancet, demonstrated that infectivity is reduced by 42 percent just with leukocyte filtration alone. So we know that there is some component of variant CJD that is transfusion-transmitted and associated with the cellular elements of blood. But there's also some that is not associated with a cellular illness.

In our final filtration design using a previously leukoreduced blood product, we look at the pre-filtration sample versus the post-filtration sample using the enhanced PTA precipitate with densitometry and basically show

oftentimes no measurable amount of prion activity or prion protein. The sensitivity of this assay approximates about three orders of magnitude.

If you look at the results of a prototype experiment in which we looked at the bioassay in hamsters, again, blood is spiked into the hamster--into hamster blood--I'm sorry. Scrapie is spiked into hamster blood, and then both the filtrate and the pre-filtration sample are serially diluted, and we look for death over time. And you can see as a result of this particular experiment, the very simple interpretation here is one to 10

9

dilution versus one to 10
5, the difference being about 4 log, the calculation used is the Karger method, and it gives us an actual level of 3.7-log reduction.

Here's an example of an infectivity study in hamsters. Again, as I mentioned before, we inject intracerebrally into the hamster. About 100 hamsters are used because they only give you about 4 mLs of blood each. And we collect about 450 mLs of blood, and then either pre-filtration or

post-filtration, subject those to groups of animals and wait to see disease.

We notice that in zero out of 38 in our most recent tally, none of the filtered product show any disease or any pathogenic prion from the brains of those animals. And prior to filtration, you can see that six out of 43 hamsters were infected. That p value is significant with a Fisher exact test.

So, in summary, the exogenous Western blot, our prototype had limited dynamic range. There was no faciltun- (?) acid precipitation employed and no densitometry. And basically what we showed was, yes, we can get reduction, but we just didn't know exactly how much.

Our final design utilized the enhanced Western blot with densitometry, and the data are currently under review and should be available soon.

Our prototype in the exogenous bioassay gave us 3.7 log, and we have ongoing studies right now which should be available to us in the spring

of '06, which would characterize this further. And the endogenous infectivity assay, as I mentioned, we had zero of 35 versus six of 43 in the controls. And the final design, again, is ongoing, and that, too, should be available to us in the spring of '06.

Some additional studies I think are noteworthy. Here's an example of an endogenous study using the Western blot. So here we inject animals intracerebrally. They become infected. We take the infected blood, pool it, and then we centrifuge it, pass the blood product packed cells through a filter, collect the filtrate. And this is in one example what we show.

Remember I mentioned that when an animal is infected via either the immune system or the brain, that type of infectivity results in a very low bio burden, and that's exactly what you see here. Before filtration, there is a very low bio burden. So the significance of demonstrating post-filtration absence of pathogenic prion is really questionable. I mean, we might only be

removing one log here. But that's not the point of this slide. The point of this slide is to show that when we recover the material off of the filter, we actually can concentrate this 500-fold and demonstrate there's considerable protein present in this sample.

With respect to product safety, we've done a variety of safety studies that are required. The release of this product in Europe is imminent, so we were obligated to do all of the testing, the safety testing.

And, in summary, this prototype filter shows that we reduce by 3.7 log in a bioassay, that the quantitative enhanced Western blot with the densitometry data, which is currently under review, is not too dissimilar to previous findings, but it does give us now a number.

The quality of the blood cells is unaffected by prion reduction filtration. Safety studies show no cause for concern, and we have also concluded 24-hour single and double isotope red cell survival studies which are unaffected by

filtration utilizing this technology. And there's one other response measure that we looked at, which was residual white blood cells, and further reduced by filtration to levels that are less than one times 10 5, with a 95-percent confidence that this will occur 98 percent of the time. And that's significantly lower than the current standard for leukoreduced blood.

I'd be happy to entertain any questions you might have at this time, and thank you for the opportunity to present this.

DR. BRECHER: Celso?

DR. BIANCO: Jerry, thank you very much. Do platelets survive the filter?

DR. ORTOLANO: We don't have a filter that's designed for platelet products. This is only for packed red cells. That's not to say we won't have it and we're not working on it.

DR. BIANCO: And the other question is: Is 3.7 logs enough?

DR. ORTOLANO: You know, I would defer that to the experts. Dr. Rohwer is going to come

up here. I'm sure that's the same question you could ask him, and he's far more qualified than I. But what I can tell you, Celso, is that from the people that we've spoken to, the experts appear to agree on this one point: that if you had to pick a number, somewhere between 3 and 4 log is a reasonable number to pick.

DR. BIANCO: Is there an attempt to do experiments in a large animal, like the sheep, where transmission by transfusion has been documented for scrapie?

DR. ORTOLANO: Well, again, we think that the hamster model is applicable. We don't see any reason to go to a large-animal model. I personally don't see any reason to do it.

We're not in the situation where it's going to bring us any closer to the clinical setting than using hamsters. And the hamster, the value of the hamster is that they're relatively inexpensive, you can do a lot of them, and that is what you need, you need big sample sizes to be able to do these infectivity studies. So I don't think

we're moving to sheep--unless Jay wants us to, in which case we may.

DR. BRECHER: What is the red cell loss going through the secondary filter?

DR. ORTOLANO: We recover about 40 grams, so it's--we do have some loss. It's about 80-percent recovery. So you have to realize that you have some loss associated with leuko-filtration alone, and that's a prerequisite to the use of this technology. So some adjustment in the standards in Europe is being considered right now, because they do have a standard, and we would probably have to do the same thing here.

But I think in the U.S., as far as we're concerned, we're listed under orphan test development, so we don't think you guys think it's all that important right now. And we are working on technology improvements so that we can deal with a non-leukoreduced product, so that we can deal with a platelet product, and hopefully in the process also improve our recoveries.

But right now I think the need is more

imminent in the U.K. and certain other European countries, and I'm really looking for some technology that could help them.

DR. BRECHER: Merlyn?

DR. SAYERS: Jerry, do you think they will lift the restriction on donors in the U.K. with a previous history of transfusion if they introduce filtration?

DR. ORTOLANO: That's a very good question, Merlyn. I honestly don't know the answer to that. Some people feel as though donor deferral--and this is hearsay, I admit, but I have heard it. But some people feel that the extent of donor deferral might be impacting the blood donor pool by as much as 10 percent. I've heard other figures down to 3 percent and some even less than 1. But if it's as high as 10 percent, in places like the U.K. that may become a necessary thing.

Now, we're never going to have, I think, the data that we need to prove that we can prevent the transmission of disease before many, many years elapse. But I think people have made decisions

based on the precautionary principle before, and if the blood is limiting, that may become part of the precautionary principle. We need blood. We have to get it. We have at least the technology that we think works, so let's not defer those donors.

DR. BRECHER: Harvey Alter has a question.

DR. ALTER: Thank you. Very interesting data. I was intrigued that in the unfiltered material, only six out of 40 or so hamsters came down. Was that because they weren't followed long enough? Would all of them have come down ultimately?

DR. ORTOLANO: I think so. We cut it off at 250 days.

DR. ALTER: Okay. There's no other susceptibility factors?

DR. ORTOLANO: None that I'm aware of.

DR. BRECHER: If there are no other questions--

DR. ORTOLANO: I think I kept you on time, Mark.

DR. BRECHER: Okay. Our next speaker is

Dr. Robert Rowher from the VA in Baltimore and the University of Maryland.

Removal of TSE Infectivity from Blood and Blood Products by Absorption

DR. ROWHER: Thank you for the opportunity to bring you up-to-date on a singular effort to develop a removal device for the non-cell-associated infectivity associated with blood. This effort is being conducted by PRDT, Pathogen Removal and Diagnostics Technology. This is a company that I helped found along with Dave Hammond and Ruben Carbonnel. Dave is a combinatorial chemist and biochemist, currently at the American Red Cross, head of their plasma research program. Ruben Carbonnel is the director of the Keenan Engineering Center at North Carolina State. He's also a combinatorial chemist. And I bring the TOC expertise to this project.

We founded this company because I had a very strong opinion that removal was probably the only thing that was going to work in terms of mitigating the risk of transmission of diseases by

blood and this was the best way to get this job done. We attracted the American Red Cross and Prometic (ph) Corporation to the effort, and it has become a joint venture of those two companies. They are the ones who are putting up the money for this. And we have recently attracted MacoPharma as our manufacturing and marketing partner in this effort.

By way of background, it was my laboratory that developed the method of limiting dilution titration which enables the precise and sensitive measurement of these very low titers of TSE infectivity in blood. And over the last eight years, we have made a number of these measurements now, and they are summarized here, which this represents about $2.5 million worth and thousands of animals' worth of data. And we are getting mean and median values right around ten infectious doses per mL during the clinical period of disease in the hamster model of the disease. This is a very small amount of infectivity compared to the infectivity in the brain of the animal at this same stage of

disease; indicated here, it is about ten billion infectious doses per gram of brain at the same stage of disease. So we're looking at a very, very small titer. And it might seem insignificant and it had seemed insignificant to the TSE field for years and years until variant CJD raised its head. And there seems to be (?) -nous involvement in that disease, and suddenly it became an issue for us. And even though it is a very low titer, when you consider that we use blood on the unit level, not on the per mL level, it adds up. So a 450-mL unit has 3 to 4 logs of infectivity in it. That's plenty to transmit this disease by the IV route.

The other important datum which I'll share from my laboratory--I can't give you the whole background of our blood studies at this time because we don't have enough time for that here--is that we've done a number of studies over the last eight years looking at the distribution of infectivity in blood, and the most important conclusion from that is that the infectivity seems to be only intrinsically associated with white

blood cells in plasma, and it's about equally distributed between those two compartments.

We have recently done an experiment--it's not quite completed--indicating that the infectivity that's associated with white blood cells is also fairly easy to wash off. And that indicates that the contact is reasonably superficial and that actually the plasma compartment is probably the most important one in terms of where the infectivity resides or with what it is intrinsically associated.

So that brings us to the question of how do we control or rid ourselves of a risk presented by these agents in blood. And if we go through the triad of classical defenses here, sourcing and deferrals, we all know that deferral has been a very expensive measure, and it's also a moving target. We now know that we've been exposed, to some extent, to indigenous BSE in North America, and presumably we may have--from that indigenous exposure, we might have cases, which undermines sort of the basic premise of the geographical

deferral policy in a way.

Screening is something we're all working on in this field. I don't know a laboratory that isn't trying to develop a diagnostic test. But my own opinion is that it's technically problematical. At ten infectious doses per mL in the clinical state of the disease, we're at the limit of detection for a lot of the conventional NAT testing, for example, and this is--we don't have the option of NAT testing in this disease. We are talking about trying to pick out an abnormal form of a protein which exists in a 100,000-fold excess in a normal form in blood for a blood-based assay based on the prion protein for example. It's a very, very difficult and technical problem chemically.

Inactivation, these agents are particularly robust, and we have these issues of risk substitution versus risk reduction that we see with the Cerus (ph) product and the Vytex (ph) product in the last couple of years.

That leaves us with this removal option.

This seems--has seemed to me, at least--to be something that's accessible and fairly low risk. And it also may have some very distinct advantages over the other methods. The thing that attracts me most is the idea that by removing the infectivity, you can actually access infectivity that might not be detectable by diagnostics. This is clearly a case for the clinical disease in blood, but it's also applicable to the preclinical disease even for brain. At some point in the infection you will get to a point--and we're talking about cattle now more than people because we're not going to do these kind of tests on people. But you get to a point where you reach the limit of detection of your assay and you are not going to be able to detect it, but you might be able to remove it if you have something with sufficient avidity for the protein.

A big problem in the diagnostic area is discriminating the abnormal form of the protein from the normal form of the protein, especially at low titer where the normal form can be in huge excess abundance. This isn't really necessary for

a removal device, and it's not even necessarily desirable. We intentionally selected ligands in this study that removed both because we want to have a way of assaying the effectiveness of the filter even when we're not removing prions. And this becomes our--the normal form of the protein can then be the surrogate marker for the infected form in our quality assurance programs related to this. And it can be more comprehensive than a diagnostic, and last but not least, it may actually be less costly than some diagnostics to go this route.

So now I'm going to tell you a bit about combinatorial chemistry. Basically this is a method where you take millions of compounds and screen them with an appropriate screening test to see if you can find a chemical structure that will bind the material of interest to you. In this case, it's the prion protein. There's really no theoretical limit to how large these libraries can be, and, in fact, the libraries that we constructed are larger than we can screen, and so we are

screening a subset of them, actually.

You want a lot of diversity. We had several different types of libraries that we screened, including a large collection of just natural commonly available materials. And the important thing in all of this screening is to devise a screening method that actually finds what you want. And in our particular case, it was very important to do this screening in the presence of plasma because in the absence of plasma we would have found thousands of more compounds than we found in the presence of plasma. Plasma is a very tough cookie to work with.

So here's our basic scheme, you know, library that had the potential for 64 million combinations. In the end we screened about 8 million of those using a bead blot type method. I don't have the time here--it's complicated, and I don't think I can get this into a short presentation. But basically this was our primary screen using brain-derived infectivity spiked into plasma or blood or red cells. We tried a number of

different combinations to find ligands that would light up the beads with a specific association with the prion protein. You then take the beads that you find, put them through the mass spectrometer, find out what the compound was, and then you can resynthesize that on a large basis, go into your secondary screening method, and then do a series of Western blot tests that are more focused to find out which one of these things work the best, and then triage that into a tertiary screen where you started working with spiked infectivity as well as Western blots, finally getting it down to seven candidates, which we screened in an infectivity assay I'll show you in a moment, and then finally to three, two of which we have now tested with our under test using endogenous infectivity. And I'll get to the issues involved there here.

So we use protein assays at this stage, infectivity assays at this stage of the screening, and we're now working in this range right here on a single-bead compound.

This is an example of the kind of data

that we generate. The experiments are done in duplicate. These are Western blots that Dr. Ortolano just told you about, but this is an undigested sample, this is a digested sample, this is brain-derived infectivity spiked into buffer. And the proteinase K digestion cannot digest the infected form of the protein; it just shifts its molecular weight. And this is the assay for the presence of the infected form.

You'll notice that with this Resin 1, the binding works both in plasma and in buffer. This is plasma here, but here we have an example of one that works very well in buffer but does not work at all in plasma. And, of course, we hadn't perceived this. This doesn't mean that this ligand couldn't be useful in some other application, but it's not going to be useful in this application.

Here is a second example using 25 percent human serum albumin. We have a resin that works very well in HSA and buffer, but here's an example of another one that doesn't work as well.

So when you get to the screening step, the

test to actually test the efficacy of this--and I guess I should preface this by saying that in our opinion, at least, the prion hypothesis has never been formally proven. We're getting closer to that, but there is no consensus yet out there in the TSE field itself that this has happened. And as a consequence, to be absolutely confident that what we're getting is relevant, we have to move from the Western blot into an infectivity assay.

The ideal assay would be, of course, to use variant CJD blood from a clinical patient and pass it through one of these devices at full scale, very similar to the leukoreduction experiment that we published in Lancet earlier this year--or late last year, I guess it was. And then if we had a way of actually testing this in a mouse model so we could get quantitative data out of it, we'd be able to do this type of measurement.

Unfortunately, we didn't have a good transgenic mouse for variant CJD. It has been surprisingly refractory to this type of application, and in any case, there is no transgenic

that's been proven to be sensitive enough to detect the infectivity in blood, period. Also, it is very hard to get a whole of blood from variant CJD patients, and short of a disaster, I don't think that is going to happen. So, as a consequence, we do have to turn back to our rodent models, and once you do that, it becomes rather arbitrary which one you pick, and so we picked the one that we have characterized so well, and that's our hamster model.

Our first tests are using brain-derived infectivity spiked into blood, red blood cell concentrates, because that is our primary target, for this device, and we're using an incubation time measurement, and I will explain how that works in a moment. And then our secondary tests will be based on endogenous infectivity in blood, and as Dr. Ortolano explained a moment ago, here we can work with millions of infectious doses. Here we are limited by nature to working with only ten infectious doses per mL.

So this is the most relevant test we can

do. This is the most sensitive test we can do in terms of demonstrating large amounts of removal. So we're doing both.

In doing these spiking experiments, we have to begin with leukoreduced red blood cell concentrates because we're not claiming to be able to remove cell-associated infectivity. That's being removed by the leukoreduction itself. We're after what's in the plasma.

We're using a hamster scrapie brain-derived spike that's been highly dispersed. This took a lot of development work, but it's important to show that you're not removing infectivity that would just be mechanically removed anyway. And we're using a large uniform pool distributed to lots of devices, and we're testing them all at once and looking for dramatic reductions.

This is the way the test works. First, we have to establish the standard curve. We do this by serially diluting the spike into plasma and then inoculating each dilution into hamsters. Each dot

on this curve represents a sick animal. The triangles are means and these are two replicate determinations. And this gives us an incubation time curve. This is days post-inoculation here versus dilution on this side over here.

Now, if we take a test group and do the same thing with it using the infectivity that's been collected after it's passed through the reduction device, we'll get another set of data here. The challenge was at this level, the 10

-3

level. It's been displaced over here, which means it actually belongs on the curve down here. If you just divide this by this, you end up with 4.33-log removal here.

We've done this--the set-up for the experiment is done this way. We actually were using four units of blood through a removal device. We're testing the device itself for the accumulation of the PrP signal, which we do by the Western blot, and the bioassay is done on the filtrate down here. And that's partly because we're beyond the sensitivity of the Western blot.

We could no longer detect that level of signal.

So here are a bunch of different resins. They're all at the 10^{-3} dilution. All of these you can see actually belong on this line. They're just spread out here so you can see the data. Here's the resin I just showed you. Here's another one, for example, and here is the least effective one here at 3.69. And this is how they line up on the curve.

If we plot this as a histogram, we get something like this, and this data, this incubation type data is probably not good enough to distinguish between these four top candidates. They're pretty much equivalent.

But it's important to remember that what we're doing here is we're challenging a much higher level than we're expecting the actual target to be in blood, ten infectious doses per mL versus a challenge of 10^{6} infectious doses per mL. And we're seeing removal at about five times 10^{4}.

Well, this is a very high level of removal, but one part per 50,000, nevertheless,

escapes, and it escapes even if we pass it multiply through these devices. So there's some part of the infectivity which is not seen by our ligand. Nevertheless, if blood infectivity is in the same proportion as the infectivity in this brain-derived spike, we still have a 4-log margin of safety. But if, for example, the bloodborne infectivity is enriched in this fraction that doesn't see the ligand, it would affect the effectiveness of the device. And this is why it's important to move to the endogenous infectivity experiment. Even though there is very little infectivity there, we need to demonstrate as a proof of principle and as a validation of relevance that it can actually remove the real thing.

So this experiment is underway. The samples we have on titration is the whole blood we started with, the leukoreduced blood, so we are repeating our earlier leukoreduction experiment. We have five resin samples that are being tested and one control. There are 800 animals involved in this. The experiment takes 550 days, and we're

about halfway through it. And all I can is it's working so far, but we really don't know until we're at the end.

So where are we in terms of the development of the product? I've just told you where we are scientifically. This is the format it will actually take as a device. The lead ligand is immobilized on a resin support. The resin is sandwiched between two membranes in a technology that has been developed at the Non-(?) Center at North Carolina State. The membranes are integrated and placed in a housing, and then the blood is passed over this filter to remove the prions.

Our partner in this has been MacoPharma, and they're experts in the construction of these types of devices, and they've been heavily involved in this end-stage development.

The characteristics, we have extremely high affinity, about 10
9 kD--I don't know how that ended up at kA there--10-9, I guess, kA, 10-9 kD. And this is a very high affinity constant for a small ligand, and we think that this has to do with

the cooperativity either at the level of the resin or at the level of the molecule. The membrane we're using has been defined. We have shown that we can remove the prions from rodent brains in both mouse and hamster, sporadic CJD natural cases and variant CJD human brains. We have shown that the product works with red blood cell concentrates, whole blood, and plasma, and we're getting about 4 logs of removal with brain-derived infectivity. And the Red Cross has conducted a very large panel of studies for compatibility with blood and blood products, and we see no problems with this.

The scale-up of the manufacturer is ongoing. We're expecting CE market in late 2005, and both the United Kingdom and the Irish Blood Services are currently evaluating the technology.

And I'm going to finish with just one other note here, which bears on this same development, and that is, we are interested in diagnostics, and, of course, these devices which are removing infectivity from blood are also concentrating the infectivity from blood. And my

own feeling is that if a blood-based assay is going to work, it is going to depend on this type of concentration. We're going to have to have the infectivity from a unit of blood in order to have a hope of even seeing it. And so this is a concurrent objective, and I had one last slide here, but we may just skip that if we are--I guess I can go to--here it is. And it's essential for a blood-based assay, and a concentration device like this could be a generic front end for practically any assay you can think of, if you can get the concentration high enough. Or we can develop novel assays based on the device itself, and I'll end there. Thanks.

DR. BRECHER: Thank you, Dr. Rohwer. I'll ask the first question.

It's very interesting that you pointed out that the prion is very loosely associated with the white cells. Presumably that would also be true for the red cells.

DR. ROWHER: We have about a 1,200-animal study sponsored by NIH, NHLBI. As a matter of

fact, NINDS also participated in this. We're looking at the distribution of blood in various components. And we've had poor luck working on just particular white blood cell types. As we purify them, we lose the infectivity, and so we have had--the evidence has been building over the years that there is something going on here. So we just focused this experiment on just those generic questions: Is there an intrinsic association with platelets, with red blood cells, with white blood cells? And though there's still about 120 days left on this experiment, it's developed far enough that the message is becoming quite clear that there is no intrinsic association with platelets, there is no intrinsic association with red blood cells. There is, again, about half the infectivity associated with white blood cells, but as part of this assay, part of this test, we washed the white blood cells just by centrifugation and PBS, you know, no surfactants, nothing like that. And we, nevertheless, washed 80 percent of the infectivity out of those fractions while losing about 8 percent

of the cells, I think, in those washes. So it's coming off.

DR. BRECHER: So the follow-up question would be: Could we simply wash our red cells with a liter or two of saline and knock down the infectivity a couple logs?

DR. ROWHER: I think that is a possibility, a definite possibility, but it's something that would have to be developed and validated. And the reason for that is it is actually hard--it is hard to get to this point with red cells. It's hard to get highly purified red cells because they pellet and you have this residual contamination by white blood cells, even after leukoreduction to some extent. And it would require endpoint dilution titrations and probably a couple of years of development to convince yourself that you had the technology appropriately applied to accomplish that goal. But I think it is a definite possibility.

DR. BRACEY: Could you describe some of the characteristics of the filtration? For

example, how long would it take? Just looking at sort of practical issues.

DR. ROWHER: Dave Hammond has been associated with the blood industry for his whole career. He's currently the Director of Plasma Research at the American Red Cross, and the American Red Cross is our partner in this. And we're not going to miss those issues.

Basically this thing has been designed from the very beginning with usability in mind. We've also consulted with the National Blood Service in the U.K. from the beginning to know, you know, just what kind of device would you want if we could produce something like this. And what they want is something that's dockable, that can be used with multiple different options in terms of leukoreduction front ends, and so basically that's what the first device will be.

Certainly, MacoPharma has other ideas about where they want to take this ultimately, and they probably will. But the first product will be quite flexible in that regard, and it fits into the

normal--the whole idea is to have it fit in within the normal parameters for leukoreduction so that it can be done in line with leukoreduction.

DR. BRECHER: Celso?

DR. BIANCO: First, just regarding a comment by Dr. Brecher, I recall that Vytex had done some--when they were working with their pathogen inactivation, had done some washing experiments, and they showed a 2-log reduction at that time. I don't remember how they did it.

DR. ROWHER: That is correct, because they had to wash the inactivant out of the blood. They had a very exhaustive washing. In fact, I suggested that to them.

DR. BIANCO: Thank you. But you showed as a slide with a unit a blood, ten infectious doses per mL having 3 to 4 logs. Actually, most of our units now are not 450 mL. They are 500 mL. So add 500 units there--infectious doses.

The filters--and I'm talking about both, as the question I asked Dr. Ortolano, reduce about 4, 5, 4.5, 4.3?

DR. ROWHER: That's on a per mL basis.

DR. BIANCO: Oh, that's a per mL--

DR. ROWHER: Yes, we're comparing

milliliters to milliliters.

DR. BIANCO: So you're adding 2 logs to that?

DR. ROWHER: Yes.

DR. BIANCO: Okay. And platelets survive?

DR. ROWHER: Platelets survive--the resin itself does not activate platelets. Whether they would be activated during passage and that sort of thing, we haven't actually--actually, those tests may have been done by the Red Cross. I don't know for sure.

DR. BIANCO: The question is that many of those filters, platelets stick to them.

DR. ROWHER: They don't stick--

DR. BIANCO: They don't--

DR. ROWHER: They don't stick.

DR. BRECHER: Jay?

DR. ROWHER: But, Celso, I can't say that they won't stick to the MacoPharma matrix that

we're putting the resin in. They may. You know, I don't know that much about that. Sorry I can't answer that.

DR. EPSTEIN: Bob, thank you for this very illuminating presentation. My question concerns the sensitivity of the readout assay in the endogenous experiment. In essence, I'm going to ask the same question that Celso asked of Dr. Ortolano. Can you just state what the limit of detection is in the final readout? I think you suggested 20 infectious units per milliliter. Because then what we really have here is an extrapolation from two experiments.

DR. ROWHER: You are taking the data off the--that was off the incubation time measurement. The limit of detection for the limiting dilution titration is one infection out of 100 animals inoculated and that's 5 mLs at 50 microliters per animal. So that's a limit of detect of 0.2 infectious doses per mL.

DR. EPSTEIN: Right. But the problem that I see is if you take that 0.2 and you multiply it

by the volume of the unit filtrate, in theory you have not ruled out an infectious dose.

DR. ROWHER: Absolutely.

DR. EPSTEIN: So the efficacy, if you will, is then a speculation that the clearance level in the spiking experiment applies in the endogenous situation. That's the very thing that we don't know. And I think that's what led Celso to query about an experiment in a large animal, either because you can use a larger inoculant or because you can do an actual in vivo challenge that more mimics the human situation.

So I am just--I understand that these are limitations we are living with now, but I just would like your comment about the limit of sensitivity of detection of clearance.

DR. ROWHER: Sure, sure. This is a technical limitation that we face right now. It's actually an economic limitation. We could always inoculate more animals. But each one of these titrations costs about, you know, $75,000, or something like that, to do. As a consequence, that

adds up. But it's not to say we couldn't do it.

On the other hand, we are working in the lab on ways of getting around this, other ways of getting around that. I don't really want to discuss that right now, but what I can tell you is that we are working with Fiona Houston, and we have our own sheep flock of infected scrapie animals, and we do a lot of work in the laboratory with infected sheep blood simply because we can get it in large volumes. And we feel like volume is an issue here, and it's an issue both for diagnostic development and for these removal studies.

The plan is that when we get a device that is fully configured and working and we're convinced that we've done what we can in the hamster model, we would put that technology to the sheep, and we will try that type of experiment in the sheep. And the Institute for Animal Health is, you know, on board for doing that.

The problem with it is that the sheep is not-- (?) had spectacular results with those studies, but it's not a well-defined model like the

hamster. It's not as predictable. And we don't know how long we have to wait, you know, for a negative to be a true negative. And we just don't have enough statistical data on that model to know what we're actually dealing with.

The intent is also to do leukoreduction experiments with sheep, and they're doing those. That's a bit of a problem because sheep blood has some significant differences from human blood, much more significant differences than hamster blood, by the way, in my opinion. The erythrocytes in particular are extremely small, and they behave somewhat differently as a consequence of that. But, nevertheless, it's probably the only model out there that would provide a direct test like this, and we fully intend to do it.

DR. BRECHER: We need to move on. We're falling further behind, so, thank you, Dr. Rowher.

Our next speaker is Dr. Alan Rudolph from Adlyfe, Inc.

PrP-Sc detection in blood

DR. RUDOLPH: Well, thank you to the

committee for allowing an opportunity to present our technology, and I'll certainly try to stick to the time that we were given.

The previous speakers have introduced the issue, and I would like to get to that in a moment, but with a brief commercial message up front about Adlyfe. We're a new company founded a few years ago, and we're in Rockville, Maryland, in the old Human Genome Sciences space. We're a small company, but we were actually started by the Department of Defense, by DARPA, to really explore new technologies and revolutionary approaches to the safety, testing, and availability of blood. And certainly the military has a long history of important blood safety efforts. And I'd like to tell you today about our particular approach to this problem in diagnostics for misfolded proteins.

The previous speakers have introduced it, but perhaps just to emphasize the point about protein folding, and what we are really talking about here is a shape change in proteins that we're trying to detect. And this problem is inherent to

a number of neurodegenerative diseases, CJD being one of them, but many of the companies like ours who are approaching the misfolded disease problem of normal proteins in alpha-helical form going to largely beta sheet confimers, aggregating, forming fibrils, plaques that deposit in brain tissue--this is a process common to a number of neurodegenerative diseases, and companies like ours and the company you will hear next from have in mind that CJD is an important first target of technologies that can measure this mechanism, but there are neurodegenerative diseases, as a-beta folding and amyloidogenesis associated with Alzheimer's, Parkinson's, and Huntington's, a number of neurodegenerative diseases in which this misfolding process is important.

As you have heard previous speakers tell you, one of the problems in detecting this process, especially in blood, is that these aggregates are in very low concentration, and the ability that we have with diagnostic technologies to look at shape change are quite limited because, after all,

antibodies are looking for specific molecular recognition sites which may not be involved in these tertiary conformational changes.

What you've heard from the previous speakers is that this looms as a great challenge for a diagnostic technology. Most of the conventional diagnostics you have heard from--the ELISAs, the Western blots--are in the picomolar range, and evidence and data from Paul Brown's work largely suggested that the infective unit, roughly in the femtomolar range, is anywhere from 50,000 to 200,000 misfolded prion molecules. And this presents a real challenge for the current diagnostic technologies to measure down in that range.

What I'd like to do is introduce to you a new technology which, when DARPA saw it three years ago, because of its revolutionary sort of approach to this problem of shape change, decided to look at investing in ways to get into this technology range of detection.

So a number of challenges you've heard

before are certainly that these are very low in concentration; it's currently unknown in the etiology of disease what is the concentration in blood as a function of time course of disease; and, in addition, the folded state of these proteins in early-stage disease is also unknown because only until very recently could we enrich them. So even understanding the physical-chemical characterization of these aggregates, which is a key component of being able to detect something, is now just being elucidated.

So our approach to this problem is somewhat similar to what you heard earlier, although the combinatorial approach for producing peptides, which was previously described, was not rational design on the sequence of the prion molecule. And, in fact, the prion molecule sequence is highly conserved between hamster, mouse, deer, sheep, elk, and human, and cattle, in cow. And so it was Dr. Cindy Orser, right around 2000, who made a rationally designed small peptide that mirrors a particular region of the protein and

undergoes this conformational change. And so the technology I'll introduce to you is a fundamentally different way of measuring protein folding in blood, and it actually mimics the disease process in the following way: These sequenced matched peptides are created with small fluorophors on their ends. When they see a seeded misfolded protein target, this protein peptide mimic, which we can synthesize, folds because it sees something that is folded and likes and aggregates to, and that folding process brings two fluorophors in close apposition, and you get a fluorescence change or an eximer formation. So it's a conformational detection that's based on fluorescence, that is seeded by the presence of a misfolded protein target, and mimics the folding disease itself in the detection scheme.

That is the basis of the transduction of our test. In the next slide, you see what is essentially the amplification as well. This is just a fluorescence spectra showing these peptide ligands. They're 33 amino acids in length. Dr.

Rowher's peptides are much smaller and are not sequenced matched to the protein per se. These are open confimers in the open form of this peptide ligand that we create, and when it sees the target, it closes and shifts this fluorescence, so you get a green to red fluorescence shift. And we can just measure this eximer formation pretty simply. That's the transduction.

In the bottom panel, you see a series of cartoons that essentially go right to left and then right to left again. And what you're seeing is essentially the mechanism that we're proposing for the amplification that gives us sensitivity to detect directly misfolded protein targets in blood. And that is, one of these targets, our ligands, sees one of this misfolded protein seeds, it undergoes a folding change. That thermodynamic event, because that is a lower energy state, a preferred confirmation for these proteins, induces other added proteins in the mixture, whether it's a reaction well or a test tube, to fold themselves. And this thermodynamic process proceeds, and as

these ligands fold, they amplify the signal which gives us the sensitivity to detect misfolded protein directly in blood.

So it's a very elegant concept, quite new in the context of diagnostics, that really mimics protein folding and allows both the transduction and amplification of a signal based on the introduction of a seed. And I think the easiest way to sort of analogize this is almost like super-cooled water, because this is a thermodynamic type of event, where the seed--in this case, if you think about an ice crystal in super-cooled water--will induce the whole reaction to go to ice. In the same way, these misfolded proteins are the preferred conformational state, the solid state, if you will, and the seed of these targets with our added ligands induces the whole reaction mixture to thermodynamically go into a folded state and be read out easily in diagnostic equipment.

To contrast this approach with the current gold standards--ELISA and Western--you certainly heard a lot from the previous speakers about their

sensitivity. One of the other features in the diagnostic challenge is that all of the antibody-based tests, which are all now postmortem tests for disease, mostly implemented in cattle and sheet, require proteinase K treatment. That is, the recognition site, the epitope for these antibodies, does not distinguish between the normal and misfolded form, so you're largely looking at a differential state.

In addition, that protein digestion step removes early stages of what we think are aggregates and infectivity. So our assay does not involve any proteinase K. It measures directly the misfolded form. I'll show you, of course, antemortem data in a moment about the test and its screening abilities in clinical states. We're currently moving into preclinical states.

The time factor is critical for those certainly in the cattle industry about holding valuable products, certainly in the blood products screening industry as well. This is a fairly simple test. That reaction time that I showed you

in that thermodynamic reaction mixture takes about an hour to proceed. So the amplification is quite rapid to a sensitive measurement.

Some historical data. We have about two and a half years of experience with this test just to show, again, the pre-symptomatic detection, in this case in the hamster model, which you have heard from before. This is not a relevant model in the context of blood screening because these hamster models are inoculated through the brain, in this case with a scrapie strain. And you can follow the time course of this disease over ten weeks when the animals become ataxic, showing symptoms at around nine weeks. And what you can see in our case is a fairly dramatic statistically significant detection of disease now in the brain in hamsters at three weeks; whereas, the ELISAs and Westerns don't detect disease until ten weeks.

There are two studies here. Both red-hatched and red solid are the infected models, and there's an increase in the infectivity as you cycle this through these hamster models. So it's

our first evidence; this data is just now coming out into press, in the peer-reviewed press, that our test is a very sensitive asymptomatic/pre-symptomatic detection, in this case in clinical cases.

We have been very active in sheep. The questions about large-animal models we do believe are relevant and important in the context of proving any test for CJD. And we have been active in sheep through the Ames, Iowa, and Pullman, Washington, USDA facilities that have herds and flocks of animals that are infected. And just as the previous speaker told you, if you look at now sheep blood--these are clinical cases of sheep scrapie--you have a higher detection, in this case the red bars, in leukocytes in our assay; serum, again, statistically significant; and plasma. The Y axis you are looking at is really measuring the ratio of these closed ligands to open ligands. So we can either measure it as a ratio, or we can measure the increases eximer formation as our ligands fold and detect misfolded protein in blood.

Most recently, we have begun detection of sporadic CJD samples. This is some recent data we have collected in five known clinical cases in red

of sporadic CJD. This is human plasma from sporadic CJD known clinical patients on autopsy confirmed by Western blot and immunohistochemistry.

We are now marching back in time with some sporadic CJD samples I will tell you about in a moment in a non-human primate study to answer the question during the time course of disease when can we detect sporadic CJD in human plasma, and we'll be soon testing the same question in variant CJD plasma in Europe in the early summer.

This is a compilation of our experience over some two years, which is mostly in clinical cases and shows detection of diseased or healthy across sheep, bovine, human--this is now the samples I showed you and increasing--as well as experimental infection in hamster and most recently in monkey and mouse. And what I want you to see is that at least in the case of clinically known samples, the fidelity of this test is quite good.

We had one miss in the bovine case. These are all blood samples, plasma or serum samples, with the exception of the hamster brain data that I did show you. And most recently, in a study that was launched by NIH and Baxter some years ago, we were getting access to samples from non-human primates that were inoculated with sCJD in the brain, and we're now evaluating those blood samples from the terminal bleeds and marching back in the preclinical bleeds. But it's our first example of some coded sampling that we're doing. Another important step in the diagnostic development of the test is certainly to move into blinded, double-randomized sampling. And our first terminal bleeds of these animals, again, we hit every one in terms of both positive detection in blood as well as detecting negatives. And we're just starting down to look at the pre-terminal bleeds just post-inoculating to answer that very important question of when during the time course with our test can we detect.

Our current estimates of the threshold are

3 femtomolar, which is right around the single-digit infective unit range. We're just now also doing titered samples of a mouse model. There is a humanized GSS mouse model that we have access to in which the known infective units have been titered through bioassay. And in that particular set of samples, our evidence indicates that we have sub one unit infective unit detection using our test, and we'll be presenting that data very shortly.

We are also very aware that a high-throughput kit is really what this community needs. The test that I've described to you is performed in a multi-well format, high throughput with standardized diagnostic technology equipment found in most diagnostic clinical laboratories. It's a fairly simple test. It doesn't require a lot of pre-handling of the sample. There is some optimization, about a 20-minute phase, to reduce background. As the previous speaker said, this is a challenging problem to pick out 50,000 molecules or less in a sea of protein.

And so with that I will end and answer any questions you might have. Thank you.

DR. BRECHER: Thank you.

Comments or questions? Celso?

DR. BIANCO: First, what does MPD stand for?

DR. RUDOLPH: Misfolded protein detection.

DR. BIANCO: Oh, okay. You don't have any--in the hamster you had some studies of brain homogenates. Have you tried to do blood--

DR. RUDOLPH: Yes, in hamster we also saw detection in plasma at six weeks, and that data will be published. I just didn't include it in this data set.

DR. HEATON: Your blood samples that you reported the detection of human samples, were they human blood spiked with human brain, or was it actually--

DR. RUDOLPH: No, these were bona fide plasma samples from clinical patients in the archives that currently exist. These were samples Paul Brown from the NIH got for us through the

American Red Cross.

DR. HEATON: So they were U.K. disease cases.

DR. RUDOLPH: Yes. Actually, no, I'm sorry. Sporadic CJD they were U.S. cases.

DR. BRECHER: Okay. If there are no other comments, thank you.

We'll go on to our last speaker of this prion section, who is Dr. Stuart Wilson from Microsens from the U.K.

Detection of Protein Conformational Disorders

DR. WILSON: Thank you for giving me the opportunity to speak to you today. That is just an introductory slide showing what Microsens--or who is involved in Microsens.

For many years, as we have been already discussing, we didn't really know whether there was a problem with transmission of vCJD through blood transfusion, but we do now know that there is a problem. What we don't know though without a screening test is how many people out there are incubating the disease and possibly passing that

disease because it can be years or even tens of years before those people develop symptoms.

We do have a problem in the diagnostic industry in detecting this disease because it is not a novel protein, it's not a virus infection that we can just develop a antibody test for. It's a self-protein.

This is sort of the way I think about the disease. In the brain we've got large aggregates of molecules that give you the protease resistance that people have been talking about, so we can treat with protease and then detect the protease resistant rogue prion that's left behind. In blood though we're not likely to have these large aggregates. In fact, the aggregates are likely to be more of this small soluble size.

I think it is interesting to think about what an infectious dose is or an infectious unit. Certainly in viral infections and bacterial infections, one virus and one bacteria does not give you an infection. You need thousands of bacteria, thousands of viruses to get an infection.

I think this is true also in prion diseases. So in terms of infectious units we really don't know in the blood how many molecules that actually represents.

The other thing I would like to comment on here is if we're looking at spiking studies using this material here, I can spike that material into plasma and I can get 10 to 4 logs(?) clearance simply by centrifuging the sample. I don't think I could achieve that with the soluble oligomers in blood.

If we look at the endogenous infection, 6 infected animals going down to zero is not 4 logs clearance. We don't know in endogenous systems what the efficiency of filtration is likely to be, and we're far from knowing that in fact. So I do bang on about protease digestion a bit, in that, all the post-mortem assays use protease digestion to remove the normal prion protein. But as I said, in blood we don't really know whether we can do that, so we don't know whether in a lot of these assays that are good for post-mortem assays, that

we can translate them easily through the ante-mortem testing.

So we do need some innovative approaches because we can't easily detect this agent. There are alternatives such as exclusion, surrogate markers and rogue prion specific antibodies. I'm calling PrPSc rogue prion for want of a better description.

We have gone down the specific ligand route, but we're not using this in our filtration device. We're using this for actual diagnostics. We call our agent Seprion and it's a platform technology. The reason why we started working on this is historically and in the literature there's lots of demonstrations that polyionic compounds, polymers can actually bind rogue prion protein.

So what we managed to do is to find conditions under which these polyionic polymers will only bind to the rogue prion protein and not to the actual normal prion protein.

I just show this because we can coat our material onto beads, our Seprion onto magnetic

beads, and then we can interrogate infected brain and uninfected brain. We can wash the material, wash the beads, and then elute the material and analyze it on a Western Blot, an antibody probing. You can see the only signal we get from the elute aggregate from our bead is from the infected brain. This is the prior protein and it's actually not present. The normal prion protein is not found from the uninfected brain, so this material, this Seprion only binds rogue prion--well, prion from infected brain.

And that material, we can demonstrate, is actually protease resistant, because we can take the material, digest it with protease and get a shift in molecular weight, though the material doesn't disappear. So our ligand binds material from the infected brain and that material is protease resistant, all the hallmarks of rogue prion protein.

Our technology has been extensively validated so there are tests out there in the post-mortem field that don't use protease. Our

test doesn't use protease. And it's had the USDA approval and EU approval for BSE, and I can tell you that if it's got EU approval than it must have been extensively validated. They insist on thousands of samples being assayed for the approval process.

But we want to move towards an ante-mortem test, and we know you guys want accept any protocols that are too involved. We want a simple protocol, and this is our protocol for plasma. So what we have here really is a front-end capture followed by a fairly straightforward standard ELISA. The front-end capture uses Seprion-coated magnetic beads and the material was captured and washed, and that can be done on an automated magnetic bead handler. And then you have the elution, denaturation and the ELISA process.

This elution and denaturation at the moment is a heating step, but it can be--other agents can be used to make the process more simple. So you can see the total time. It's not much longer than a standard ELISA with a little bit of

front-end sample processing, and you can actually handle hundreds of samples, depending on the automated platform, at a time.

We also do have a whole blood and a--I'm calling it a crude non-red cell fraction, but that really depends how you prepare the blood, because for this protocol, the difference between this and the plasma protocol really is just for the non-red cell fraction you have to prepare the non-red cell fraction, so it depends how you do that. And you've got a cell lysis step, DNAse treatment. Then you're pretty much into the plasma protocol. Again, the whole process is easy to alternate.

So how do we get on with this protocol? When we first started working in this area we were actually using large volumes of blood because we were told that the level of the prion in the blood would be very, very small, very, very low. But to our surprise we subsequently discovered that there is more of this material in blood than we originally thought. But some of these results I'm showing you now are on large volumes. We can

distinguish signals from scrapie infected and uninfected animals using 5 mils. of blood.

We've done some blind studies. This is a whole blood result where we took the blood from the animals before they were slaughtered and then investigated by Western Blotting. I should say the reason why we're doing all this scrapie studies, of course, is because it's an animal model for vCJD, and I was going to say I will give my left arm for vCJD blood, but perhaps I won't go quite that far, but it's a very precious sample and very hard to get hold of. So a lot of our work is on the scrapie model.

So we take the blood from the animals before slaughter, and then the brains are investigated by your standard techniques of Western Blotting, and you can see this is a blind study. You can see that we picked up the two positive animals. Got pretty good results there. We picked up one position that the Western Blot didn't actually pick up, but there are problems with the Western Blot standard, as we know.

Now, you guys are not necessarily interested in the clinical phase of disease. If someone's sick, they're not likely to be giving

blood, so we've been looking at the pre-symptomatic animals. This is a flock, exposed flock. Most of these animals eventually go down with scrapie, compared to an unexposed flock. And what we found is that we can actually detect disease early on in the process. That's just another study showing the same of spread of signal from exposed animals, high and low signals compared to the low signals from the non-exposed. When we actually go forward and look to see when these animals develop disease and see when they first became positive by our test, you can see that sometimes in some cases we're picking up the disease maybe a year before they actually develop their symptoms.

We only have really only one vCJD result, and that's shown here. We haven't been able to get any blood that's controlled by the authorities, but we have had people sending in their samples for testing by their physicians. And this is just one

experiment from one particular day, again, using large volumes of blood though, so it's not all critical. And you can see that on that day we received blood from a suspected iatrogenic patient, a suspected vCJD patient, their mother's as controls and some other controls there.

The only sample we actually picked up with our test was the suspected iatrogenic CJD patient. She went on to develop disease, the suspected vCJD. As with many or most of the samples we receive, was not actually infected, and recovered from whatever ailment they had at the time. So that's the only result really that we have today on detecting disease in human blood.

It's just worth going back to this plasma protocol, just to emphasize that this is our preferred protocol, using small amounts of plasma, and we're working on this protocol now, so just a little bit of current data. We're still comparing our model system, the chief model system, and we've actually started getting some human plasma samples now from the National Blood Service. We're looking

to see what the specificity of our assay actually is on these samples, and it's quite comforting to see that so far we've tested 60 blood donated plasmas and they all fall within the same signal range as the unexposed sheep samples that we're still investigating.

If we look now at the suspect sheep though, the ones that actually have clinical symptoms, and the post-mortems will be performed on those animals to confirm that they have disease, you can see there's a whole spread of signals, and some quite high signals in those animals. So, again, just showing this distinction between exposed animals and unexposed animals and what we hope is human plasma samples that are not infected.

Just to summarize that, we have detected the prion protein in the blood of symptomatic and asymptomatic--that's important for blood screening--scrapie infected sheep, and we believe in the blood of one iatrogenic CJD patient. It is a small-volume blood assay. We can use it on plasma wide cell preps, whole blood. We have

begun, and we have had some independent evaluation of the technology and the protocols are pretty straightforward and should fit within the screening protocols of the blood screening labs.

Thank you.

DR. BRECHER: Thank you.

Questions, comments?

[No response.]

DR. BRECHER: Thank you very much.

We're then going to move on to a slightly different approach, pathogen reduction, Larry Corash from Cerus Corporation.

Status of Pathogen Reductions

DR. CORASH: Dr. Brecher, members of the Committee, thank you for the opportunity to review the status of pathogen-inactivation technology for platelet components. Pathogen inactivation, treatment of the labile blood components, which is platelets, plasma and red blood cells, does represent a paradigm shift in blood safety.

Today I'm going to focus my comments on platelets because the general topic of pathogen

inactivation is too broad for the current forum. These are the topics that I'm going to focus on today because I think that they're the most relevant and important issues to consider. At the end of your printed materials, there's a list of key publications that expand on the details that I'm going to be covering.

Over the past three decades testing and donor screening and multiple tests have been implemented, have certainly improved very substantially the safety of blood transfusion of the labile components. But pathogen inactivation can be thought of as a prospective and a complementary strategy to further improve and deal with some of the issues that have not been completely resolved, to interdict pathogens that we currently don't test for, to deal with the low-burden pathogens during window periods like HBV and West Nile virus and CMV--recently HBV has been a considerable issue in Japan--deal with bacteria in all the types of platelet components, both single-donor and whole blood, deal with emerging

pathogens when we may not always have a test available--West Nile was an example, and then a test was of course put into place, but there may be issues with low pathogen burden detection--and inactivate residual CMV and also leukocytes in patients who are not always identified as being immune suppressed, and we know that current testing for CMV using serology or leuko-filtration does not interdict all transmission of CMV disease.

The technologies that have been developed and are available in various stages of development today are amotosalen plus UVA light, which has received a CE mark and has started in clinical practice in Europe. It's under a U.S. review right now for a PMA application, and the Japan Red Cross is in the midst of evaluation studies. Another technology is riboflavin with UV light. It's in development, in Phase I of clinical trials.

Because I am most familiar with the amotosalen technology I'm going to focus on that today as an example of where this technology can go and where it fits.

Now, a large number of studies of inactivation of infectious pathogens have been published, and I refer you to the list of

publications. It's effective against the envelope viruses, the ones that we commonly test for today that you see on the first line, like HIV, the retrovirus HTLV-1 and -2, hepatitis viruses, hepatitis B virus. It's effective against the envelope viruses in the herpes family, the CMV, EBV, HHV-8, effective against West Nile virus, the coronaviruses, SARS and also vaccinia. It's effective against some non-envelope viruses. We've recently completed studies showing effectiveness against parvo B19 and adenovirus and reoviruses as models. It's effective for inactivation of bacteria, a spectrum of protozoa including T. cruzi and babesia and plasmodia and leishmania. It effectively inactivates leukocytes and completely inhibits proliferation in cytokine synthesis and activation antigen expression. And we believe it should have effectiveness against bioterrorism types of agents.

There are some limitations. It does not inactivate certain non-envelope viruses, the most notable being the hepatitis A virus that has an extremely tight protein capsid. Fortunately, this virus is not a significant pathogen for the labile blood components. It does not inactivate bacterial

spores. It will inactivate these bacteria when they germinate. And as I think very well described by the previous speakers, does not inactivate prions, and that's because this technology is nucleic-acid targeted and the prion or protein infectious particles don't contain nucleic acids as best demonstrated.

843 patients have been transfused in clinical trials performed to date in the United States and in Europe. I'm going to focus on the two clinical trials, the largest ones that are indicated in orange, a trial in Europe that we call euroSPRITE for buffy-coat platelets derived from whole blood, and a trial in the United States that evaluated bleeding that's called the SPRINT trial. The details of both of these trials are described

in the publications listed at the end.

The primary endpoint in the EuroSPRITE trial, which was conducted in four European countries with whole blood buffy coat platelets, was the one-hour count increment. We also looked at bleeding in clinical hemostasis, but the trial was designed with sensitivity and power for the one-hour count increment.

Now, the platelet count increment is a surrogate measurement of platelet efficacy. The actual clinical measurement of course is prevention or treatment of bleeding. In this study though, over the range of doses that were transfused in the four different countries involved, we showed that the one-hour count increment, when adjusted for differences in platelet dose, were statistically not different between the test product which was the amotosalen-treated platelets and the control conventional platelets prepared in the method at the study center that was in current use.

In the United States we conducted a trial in 12 centers of which the primary endpoint was the

prevention of bleeding, and the type of bleeding that was most relevant for platelet transfusion in patients with thrombocytopenia is known as Grade 2 bleeding by the WHO criteria. We also evaluated patients for higher-grade bleeding, Grade 3, where red cell transfusions are required, Grade 4 bleeding, which can be disabling or fatal. We looked at the numbers of bleeding sites and the proportion of patients whose maximal bleeding grade was Grade 2.

This study enrolled 645 patients, took about two years to complete. It's the largest study conducted to date of hemostasis with transfusion support of patients with thrombocytopenia. 75 percent of the patients in this study were undergoing bone marrow stem cell transplantation. That means that they had profound periods of thrombocytopenia requiring platelet support.

What you can see is that there was only a 1 percent difference in the incidence of Grade 2 bleeding. This was a non-inferiority equivalence

trial. Therefore, equivalence was demonstrated for this endpoint. There was no difference in Grade 3 or Grade 4 bleeding. The rate was slightly lower in the patients getting the treated product but not reach statistical significance, and there was no difference in the numbers of bleeding sites or the proportion of patients with maximal bleeding grade.

Another important aspect of platelet transfusion is obviously the prevention of bleeding. Most platelet transfusions these days are given for prophylaxis based on the morning platelet count. So time to the first Grade 2 bleeding event is important, and in this study, the median time to onset was 8 days for both groups, test and reference, and was not different.

Red blood cell transfusions are given to obviously treat bleeding under certain occasions. They are also administered to these types of patients for correction of anemia. That's really the most frequent cause of red cell transfusion. These patients undergo bone marrow suppression because of their disease and their therapies.

Over the entire 30-day study period the mean number of red cell transfusions administered was not different between the test and the

reference group, but the median was different when one uses a Wilcoxon non-parametric test. We are now reviewing the relevance of these in our discussions with FDA.

Safety is obviously an important aspect of using this type of technology. Adverse events were accessed exhaustively in this patient population. When we look at all adverse events by system organ class, we detected a difference in two categories, the skin and the class known as infections. These were low-grade events, Grade 1 for the most part, with a few Grade 2, and we're reviewing and discussing this with FDA.

When we looked at severe adverse events, Grade 3 and Grade 4 through the various organ systems, including skin and the infections class, we saw no differences.

This technology was implemented into European clinical practice in 2003. You see an

indication of the various centers in red where it's entered routine practice, and in yellow where process validation and experience studies are going on. Approximately 12,000 doses have been transfused with these treated platelets as of March of this year.

This is the system which is being marketed in Europe. It's compatible with whole blood derived donor platelets and also single donor apheresis platelets. It's a series of integrated plastic containers. It's used in the blood center. The platelets are processed within the first 24 hours of collection. That's important to prevent bacterial replication. It is used in combination with a UVA illumination device which gathers data and is connected to a center's host computer. The same platform is also used for plasma. I'm not going to discuss that today.

The process takes three minutes for illumination and then there's a four hour incubation step to reduce the level of residual amotosalen. The product is then available for

release for transfusion or storage for up to five days in some geographies, and now seven days in other European geographies.

The focus of our European post-marketing studies have been in four major areas. Number one, looking at inactivation versus bacterial detection because bacterial detection had been going on in Europe for a number of years, looking at affective shelf life. We instituted a hemo-vigilance study in a broader patient population than we had seen in our clinical trials. The goal is to look at 5,000 transfusions. We have just done an interim analysis of the first 2,512, not seen any unusual reactions and very good acceptance by patients and the blood centers.

We are looking at platelet utilization and also a pediatric transfusion experience in our clinical trials. We only enrolled a small number of patients. This is an area of interest in the transfusion medicine community. At the University of Ghent we have done 300 transfusions in 42 patients, and those data have shown very acceptable

therapeutic count increments in hemostasis and no unusual adverse events.

I'm going to spend a little bit more time today on two of these topics, and that is the bacterial inactivation and the platelet utilization data.

Three European studies over the past few years of more than 175,000 platelet components have shown that only a minority of contaminated products can be detected and interdicted before transfusion. In the United States the bacterial detection methods are not particularly well suited to whole blood platelets at this time, and so we did some studies in Europe to compare bacterial detection with pathogen inactivation, working with Dr. Walter Nussbaumer at the University of Innsbruck in Austria.

We compared paired single-donor platelet units and then we purposefully contaminated them with the 7 organisms that you see listed at the bottom. These are bacteria that are associated with bacterial transmission through platelet

components. These products were then cultured after one day of storage, using a sensitive commercial culturing system that's in common use in Europe, and then we measured basically detection and the rates of growth. These units were contaminated at 10, 100 and 1,000 CFUs per unit of platelet product. This is the level of contamination that one sees with community acquired bacterial contamination.

Where you see a zero or a completely flat bar, that means no growth has been detected. When you see high bars that means that the growth occurred but was delayed for detection. These were cultured for up to 50 hours after being sampled on day one. The low bars indicate that there was rapid growth and detection. Five of these strains did not have detection at various levels, primarily at the lower levels, although some at the upper levels of contamination at the time of release, so they would not have been detected.

In contrast, the units that were subjected to the inactivation procedure and stored for five

days and then cultured, all showed no growth at all levels of contamination.

At the university clinics of Muncodene [ph] in Belgium, we were very interested in the affect of this technology on platelet utilization. This center complete switched to pathogen inactivation in 2003. They have a very extensive database on their products and the patients that the products are used for. And so they compared data from 2002, the year before the technology, to the year after the technology. There were 2,349 transfusions of platelet products in 2002, and 2,965 with the pathogen-inactivated products in 2003. The number of patients supported went up in 2003 because the center became busier in some areas. The units per patient increased by 8 percent after the introduction of the technology. The patient populations were made pretty much the same.

There was no impact on donor recruitment or on the availability of product in the center after the adoption of this technology, although

they did transfuse 8 percent more units on a per patient basis.

So in summary, pathogen inactivation has a broad spectrum of activity. There are some limitations. It is a prospective approach to safety because it is widely reactive with a very broad spectrum of both DNA- and RNA-based pathogens, viruses, bacteria, protozoans. It did address some of the limitations of bacterial testing. We believe components could be made available earlier, and it is compatible with whole blood platelets, and it has now been implemented into European clinical practice where it does allow for earlier release of products.

Thank you for your attention.

DR. BRECHER: Thank you.

Harvey?

DR. KLEIN: Larry, would you comment on the 7-day platelet storage in Europe that you mentioned in passing? Do you have data on those?

DR. CORASH: Yes. We have one pilot study which was conducted in Copenhagen, where we

specifically attempted in a crossover study to transfuse 5-day platelets and 7-day platelets in a series of bone marrow transplant patients. We saw acceptable hemostasis with both products. The study had an interaction effect because the interval between when the two transfusions could be accomplished was highly variable, and so those data have actually been submitted for publication, but they did show that we get count increments, although they were different in one period of transfusion versus the other, so the analysis was a little bit complex, but hemostasis was comparable.

We have a four-center study now which is being led by Chris Prowse from the Scottish National Transfusion Service, looking specifically at 7-day platelets now in a slightly different design, a parallel group design, so we don't have the confounding crossover issue and the intervening period of transfusion support, that is being launched. And we have a number of other centers that are using the product at 7 days and making observations.

DR. KLEIN: These are whole blood derived platelets?

DR. CORASH: Both whole blood and single

donor.

DR. BRECHER: Celso.

DR. BIANCO: Larry, I recall that there was a lag for sometime in the developing of the process for platelets, and from public statements made by you and others, the need for additional clinical trials in the United States at that time was mentioned before licensure was--you want to give us an update on that, whatever you can talk about?

DR. CORASH: We're in the process of discussing this program with the FDA to get clarification on what would be required for eventual approval in the United States, and those discussions are ongoing and I can't comment on them at this time.

DR. BRECHER: Larry, two questions. Besides the expense of needing 8 percent more platelets, which you did state, what's the cost in

euros per product?

DR. CORASH: Well, that's a little bit difficult for me to address, but I would say that the price of--it's difficult to address because different centers based on volume, et cetera, are negotiating different prices. But we believe that the product is available for somewhere between, I would say, in dollars, 65 to $75 per treatment. I would also say that at the center in Muncodene, they have replaced bacterial detection, they have replaced gamete irradiation, and they have replaced CMV serology for their transplant patients. They believe on this basis that their net cost is closer now for them to about $20 for the product.

So there is the potential to positively impact some of the economics around this product.

DR. BRECHER: We'll hear this afternoon about 7-day platelets, but the current thinking, as I understand it, from the FDA is to compare survival with fresh platelets, using radio-labeled studies. Have comparisons been made with fresh platelets?

DR. CORASH: We haven't done those types of studies with this product yet because in Europe where the product is being used, there was interest

in actually looking at hemostasis in a clinical setting. Those radio-labeled studies first of all are not very applicable to whole blood platelets. In Europe whole blood platelets are a very important part of the product mix, and so they wanted to go ahead with actual clinical studies.

DR. BRECHER: Other questions, comments?

[No response.]

DR. BRECHER: If not, how about a 10-minute break?

[Whereupon, at 10:35 a.m., there was a brief recess.]

DR. BRECHER: We're going to move on to some additional orphan test development, first going to hear from Dr. Sanjai Kumar from the FDA on malarial detection.

Orphan Test Development

Malaria detection

DR. KUMAR: So what I'm going to do this

morning is review different malaria parasite detection technologies and how this can help us improve blood safety from transfusion transmitted malaria.

So for those of you who don't think about malaria every day, here's the life cycle of the parasite in a nutshell. Here is the human host and you have mosquito host here. So just to make it very brief here. So the infection initiated by inoculation of infectious sporozoites here. It goes through this liver stage cycle, which is asymptomatic, and then through erythrocytic stage cycle, and that's the stage that is responsible for the most of--not most--all of the pathological and clinical problems associated with malaria. And then the gametes here have picked up the mosquito again and go through mosquito stage cycle here, and the cycle continues here. And here is a real life picture of the malaria parasite, electron micrograph here. But none of that is important for what I'm going to talk about today.

So if you look at a malaria transmission,

global transmission over time, so what is happening now is the disease has basically contained itself within the Tropics of Cancer and Tropics of Capricorn here, so become a tropical disease over time here. But if you look around in the '40s here it was very much a part of the American landscape. This is the U.S. south here. And then it spread across to Europe and in the larger part of South America also. But with time, with the industrialization and the growing wealth, it has become the disease of the mostly poor countries only here, Sub-Saharan Africa here, Central and South Africa, India here, parts of China and then extends here, in Papua New Guinea here, just up from Australia. So that tells something about the disease itself.

So now let's look at what are the facts about malaria. There are four species of human Plasmodium that cause disease in human. Majority of deaths come from falciparum malaria, but there is something astonishing here. There are more people who are infected with malaria parasite today

than ever before in the human history. By conservative estimate there are about 300 to 500 million new infections every year, and about 1 to 3 million deaths, mostly in children. There's a latest report that say that these numbers are probably twice as much what I have projected here. And in some parts of Africa, at any given time, 90 percent of the children are infected with malaria parasite, and about more than 40 percent of the human population lives in parts of the world where malaria is transmitted.

So what does it mean for us? People tell me why is it a problem for us? The disease has been eradicated here. So I'm going to tell you what it means for us. More than 27 million Americans who go to the parts of the world where malaria is transmitted, and at any given time there are thousands of American soldiers who are deployed in the malaria endemic areas, so these people are at a constant risk of contracting the disease.

There are 1,500 annually imported cases of malaria. This is CDC data. And most important for

this talk here, for this group, in the last four decades there have been an average of 3 cases of transfusion transmitted malaria every year. That amounts to about 0.25 cases per million units of blood collected.

And what's more important, probably where it hurts us most, is that about 150,000 donors are deferred due to potential exposure of malaria. And you get the sporadic cases of transfusion transmitted malaria.

So how do we control transfusion transmitted malaria? Mostly through the FDA Guidelines that are currently in place. There is a three-year deferral for people who have got clinical malaria, or people who are prior residents or established prior residency in malaria endemic countries. That endemic includes everyone, immigrants, refugees or expatriates who lived a long time in malaria endemic areas. People who are non-immune to malaria or come from the parts of the world where malaria is not transmitted, when they come back from a visit they're deferred for one

year.

How do we define malaria endemic areas? Basically this information is based on CDC information, which is an accumulation of many places including WHO and other nonprofit groups.

So just to briefly mention here, because question may arise, that we are in the process of modifying these guidances because it's been a long time now, it's more than a decade old now, and we are taking a lot of science into account, and we are also relying upon the more updated version of malaria transmission. So like, for example, somebody visited a country--malaria is a very focal disease actually, so in a large country malaria may not exist in most parts of the country there, so under the changed guidance there is no need for somebody who has just simply visited a malaria endemic country; it will be more relied upon the CDC-based information for local transmission for malaria, that is to say, where the person actually went.

So what are the issues related to

transfusion transmitted malaria? Currently there is no approved test that we can use in this country here to screen for malaria parasite in donor blood, and the only method we use for blood safety for malaria is the donor deferral, and that's based on mostly a recent history of travel or past residence in endemic countries.

And the majority of cases of transfusion transmitted malaria occur when the donor deferral processes, those that are in place, are not implemented properly. Either the donor forgot to tell where he had been or whether had malaria, or absent part of the blood screener.

But I will say here that in some European countries they use an antibody-based test to define whether a person has been really exposed to malaria or it is just simply based on a oral history. So I will go into that a little more.

So what is the rate of transfusion transmitted malaria? There's a CDC slide from (?) here. So between 1963 and 1998, from as far as the information is available, there have been 91

cases, 10 deaths and 4 species of Plasmodium falciparum have been implicated here. That's something for you to keep in mind. Because all these parasites have very different infectivity rate chronicity in the human host and so forth, and all of those are bearing in our different policies.

But something that happened, we must be doing something right. The pattern of transfusion transmitted malaria in the U.S. is changing. In the last one decade, if you went back and looked at every single case of transfusion transmitted malaria, the rate has fallen very dramatically from 0.25 cases per million units collected. It has come down to 0.04 cases. So that is to say in the last 10 years there have been only 6 cases of transfusion transmitted malaria here. From the previous incidence record it would have been somewhere close to 30 cases probably.

And also something else has dramatically changed here. If we look at the prior history of people who caused transfusion transmitted malaria, half of them were travelers and the other half were

immigrants who had lived for a long time in malaria endemic areas. Now, in the last 10 years, 5 of these 6 cases were caused by people who were born in malaria endemic country. So it has become a disease caused more by the long-term residency.

So what are the methods to detect malaria parasites? I mean that's what it comes down to, if you can have a blood screen test in place. So blood microscopy method, take blood smear, QBC method, antigen detection so you can directly detect the presence of parasites by looking at some of the malaria parasite antigens. This method is used (?). Then the DNA-based test here, PCR test, TaqMan, real-time PCR, and then more recently, microarray. And then one could use indirect demonstration of parasite exposure like what Europeans are doing, basically using an ELISA.

So talking about the advances in malaria parasite detection, this is a paper published by Ronald Ross in 1903, more than 100 years ago, in the Lancet. And Ronald Ross was the person who got first Nobel prize in medicine for describing the

mosquito stage of malaria cycle, and here is the title of his paper, "An Improved Method for Microscopical Diagnosis of Intermittent Fever." So the method he described more than 100 years ago is still in place in reality, so we're still waiting for that improvement since then, because every single case of malaria that is detected so far is basically still being done using this method. Not that people have not tried.

So basically this is the take blood smear method of malaria parasite detection. There's no need to go into detail, but basically method is highly sensitive and you can detect up to 5 parasites present per microliter of blood. In most cases this is sensitive enough to pick up the clinical cases of malaria. Usually you need the threshold for clinical disease much higher than that.

So this is the work from our own laboratory. This is the two-step PCR here using the 18SrRNA Gene as amplification template for falciparum malaria, and we did this experiment by

spiking normal human blood, and without going too much into detail here, the bottom line is, if we look into lens six here we can detect a single malaria parasite present in 1 microliter of blood. So probably this is as sensitive as it gets. You can't detect anything if the organism is not present there.

This is looking at the TaqMan assay here. We can, again, if you go into later cycles, higher amplification cycles--we're not in the best part of the curve here--but in enough amplification, again, we can detect single parasite by this method.

This is a microarray test here. This is hi-fi(?) labeling, and again, without going into detail, we can pretty nicely distinguish all 4 species of malaria parasites by this method here.

So then what's the problem? I mean we can detect a single organism. Not a problem, is it? All of these are basically DNA amplification methods. Those of us who work with this every day, we know the hemoglobin is a big inhibitor of DNA amplification reaction. So here we can detect a

parasite present in up to 5 microliters of blood. But what we need to detect something is in a unit of blood, so just go figure. I mean how can you look for it? It's like looking for a needle in a haystack.

So what are the issues here for a test to become a blood screening method? Only a few parasites could cause transfusion transmitted malaria that could be present in 450 mil. of blood, and both the microscopy and DNA-based methods are highly sensitive but not suitable for large blood volumes. And then here the antibody-based test, which has not direct parasite demonstration, but still you could use them to shorten the donor deferral periods here.

So the test Europeans use, they detect antibodies of falciparum and vivax malarias only, and again, the test is not 100 percent sensitivity. So if you remember the slide I showed you, in the U.S. at least all 4 species, falciparum caused transfusion transmitted malaria, so we determine at this time that does not serve our purpose

completely.

So at this time what we have taken upon ourselves here to develop a blood screening test where you can detect all 4 of the species of Plasmodium using a combination of recombinant protein, so this work is ongoing in collaboration with CDC.

I guess essentially that is where I will stop now. These are the people who were part of the work that's going on in the lab, and also in policy formulations. And I would like to thank Dr. Jay Epstein, who is very intimately involved in discussions and with the policy formulations.

I'll take questions.

DR. BRECHER: Thank you.

Comments, questions? Celso.

DR. BIANCO: Sanjai, thank you. You said that just a few parasites are enough to transmit malaria.

DR. KUMAR: Yes.

DR. BIANCO: Do you see any of those tests being sensitive enough to screen a blood donor to

say that that donor is not going to transmit?

DR. KUMAR: Well, there were some (?) done with the prisoners in the '30s, and part of (?). So they have shown that 100 blood state parasites can cause infection in almost 100 percent of inoculated volunteers, I mean if you call them volunteers. And the thing is that blood is not sensitive enough, but as I said again, also, you cannot detect what is not present there so the best--I mean you can talk about all these copy numbers, genome copy numbers (?). Some of the genes have thousands of copies, but if something is not present in the volume you can't test.

So what we need is something--I'm not saying the technology would not provide the answer with time. What we need is something that can detect, that can (?) the parasites present in unit of blood, so you can concentrate that. The tests are sensitive, but if the sample you test, if organism is not present there, the sensitivity of no use.

And that's where probably antibody-based

test could be more value to us, at least for now. I'm sure with time technology will give the answer. But this problem is not only unique to malaria. This is the same problem associated with a few pathogens that can cause transfusion transmitted disease. And you cannot use large blood sample to use your test. It's the same problem everywhere.

DR. BIANCO: And one more. There are some very old stories saying that during the process of blood donation your spleen contracts and it will release things that you don't see in a pre-donation sample. Is there a basis for that?

DR. KUMAR: Well, again, I mean, there is something you could turn to. Sir Ronald Ross, more than 100 years ago he had very beautiful (?) of the splenomegaly and the parasite burden. That is something people don't use any more. But what I would say, the biggest problem is the chronic areas, people who either just because of the virtue, the nature of the parasite etiology, some of them are chronic infections and the others are asymptomatic because of the partial immunity. We

have no data on what is the parasite burden on those asymptomatic areas. So maybe if the opportunity comes we would like to go back and look what are the--I mean maybe they don't carry very low parasite burdens, maybe we'll find with these tests, but it's difficult to be sure about that until we have that answer.

DR. HEATON: You mentioned that 5 out of the 6 U.S. cases were endemic in that they were people who had previously had malaria. Do you have any idea of the parasitemic load of the donors at the time that they transmitted the transfusion--

DR. KUMAR: I don't. I mean it's probably the most CDC could do and go back and draw the sample, but I mean that's a good point. Maybe if those blood smears are available, those could be looked into. But the data is not available in terms of published literature.

DR. HEATON: One might reasonably presume that the level of parasitemia would be fairly constant, so it might be worth your while getting donors back like that to look at their loads

DR. KUMAR: I mean what we know is that we know how few blood (?) parasites can cause (?) infection. What we don't know is what is the usual

parasite, or something in the parasite burden presenting in the chronic donors.

DR. BRECHER: Art?

DR. BRACEY: You mentioned that some European nations are testing. What's the data in terms of the specific issue in the states, you know, other than Houston, and there are lots of people that visit Cancun and go to the ruins and are therefore rejected. Is that data something that's potentially useful in making policy?

DR. KUMAR: The implementation of the test is coming more to all European countries now and to Australia, but the data is very limited. It's only the last two or three years. I mean somehow those countries have been lucky. They have fewer problem of transfusion transmitted malaria, and I don't know why. I mean they have immigrant population, they have the travelers.

But the paper, the basic science behind

that paper, I mean when they published that paper, along with the shorter case of falciparum that has slipped through the system using the system in place, then conflicting reports will show the tests are not good enough, have not been validated enough to be put in place. So we have been watching those reports very closely, but at this time we cannot really determine that that's what we need, especially even in the last 10 years when there have been only 6 cases, 1 of those was Plasmodium malariae, and that parasite did not present in that detection system. So knowing there is difficulty in turning to something which could get into trouble.

DR. BRECHER: Okay. Thank you. In the interest of time, we're going to move on.

DR. KUMAR: Thank you.

DR. BRECHER: We're now going to hear from Dr. Robert Duncan from the FDA on Chagas, Leishmania and bioterror agents.

Chagas, Leishmania, and Bioterror Agents

DR. DUNCAN: Good morning and thank you

for letting me speak on these issues. I'm going to talk about these three different cases in order of the amount of information that's available, so first of all, in Chagas disease.

A little bit of background. Chagas disease is caused by a protozoan parasite that's present in the blood. You see in the picture there one parasite in a smear, a blood smear from an infected individual. It's a small protozoan parasite, but the other facts here are important for understanding the risk of transfusion transmission of this disease.

It's a chronic, asymptomatic infection, so people who have acquired the disease early in life might not even remember that they acquired the disease, but they're carrying the parasites pretty much for their whole life. Beyond the acute phase it's difficult or impossible to treat, and in some cases leads to serious clinical symptoms late in the disease.

It's endemic to portions of Mexico, Central America and South America, areas from which

there's quite a bit of immigration to this country.

The transmission is primarily with an insect vector, but it also can be transmitted congenitally, with an organ transplant or other forms of blood exposure like transfusion or laboratory accident.

This is just a quick scan of aspects of the disease. The reduviid bug that is responsible for transmission in the upper. This girl has the acute infection where she's been bitten near the eye by the insect and has a swelling that might last weeks to months at the site of the bite. That's the acute infection before the parasite becomes systemic. And then this next picture below is the amastigote stage in the parasite, where it becomes intracellular in the deep tissue, in muscle tissue, which would include, for example, the heart, where it causes some of the complications later in disease.

The issues for donor screening, worldwide there are 16 to 20 million people that are infected, mostly in Central and South America.

Blood transfusion transmission is recognized as a problem in these endemic areas. So in most areas there is testing of blood that's collected. There have been various reports of the probability of an infected unit causing infection in the recipient that ranged from 1.2 percent up to 48 percent, depending on the time and the geographic area.

This slide actually needs to be updated. There is currently 7 cases of transfusion transmission documented in the U.S. and Canada, and I'll go into little more details in a later slide. There are also 3 cases that resulted from solid organ transplantation. There's a wide range of seroprevalence estimated in the U.S. donor population from about 0.01 percent to 0.2 percent, and that high end, the 0.2 percent is probably rare and just local foci of high concentration of immigrants. I mean the broader screening results are in the 0.01-0.02 percent range across the U.S. population.

But we also are experiencing increasing rates of immigration from the countries that are

endemic, and that's the main reason why it's a concern.

And these are the cases of solid organ transplantation. One organ donor actually donated organs that were transplanted into 3 recipients, and all 3 became infected. And this is a blood smear from blood from that donor, and you can see 4 parasites present in one small area of the blood smear, which shows they probably had a fairly high parasitemia.

This is the history of transfusion transmissions from 1987, and you can see from this overview that primarily the donors were from--originally lived for an extended period of time in an endemic area. Their current place of residence was all over the United States, so you can't really gain any benefit from focusing your screening for Chagas in particular areas. And the rate of transfusion transmission, in terms of 1 every 2 to 3 years seems to be continuous, which might suggest that here in 2005 we're due to find another one occurring.

The other thing to make note of is that these detected cases, they're detected by clinical presentation of the recipient coming in with fever,

and someone having the wisdom to look for Chagas disease. In many cases a transfusion transmission would probably result in a very low-grade, non-specific kind of clinical presentation, and may never be diagnosed as Chagas disease or may never even be symptomatic to the level that causes a person to come in for any kind of treatment or any kind of diagnosis.

So we would look at these as just the tip of what might be a much larger amount of transfusion transmission that's going on. I didn't show the worst case pathology in the disease. 10, 20, 30 years after infection a person might have heart complications or digestion system complications, so that's the kind of background where the concern is, that people who are getting infect through blood transfusion, it's not detected acutely, how many of those are going on to heart complications much later in life.

This is a history of some of the policy issues related to Chagas screening. 1989, FDA brought to the Blood Products Advisory Committee the issue of Chagas screening, and at that point it was recommended that there be screening for Chagas disease of U.S. blood if there was a suitable test

available. In 1995 the question was brought again, and there was unclarity at that time, but basically the conclusion was the same, that there can't be screening unless a suitable test is available. 2002, I made a presentation to the Blood Products Advisory Committee outlining FDA's current thinking on what the requirements would be for a suitable Chagas screening test and what the regulatory pathway should be.

Since that time we've had multiple pre-IND meetings with companies that are developing Chagas screening assays. There are several that are a little farther along. There's a T. cruzi lysate, the parasite lysate-based ELISA test, and there's another recombinant antigen-based serological assay. Both have published results that indicate

high sensitivity and specificity. But the final conclusion on any of those tests would depend on the results of clinical testing.

The big question that remains is a confirmatory test, because any blood screening test has to be of a nature with high throughput so that many units could be screened rapidly, and there are certain sacrifices in terms of sensitivity and specificity that have to be made to achieve that kind of throughput. So in any blood screening test there is expected to be a confirmatory test.

And FDA has requested that any biological license application for licensing a blood screening assay for Chagas disease should be accompanied by validation of a confirmatory test.

At this point the Radio Immune Precipitation Assay is recognizes as the most specific and sensitive test, but the methodology is very difficult and hazardous. And manufacturers are resistant to use that technology for a licensed confirmation assay.

So at this point it's a major need. Other

methodologies need to be developed and validated that can confirm a screening test with some kind of technology that is different from the recombinant antigen or whole lysate-based ELISA type test, and there are some methodologies out there. We're looking into doing some exploratory work in our laboratory as well.

So then I'll move on to the next disease, leishmaniasis. It's caused by infection of a very closely-related parasite, Leishmania. There are multiple species of Leishmania that cause different disease in the end. In the picture in the upper left, the young boy has a visceral disease that's caused by one species of the parasite, which is fatal if not treated. On the right, cutaneous disease caused by other species. Though it's painful and disfiguring, it not life threatening.

There is also an infect vector that's involved, and the bite of the insect transmits the parasite. The disease is found in the Middle East, Asia, Africa, Central and South America and along the Mediterranean coast of Europe, which that kind

of distribution creates real problems for any kind of geographically based deferral policy. I'll go into that a little bit in the next slide.

But I also want to mention that Leishmania transmission by blood transfusion has been demonstrated. There are 15 cases that have been reported, and that's worldwide over many years. So the transmission rate is not high, but it is possible. And in all of the cases there were circumstances were the patient was living--I mean the recipient was living in a non-endemic area, and the donor was traced to having been in an endemic area. So otherwise in endemic areas it's very hard to document.

So one of the reasons this issue became intense for us is when a large number of potential U.S. donors were exposed to an endemic area, and in particular in this situation that we're talking about, troops that were stationed in Iraq, the environmental conditions under which they were exposed were much different from a normal traveler. They were sleeping on the ground, exposed to a lot

of insect bites. At the time, in December of 2003, when I made a presentation to the Blood Products Advisory Committee, there had only been 300 cases, that we made the decision about deferral based on, and up to today there are more than 900 cases of leishmaniasis in U.S. troops.

At that time the Department of Defense and the American Association of Blood Banks had already made recommendations for deferral for travel to Iraq, and it's important to note that Afghanistan is also an endemic area for leishmaniasis, but it's also a malaria area. So anyone traveling to that area was already deferred based on malaria exposure.

So at that time the Advisory Committee voted unanimously to support our recommendation for one-year deferral for travel to Iraq, and a lifetime deferral for diagnosis of leishmaniasis, and that was consistent with the Department of Defense and the American Association of Blood Bank's recommendations.

We also recommended at that time, and got

support from BPAC, for not extending the deferral to other endemic areas because the risk benefit analysis was not favorable, that it was--I mean that it was the particular epidemiology and exposure in Iraq that made a difference, and that's why we focused the deferral there.

This is a session about orphan testing. What's the status of testing for leishmaniasis? There are no assays approved or proposed at this point in time. There are diagnostic tests, but many of them are restricted to only certain species, and their sensitivity and specificity is inadequate for donor screening, especially balanced against the transmission risk. The transmission risk is low. As I said, there were only 15 cases documented worldwide, and all of those cases have been associated with visceral disease. So that the large percentage of people infected with leishmaniasis primarily have the cutaneous form of the disease, and at this point the possibility of transfusion transmission of cutaneous disease is uncertain.

And the endemic countries where leishmaniasis is endemic are not screening their blood either, as opposed to in the case of Chagas

disease, where there is donor screening in endemic countries.

So that's the status at this point of Leishmania screening. I'll come back to it in a summary.

And then we have a brief statement about bioterror agents in blood donor screening. There are no approved assays for any of the recognized potential bioterror agents, and there are currently no submissions for blood screening for bioterror agents.

One of the reasons--I mean I think one of the primary reasons is that most of the bioterror pathogens, by the time they reach a bacteremic stage, the person is already so sick that they would not present as a blood donor. There are very few--some of the viral agents have a potential brief asymptomatic phase, and those would be the only ones of major concern.

But in terms of preparedness it's important that we at least be prepared in the event of an attack, so we've developed some research assays that I just mention. In our laboratory we've developed a multiplex real-time PCR assay. This printout from a smart cycler is showing

simultaneous detection of Bacillus anthraces, Leishmania donovani, and Yersinia pseudotuberculosis, which is very closely related to the bacteria that caused the plague. The fourth peak on the scan there is the Human Ribosomal RNA internal control, and this--we've been able to demonstrate this assay effective down to blood spiked with 50 colony-forming units per mil.

A second research assay is a microarray, and on this microarray we've printed both bioterror and some other common blood-borne pathogens, and we have spiked blood in a similar way, and shown detection of Bacillus anthraces, Francisella tularensis, the causative agent of tularemia, and Yersinia pseudotuberculosis at 50 colony-forming units per mil.

So just to summarize, for Chagas disease blood-screening tests are in development and I would say within the next year or two it will be a major question of the possibility of universal screening for Chagas disease.

Leishmaniasis, there's no test visualized, but we are maintaining vigilance for shifting epidemiology such as the invasion of Iraq, and making appropriate adjustments to deferral policy.

For bioterror agents, blood screening is not a likely mode of early detection, but we have preparedness currently at the research level.

And I'll take any questions.

DR. BRECHER: Thank you.

Questions? Mike?

COMMANDER LIBBY: I would like to add that of all the cases that we've seen of leishmaniasis in our troops, only 4 cases have been visceral. I think it's important to note since it is a transfusion-related disease. I think that's all. Thank you.

DR. DUNCAN: Thank you for making that

point. I forgot to mention that.

DR. BRECHER: Celso?

DR. BIANCO: Thank you. Are there studies here in the United States with chronic recipients of blood products of red cells, people with sickle cell, thalassemia? Because despite the prevalence with the current not-licensed assays of several studies that have been done, look-back studies and all that were unable to detect transmission. But I haven't seen any serious study of a large population of chronic recipients in one of the areas where you have high prevalence to antibodies to document that. Are you aware of any?

DR. DUNCAN: I'm not aware of any studies in this country. There's been one you're probably aware of--I think it was in Brazil--of chronic recipients of blood, and it showed higher prevalence of Chagas disease in those chronic recipients.

DR. BIANCO: Yes. But even in Brazil what is very interesting is that the studies in the '50s, '40s, '50s and '60s, showed higher rate of

transmission than more recent studies have shown, and I certainly don't know that.

The other thing that I wanted to comment and that may help us, yes, we hate RPA(?) It's a complex assay and all that, and I wish you guys will not force any of those diagnostic companies to use it as a standard confirmatory, but there is a technique if you have two assays, of using both assays in a confirmation that has been at least used in HTLV and other assays with low specificity that could be useful. Just a comment.

And one more point in relation to the Leishmania. I don't know with those assays, but many of the older assays for Chagas have substantial cross-reactivity with Leishmania, and that could be very helpful in that sense, that is, you don't wait it to be that specific that eliminates Leishmania. It could be very useful in this situation.

And last comment in terms of bioterror. Your statement is correct, that is, blood, blood donor and all of that, we have to agree with you

entirely that any screening test will not help. And the major concern that we all have with a bioterrorist attack is the social disruption and what it does to our donors so they don't show up, and for that we haven't found a solution yet.

Thanks.

DR. BRECHER: Merlyn?

DR. SAYERS: Thanks. Do Trypanosomes have any affinity for the leukoreduction filters that we use?

DR. DUNCAN: Well, leishmaniasis is completely intracellular. I mean Leishmania is completely intracellular, so leukoreduction would probably almost completely eliminate Leishmania transmission possibility. I mean I've heard it anecdotally that in Greece, which is an endemic area, once they instituted leukoreduction, they didn't see any more transfusion transmission.

For Chagas disease is has a extracellular phase, and that's one of the major potential sources of transmission. Whether it would be trapped by the leukoreduction filter or not, I

don't know.

DR. BRECHER: Matt?

DR. KUEHNERT: Just a point of clarification. I'm not sure what the answer is, but you have in one of the slides that one-year deferral for travel to Iraq and lifetime deferral for diagnosis of leishmaniasis. Is current DOD and AABB recommendations--and I'm not sure if that's the case for the latter, the lifetime deferral for diagnosis of leishmaniasis. I just wonder if you could clarify that.

DR. DUNCAN: As far as I know, you're correct, that the Department of Defense has lifetime deferral for diagnosis and the AABB recommendation does not include that. I was just kind of jamming everything into one sentence.

DR. KUEHNERT: Okay. Just wanted to clarify that. Thanks.

DR. BRECHER: Last comment. Jay?

DR. EPSTEIN: Rob, you cited the Schmunis paper in 1999, 1.4 percent to 48 percent transmission risk from a single unit. That's a

rather broad range, and it doesn't seem biologically plausible to be that variable. I just wonder, does that suggest that it was a methodological limitation in diagnosing the transmissions? Which number is the right number? And if it's really the lower figure because of false positives, then that would suggest why we don't see it here, because maybe we're at the low end of that spectrum. But just any thought why that range was so large, and was that commented in the paper?

DR. DUNCAN: I can't really answer your question as clearly as I would like to. My reading of it was that they were collecting together data gathered from a number of different sources, and it's a certain amount of lack of control. Without really giving you good hard response, the impression that I came away with was that there's wide variability because the methods were variable.

DR. BRECHER: Okay. Thank you.

We'll now turn our attention to nanotechnology and Dr. Dimitra Georganopoulou from

Northwestern.

Application of Nanotechnology

DR. GEORGANOPOULOU: Good morning. I would like to first thank Dr. Holmberg for the invitation and the opportunity to show to you some of the stuff that we're doing in Chad Mirkin's group.

I would like to apologize for not providing you handouts, but most of the results that I will show are pending publication.

So I wanted to present a new method we've developed in the last three years that we call the Ultrasensitive Detection of DNA and Proteins, using nanoparticles [inaudible].

So as you know, so far the standard [inaudible] have specific tags attached to them. There are either radioactive or dyes that are used for colorimetric detection, chemiluminescence. The one that is the major player at the moment is fluorescence, and [inaudible] nanoparticle base definitely can be advantageous to provide a diagnosis.

So we want to introduce some potential advantages that nanoparticle assays have, and that includes selectivity, sensitivity, multiplex and

capabilities, ratioing capabilities, and of course cost.

So for nucleic acid detection, to begin with, we know already that we have very sensitive assays from the polymerase chain reaction that can go down to very low concentrations, and of course at higher concentrations we have the established molecular fluorophore-based assays and gene arrays.

The problem that we have though with PCR is that there's a lot of limitations that include speed, complexity, multiplex capabilities and increased cost. So we were thinking of introducing a way of removing these problems. And what we have been doing in the last decade in the working group is coming up with new methods for detecting oligonucleotides in (?)genomic DNA targets.

We started back in '96 and '97 when we were able to detect oligonucleotide targets in solution with a simple colorimetric test when you

had a gold nanoparticle A that was half complementary to the target, and B, that was half complementary to the other half, and then when you brought these three components together, the solution went from red to purple, and you could have an easy colorimetric litmus test.

You could do the same thing on the surface, and you can have different shape or size particle. There is spherical nanoparticles, spherical, triangular, or silver or different composition. And you can see the new radiant white light. You can have different kind of wavelength excited. You can also see the same thing when you can have the linker being detected when gold nanoparticles would attach and bridge to electrodes, and you can have electronic detection out, or you can take advantage of the fact that you can have different dyes attached on the gold nanoparticle, and then when you irradiate laser you can have some (?) signatures out.

The two methods that I'll be talking about today will be the bio-barcode assay that I will

explain in a second, that has a readout called the scanometric assay. The way it works is you have, let's say, a green oligonucleotide that has a half complementary capture strength on the surface as light, and the other half complementary is on a gold nanoparticle. When you have the green target present, the gold nanoparticle will sandwich the DNA linker on the surface. Then you apply a silver ion solution and you silver amplify the whole thing, and you can see scattered lights. You would see spots that have been (?) with microarrayer.

This actually was started--this scanometric assay was developed back in 2000, and now the method that we have to have the readout is a scanometric device that is already commercialized by Nanosphere.

The way it would work is this is how the platform works. You have the glass slide with the captured DNA strand spotted with the microarrayer, and then you have your linker being introduced through the ports, and then washed away, and then you have the silver ions being introduced and gold

nanoparticles. You have the amplification. You remove this gasket. You add it very simply in the scanometric reader device, and you see the spots of silver amplified on the DNA slide.

So the problem though that is there for proteins is that we can't have access to the concentration rates that PCR gives for DNA oliganucleotides or genomic DNA. So you know that for milli--micromoler or even picomoler you can have a small molecule of micromoler detection or even ELISA that can go in the best case scenarios or high fentomoler concentrations. But there's nothing really that can address this concentration range from conventional methods.

So this is the method that we came up with, and I'm going to take you through this. We have a very simple idea of having a database of oliganucleotides, short oliganucleotides that will resynthesize in lab, and then we assign one random oligonucleotide to a protein B, and we will call this green oligonucleotide the barcode DNA.

Then what we have, we have a gold

nanoparticle that is functionalized with a polyclonal antibody specific for protein B, and then further functionalized with a black DNA that has a thiolated(?) end to it that would attach to the gold nanoparticle, and is complementary to the green, which we call the barcode DNA. So we have a first probe ready that has the antibody specific for protein B, and then a captured strand and a barcode DNA also assigned to protein B. There's no natural significance. We just randomly assigned this.

So the first probe is the gold nanoparticle with its specific antibodies, and then the second probe is a magnetic microparticle that has also a monoclonal antibody, this case, also specific only for the choice of our antigen. So what we have chosen so far, for example, for a (?) protein, the way it works is you would have a huge access of magnetic particles that have been functionalized with monoclonal antibody in solution that would scavenge away all the target proteins that are present.

One of the ideas from this method is that you have a huge access of microparticles present, so therefore it's almost like a homogeneous

reaction. So you can scavenge the entirety of the proteins that are present. So the first binding event is taking place, and then we introduce a second gold nanoparticle probe, and that would make a tri(?) structure that would sandwich the antigen between. Remember, this is a monoclonal antibody, and the blue one on the nanoparticle is a polyclonal antibody.

So you would apply a very easy magnetic field that would take these structures to the edge of their (?) and also the ones that do not carry any p24. You would wash away any unreacted nanoparticles.

And then this is the second very key component of this assay by one step, adding just water, you would dehybridize the barcode DNA and isolate in solution. So this is the key component. Instead of detecting one antigen, you can now detect hundreds or thousands, depending on how you

made this nanoparticle probe of DNA oligonucleotide strands in solution. So there's already one amplification step, going from one antigen to hundreds or thousands oliganucleotides, but also you can now do the various methods that are out there for DNA detection, and calculate how much initial antigen was present. The method that I'm going to talk to you about today is the scanometric assay, the one I explained earlier, when this oligonucleotide will be captured on a glass surface and then sandwiched in a gold nanoparticle, silver amplified, and then just measure the scattered light.

The interesting thing from this method also is that you can have an increase of 10 to the 5th amplification from the effect of the silver amplification.

The data that I am showing you today is based on the human immunodeficiency virus, and you know already how the usual tests are now done with the two unspliced viral genomes with PCR, real-time PCR.

Instead of course we wanted to target the protein, and the one we chose was the capsid protein. That was very interesting because there

was already an internal amplification. For each virus you have 2,000 copies, so not only that, the capsid protein itself is very good because it has relatively few mutations.

Initially what we did we made a calibration curve in a spiked dilution series and buffer. And you can see the result that I was showing you, for decreasing amount of antigen, initial antigen introduction, the response of the release barcode DNA goes down accordingly. So you can see here this is the antigen, initial antigen introduction and this is the intensity from the released barcode DNA, and then detected with a scanometric assay.

So then what we wanted to do, in collaboration with Dr. Wolinsky from the Department of Infectious Diseases, he had some samples from 16 patients. You can see here that they did also PCR for the viral RNA but also for the p24 ELISA. You

can see that you cannot detect anything from the 16 samples with ELISA because the detection limit is too high.

But what we could do, based on our scanometric method following the bio-barcode assay, we will get a normalized intensity and with correlation of the patient sample you can see we could have a very good correlation of what they have with a real-time PCR, and this was done in 3 hours in a much simpler and much cheaper way and less cumbersome than what the PCR involved.

So really what I've showed you is that with the method that we've proposed today, the bio-barcode assay, we're able now to address this area of concentration that were not being able to be attained with the normal standard methods that are out there. What that does, it opens up the field for detection of markers that cannot be detected so far because the concentration for most of these markers is too low for standard methods. So you can see here this is for molecules per drop of blood. With the bio-barcode technology what

we're doing now in the lab, we're detecting markers that are indicative of Alzheimer's disease, for the mad cow prions, ovarian, breast, for many other cancers, pulmonary disease, cardiovascular disease, and of course the HIV results that I showed you.

What that does, allows you to detect thousands to tens or maybe even down to one molecule per drop of blood.

So finally I just want to give some acknowledgements. These are some of the people that are in the bio-barcode group, and then this is the main funding sources that we have in the lab. And these are external collaborators that provide most of the antibodies.

Thank you again. Any questions?

DR. BRECHER: I think we have time for one or two comments, questions.

[No response.]

DR. BRECHER: There's none. Thank you.

We'll move on to our next speaker, which is Dr. Eric Delwart from Blood Systems Research Institute.

Detection of New Pathogens through Surveillance of Bank Samples

DR. DELWART: Thank you. We've heard some

pretty amazing talk this morning about methods of detections for virus, highly-sensitive detection methods.

What I'm going to talk about though is the discovery of new agents, which is nowhere as sensitive, but will allow you to see things on the horizons, either emerging viruses, or really viruses that have been here all along that we just haven't seen or detected.

These technologies are using nucleic acids only, and they're becoming I think easier. The advantages here is that viral replication(?) is not essential in tissue culture for discovering these new viruses and serological reagents are also not required. A good source of material for discovering new viruses, obviously, is samples of patients with unexplained symptoms of possible infectious origins, and even more useful are epidemiologically-linked disease clusters.

When you use these techniques you can either take sort of a blind general look at nucleic acids using nonspecific systematic shotgun sequencing type of approaches, or you can narrow your discovery to viruses anticipated to exist or to be emerging. Here's just an example of a slide

I put together a year ago showing the phylogenetic relation of the HTLV family, and you can see that there is three groups of these T-cell (?) in animals and humans, but HTLV-I and -II only belong to two of these families. So people anticipated that HTLV-III would be discovered soon. And beautiful work at the CDC just recently identified HTLV-III in humans, as well as HTLV-IV, a brand new lineage. I'll briefly touch on the technique that they use to discover that.

Now, of course, new viruses may be part of highly prevalent viral floras with no identified clinical consequence, and TTV and GBV are a fine example of that. I think these viruses were originally thought to be pathogenic, but further study showed that they're extremely prevalent and

they don't essentially cause disease with the caveat that such otherwise harmless infections may really be pathogenic in some genetic backgrounds of in immuno-suppressed individuals.

So I'll try to lump viral discovery methods using nucleic acids into two broad groups. One is methods that depend on nucleic acid level similarities to known viruses, and then the second group is methods that are independent of detectible nucleic acid similarities to known viruses. The first group of methods which has been used many, many times for many (?) viruses is really reliable for known viruses or the closely-related species, and they can target either specific viral groups, the consensus PCR methodologies, or you can go using microarrays, try to identify new viruses related to older known virus families. The limitation of this technique though is that they will fail to identify highly divergent viruses or new viruses whose nucleic acids will not anneal to the oligonucleotide probe or primer.

I'll quickly go through the consensus PCR

that works, is being used very successfully for discovering new retroviruses. For example, this is what was used by the CDC to discover HTLV-III and -IV. It's been used many times for discovering new herpes viruses, picornaviruses in seawater, and recently for new coronaviruses. Very simply, you align members of that viral group and you look for the most conserved region, and you design primers that will stick over the most conserved region of the gene.

Some examples of viruses discovered this way, picornaviruses in seawater, many herpes viruses, coronaviruses, and there's a whole list of viruses used.

Virochips have also been used. There's a group in San Francisco who's very good at this, basically to scan GenBank for all the full genomes to look for the most conserved region, and they put many, many, oliganucleotides on their virochips. What they do is they take--in the published case, they've taken tissue culture material, but you can also take uncultured material. First you label the

nucleic acids and you hybridize that to your probe--so sorry--to your chip. After growing the virus in tissue culture, they identified it as a coronavirus within 48 hours, using this microarray.

They were also able to actually scrape off the red dots on their chip and to actually sequence what had hybridized to their coronavirus, previously known coronavirus oligonucleotide.

So the second method is the one we've been using, the methods that are independent of known similarities to known viruses. And broadly speaking, what you do is you come up with a method of sequence-independent amplification or concentration of your nucleic acid. And there are many ways to run a bad PCR. In effect, that's what it is. You take a nucleic acid. You don't know what you're amplifying. You just want to amplify everything so that you can then subclone it and generate plasmids from every molecule which you would then sequence and search for sequence similarities to known viruses.

The advantage here is that theoretically

you can detect all viral groups. I think another advantage also is you can detect some more highly divergent viruses than you can by using nucleic acid relationship, if you want, techniques because you can translate the sequence that you get into amino acid sequence, and that allows you to find a homology to new viruses over longer evolutionary periods than you can using only nucleic acid hybridization techniques. And also these techniques are possibly now to decreasing cost of sequencing, this huge overcapacity for sequencing, an increased and improving sequence similarity search algorithms. That is, when you have a sequence you want to see if it looks like a known virus.

As an example the (?) people (?) Seawater, a very nice publication. Background is 3 million viral particles per mils of seawater. The next time you go swimming, keep that in mind. And people basically did a shotgun sequencing analysis of seawater, and they came out--the technique they used actually quite simple. They

just filtered to remove bacterial and large particles. They banded for viral particle of the right densities. They did, only had DNA viruses, that discerned the DNA viruses. They shared that, added linkers, a few cycles of PCR, and sequenced just 1,000 plasmids. And they took that sequence, did a blast against GenBank.

What they found first of all, was that three-quarters of the sequences had no homology in GenBank. They were just hitting new organisms. Among the sequences, there was homologies that--a mix of viruses, mostly bacterial phage, a lot of mobile elements. From the available repeat sequences the estimate is about 5,000 viral species in seawater.

Now, the method we've been using is that developed by this group at the NIH, and I'll get into the details of this technique. When they developed it and they used it first, they picked up two novel bovine parvoviruses in a serum. They were using it as a (?). So when you use bovine serum and tissue cultures, keep in mind there's a

lot of stuff there.

So what we did is we used this technique first on 6 controls which were HCV RNA positive plasma from blood donors. And then we also got 25 plasmas from people who thought they had been infected with HIV because they came in with very acute symptoms of primary HIV infection. But as people would IV use in a sense, but these people were not infected with HIV as confirmed by repeatedly HIV RNA negative testing. The method used in that published paper is quite simple.

You first take the plasma or the serum--but this technique works only with material that has very low level of human DNA like plasma or serum. First you filter it out to remove any residual cells. You remove contaminated human DNA by DNAse digestion. And presumably, the viral nucleic acids are protected within the capsid of the viral particles.

You then take--you use random primers for reverse description. Then you do the second strand synthesis, which should be here. You then cut the

DNA here, the randomly generated double stranded DNA with restriction fragments, with restriction enzymes, you add linkers. And you use those linkers now, the sequence of those linkers as PCR primers, and you basically amplify whatever is in between those restriction sites. You take this material, this randomly-amplified material. You run a gel. Take a look at what you've got. You then would subclone into plasmids. You will sequence those and you will blast those against the GenBank database.

What you expect when you do that, if you have a sort of dominant viral species in your plasma, you expect a low complexity amplification product. On the other hand, if you just have a lot of low-level though diverse mix of nucleic acids, you expect a smear on your gel. Here's the results we got. Here we're looking at the 6 hepatitis C RNA positive samples. In 5 out of the 6 we got distinct bands. In the 6th one we just got a smear. Out of the 25 plasma from people with acute infection, what we got were bands in 3 of them for

which we did this experiment looking for RNA viruses, and looking for DNA viruses we got bands in a different 3 individuals.

What we did next is we basically cut out those bands out of the gel, mix them all up, subclone them into a plasmid and sequence. And we did a minimum amount of sequencing, about 10 to 20 subcounts per sample. What we got out of these, we didn't bother with the smear one, we just took the 5 ones from the hep C control. We got hepatitis C sequences from 4 out of the 5, from three different genotypes of hep C, and here is the genome of hep C. And those little bars here and the number next to them represents nucleotide similarity, represents a fragment of hep C that we got. So the controls work quite well considering the minimum amount of sequencing that we did.

Now, for the mystery samples, the one from people with acute infections without obvious cause, when we did the RNA extraction for RNA viruses, we got GGBBC in 3 of them. And these are the fragments from one, multiple fragments from another

patient. And one we've just got a little, just one subclone to a piece of GBD. But they're very easy to see, the homology is so, you know, it's over 90 percent.

And in another patient, looking at DNA viruses we got a piece of hepatitis B. Now, the patient, a little background, GBBC is highly prevalent flavivirus. The early link to transfusion hepatitis had not been confirmed.

So then we look at the other sequences that we got, which became much more interesting. We saw in two subjects DNA fragments that we subcloned that were similar to TTV but really quite divergent. TTV is recently discovered in a blood donor. It's a small circular single-stranded DNA genome, no link to disease, very prevalent in humans, and it's also present in all known--in all tested non-human primates, possible co-speciation of TTV in primates has been going.

We also had a piece of this genome. It was quite straightforward to amplify the entire genome, especially if they're circular DNA genomes,

basically outward pointing primers, and we sequenced them. And we noticed in these two patients these TTV like viruses were quite smaller than the known TTVs or TTMV, which is a related species. We did a follow genetic analysis of these viruses and we found out they were really quite divergent from the known TTV in humans, which are here, which is a very quite divergent group of virus into TTMV. So we got a new outlier, if you want, of the TTV group, and these are the sort of TTV you get in other species.

Most interesting though, we get a sequence which gave a hit to parvoviruses. At first when we just did a nucleic acid homology search, we saw no homology at all. It's only when we did amino acid level similarity that we saw this similarity to known parvoviruses. This just highlights the fact that if we would use a virochip, for example, in this sample, we would not have picked up this virus because it is just too different at the nucleic acid level. You really need to translate the sequence.

Again, we had a foothold on a genome and we went on to sequence the entire thing or just about except for some of the edges, and it looked

just like the typical parvoviruses, with the two remaining frames up here. This is B19, for example. We did a phylogenetic analysis of this and what you'll see, it's a quite different parvovirus. It's got a very deep branch. It is part of the group that has B19, V9, A6, the sort of human parvovirus group. It's also in the same group as these non-human primate parvoviruses, but it is extremely divergent.

A little background. Parvo B19 is a common innocuous infection in infants. It can be pathogenic, resulting in severe anemia as a group of people. It is resistant heat inactivation. We heard this morning that chemical inactivation may work, and the plasma pulls off screen(?) by PCR to exclude high level B19 DNA positive donations.

In collaboration with a group in England we provided them the sequence. They designed a very sensitive PCR assay and decided to look at

some of their plasma pools. Was this virus just an odd case of zoonotic transmission to this person or is actually quite--is it found in other humans? And looking at plasma pools they found other positives. 8 out 43 from this manufacturer, 2 out of 3 from this manufacturer, and some manufacturers seemed to be free of these new parvoviruses.

The detection of this virus was confirmed by sequencing the amplicon that they used, so it was not B19 and it is indeed this new virus, parvovirus, and interestingly they found another variance that's about 10 percent divergent from the one we had picked up. So it looks like maybe this group of parvoviruses is like the B19 group. It has its own variance.

Together with Focus Diagnostics, they are now trying to express the glycoprotein to develop an antibody test to determine seroprevalence which will be essential for determining just how common this infection is, but it is quite hard to develop these serological assays.

So to finish, from 25 patients with acute

infective symptoms, we found sequencing only about 100 subclones. I mean this is minimum sequencing. And similarity surge we found one hepatitis B, 3 GBV's, 2 highly divergent TTVs, and a new parvovirus, looking at really only 6 patients.

We had some hits that are worthy of follow-up. We had a hit to an RT sequence and to adenovirus. Now, (?) sequence had no hits to GenBank, so we're quite puzzled by those, and we think it could be very interesting, either completely very highly divergent viruses that are so unrelated to what is currently known that we're not getting them by homology, or they could be part of a viral bacterial flora. It's not excluded that these are bacterial DNA. Clearly they're not human DNA. The human genome has been done.

Maybe by increasing (?) with this sequencing of this non-human nucleic acid in sick patients will yield a more concerted region of highly divergent viruses. This could aid in the identification as viral sequences, and maybe just getting more sequence (?) to build larger

configs, and again, to see does that look like a virus? Is it (?) size? Does it have (?) and so forth. By doing this on many patients we could determine if unidentified nucleic acids were actually prevalent in healthy or sick subjects.

So good molecular methods are available to both closely and distantly related new viruses. Transmission of those viruses through blood transfusions could then be tested using the sort of linked donor recipient sample banks that Mike Busch described this morning.

And again, the hard part I think now is when you find a new virus is, is it pathogenic? Is it a concern for a group like BPAC or not? We used to have disease in search of viruses. Now we're going to have viruses in search of disease. These are going to be the hard questions.

A little background. There was another group--this is such an obvious way to do it--I mean there was a paper in I think Nature a year or two ago, this father-son group planned to take hundreds of liters of volume of plasma from clinical labs

and just basically pool them and centrifuge them for viral particles, and then, you know, within 7 days we'll sequence a known subclone and do the human virome to identify all the viruses. But what we did was a very small scale, 100 subclone version of that.

We have future plans, of course. Find different methods to randomly amplify nucleic acids, to purify viral particles in general for their amplification, analyze different cohorts, improve the secret search algorithm. If you're looking for (?) homology the computer work is actually quite complicated. You determine the prevalence of these new agents in the course of pathogenicity of these viruses. We have access to many interesting samples from different groups who collect plasmas and other bodily fluid from people who want to explain disease or who die from unexplained death.

Particularly interesting is the (?) culture supernatant from Diagnostic Labs, people who do that for a living. Sometime they claim to

have an agent that causes cytopathic effects but they don't know what it is. These supernatants are great starting material for discovery of new viruses. The bio-informatic, we're getting a group to help us deal with the increasing sequence load.

And finally, the people who made this possible: special thanks to Sally Baylis in England, who tested the plasma pool and found that this parvo virus is actually common; the phylogenetic analysis with Peter Simmonds and Vladimir Lukashov; and the sample from the acute infection cohort, Rick Hecht in San Francisco; and the bio-informatic, Robert Shafer; and Morris Jones did most of his work in quite a short amount of time, and we were quite surprised by the rich yield of viruses that we got.

Thank you.

DR. BRECHER: Thank you. Thank you for giving us one more thing to think about before we go to the beach.

[Laughter.]

DR. BRECHER: Celso.

DR. BIANCO: Fascinating technology and beautiful. But my question is--maybe you don't remember, but in '92 we had the infamous idiopathic

(?) for positive lymphocyte opinion, and it was a whole hysteria of AIDS without HIV or something like that. As you look for all those things and you have absolutely no idea of they have a pathological role, how are we going to prevent a crisis in the whole field that we are.

DR. DELWART: You mean the fear of the--

DR. BIANCO: Fear of the new agent that is going to destroy the world.

[Laughter.]

DR. DELWART: I agree. But maybe a whole vial of flora that just hasn't been identified because they don't cause symptoms, and just sort of a shotgun approach or some name for these meta genomics, will discover all these viruses. I think people should not be that concerned until the pathogenicity is proven. We may also discover a lot of new pathogens especially if you look in clinical samples from very sick people. There may

be a lot of viruses that have just not been seen before. The technology will allow that. The impact on blood safety really depends on the prevalence of these agents, and I think developing serological assays for these new agents, PCR assays, real-time or whatnot will help reassure people. But, you know, just ignoring them or wishing them away is--now with the technologies is almost--it is tough.

DR. BIANCO: I though that you were also going to include the repositories. I think that the major question that we'll ask about each one of those viruses is, is it epidemic or is it a stable prevalence?

DR. DELWART: I think the accumulated samples over the years can get to that.

DR. BRECHER: Okay, thank you.

We're now going to move into the easy part of this session, which is directing some recommendations and discussion. This is a carryover from yesterday afternoon's presentations and this morning's. Matt had kindly put some

preliminary thoughts on paper, so maybe we can get those projected. Matt, if you could walk us through that.

DR. KUEHNERT: Okay. This looks a little long because the font's big, maybe. But starts off with a little preamble about why we think this is a problem. I'll just read through the whole thing and then we can get comments on it.

The Institute of Medicine and its report on microbial threats to health has identified blood, organ and other tissue safety as critically important challenges to optimal health care. Blood safety is a shared responsibility among the public health service agencies and the blood community, including collection centers, transfusion services, front-line clinicians and recipients themselves.

A comprehensive national program for surveillance of blood and blood products, i.e., hemovigilance, is lacking in the United States, although existing fragmented elements for such a potential program now exists.

We believe that PHS agencies including

CDC, FDA, CMS and AHRQ and the blood and plasma community need to work together to develop a coherent framework for hemovigilance including communication, surveillance and risk assessment, to respond to known and unknown threats to transfusion recipient health.

Now we get to the details.

The following actions to achieve these objectives are recommended:

No. 1. Implement a plan to coordinate public health response during routine situations and emergencies;

No. 2. Integrate and standardize adequate tools for national surveillance of the blood supply, including (A) monitoring and analysis of data on currently screened pathogens; (B) use of repositories for pathogen and disease discovery; and (C) representative monitoring of transfusion related adverse events.

3. Develop a decisional process for prioritization of blood safety activities and resources among and within PHS agencies including

epidemiologic and laboratory response.

Formation of an HHS task force, including representatives of the blood community and PHS agencies, would be an important first step for identification of existing and lacking activities in relationship to these objectives.

DR. BRECHER: Jerry?

DR. HOLMBERG: Matt, I think that's very good. The one thing that I keep coming back to is the hemovigilance versus the biovigilance. And already this morning we've heard about the transmission of Chagas in organ transplants. I think we--you know, West Nile, like the last couple years has been transplanted by tissues, tissue and organs. Also I think the rabies cases in both--I think it's in Texas and Germany--I think gives us a lot of credence to the idea that we need to go more to biovigilance, and I just throw that out as a question.

And then also, if we do go out to a biovigilance, would you want to increase the agencies to include HRSA for the bone marrow and

tissues?

DR. KUEHNERT: The bone marrow and--

DR. HOLMBERG: Organs and tissues.

DR. KUEHNERT: I think it's a good point. I sort of wasn't sure how far the Committee wanted to go so I sort of waded into it with the IOM report at the beginning and then backed off from it. But certainly if we wanted to integrate those, that could easily be done to include organs and other tissues and then refer to it, you know, if you want to use the term biovigilance or another term, that could be done. It's just that my concern was that the Committee's charter doesn't extend, to my understanding, doesn't extend to those, and I wasn't sure whether that was appropriate or not.

DR. HEATON: I would very much support extending the purview to cover biovigilance. I mean we're seeing--West Nile virus was first identified in organ transplants. It wasn't identified in blood transfusions. So we're beginning to see an increasing pattern now to the

more esoteric organisms being transferred either by tissue or by organs, so I would very strongly support extending this to biovigilance and a recommendation if we need to make such, the purview of this Committee be expanded to pick up tissue, organs and cell therapy.

DR. BRECHER: Celso.

DR. BIANCO: Matt, the way it's written gives the impression that it is just coordinating available information. You don't think that we need new resources to be able to do that?

DR. KUEHNERT: New resources you said?

DR. BIANCO: Yeah. Money.

DR. KUEHNERT: If you scroll down I guess that--I think if we go to the specific action items, we may want to then alter No. 3 which alludes to a decisional process for prioritization of blood safety activities and resources. And you're right, as that reads, it seems to imply that we only are asking for prioritization of existing resources, so I guess that could be changed to include "additional." I would say that that would

be essential, and maybe that should be added explicitly. None of this really can be done with existing resources, or very little.

DR. BRECHER: So why don't we insert the word "additional" now?

DR. KUEHNERT: Okay.

DR. BRECHER: So just "additional" before "resources."

Karen?

MS. LIPTON: I wanted to return to the issue of biovigilance first as hemovigilance, and I too feel very strongly we need to include anything related to cellular therapies, tissues, organs, because I think we run the risk that people will start developing other systems to do this, and I think that what we've seen is we should be taking the lessons from blood because if they had been taken more seriously I think some of these issues wouldn't have--some of these transmissions wouldn't have happened in tissue, for example.

So I would strongly encourage us--I mean even if it's not, you know, within our charter

right now, to make that recommendation that all of these activities be coordinated.

DR. BRECHER: That sounds like a separate resolution after this one, that the charter of this Committee be broadened to include cellular therapies and organ transplantation.

MS. LIPTON: That's one piece of it. But even without broadening our charter I think we can make this recommendation as being one that's not just limited to hemovigilance.

DR. BRECHER: Let's do that and let's be specific. Where would you change it? National surveillance of the blood supply and tissues for transplantation?

MS. LIPTON: Well, you're probably going to have to say blood, cellular--let's see, what would it be? Blood, tissue, organs, and then what would you think--blood, tissue and organs, and that would then really include cellular components.

DR. KUEHNERT: See, we sort of tried to coin a term. I don't know if it's going to catch on, but, you know, blood, organs and other tissues,

which the acronym is BOOT, but be that as catchy or not as that is, EIS officers have as a symbol sort of a shoe with a hole in it, so this is just an extension of the shoe. But anyway--

MS. LIPTON: One thing about that. My caution about that is that people who are in the core blood and cellular therapy fields are not necessarily going to recognize that they've been included in that.

DR. KUEHNERT: If it's blood, organs and other tissues, they wouldn't--

MS. LIPTON: Yes. I don't think they really call it--they really don't think of themselves as being other tissues.

DR. KUEHNERT: Oh, cellular therapy.

MS. LIPTON: I mean I think that from because of the FDA regulations with GTPs they do, but I think as a discipline and as a field they probably do not.

DR. BRECHER: Jeanne?

DR. LINDEN: Just along the same line, the way the regulatory structure is, organs, blood and

tissues are three different things. They're not--organs and blood are not other tissues. So I mean it's really blood, organs, tissues and cellular therapies. I mean I think Karen's right, in whatever order you want.

DR. KLEIN: I mean that's fine. I think however it's described is fine. It's just that I'm not sure if we want to align ourselves along--I mean I think there's an argument to align ourselves along regulatory lines but also align ourselves along pathogenic transmission lines too, and so--I mean the--however that is--I mean there are some things that are just as transmissible across organ transplanting as blood transfusion, as other tissues, and then some things that aren't. So I don't know how to resolve that, whether we want to go along regulatory lines or not. Certainly that goes--then allows us to be aligned as far as agency activities.

DR. BRECHER: Jerry.

DR. SANDLER: When the agenda was planned for today pathogen inactivation was put on the

agenda. Pathogen inactivation is getting subordinated quite a bit, one, in the document that we have, and two, by expanding to tissues and organs. I'd like to just go back to the charge of the Committee, the Advisory Committee on Blood Safety and Availability. When we consider our specific charge, pathogen inactivation becomes a very major alternative to a lot of this, or certainly a partner in all of this. When we go to tissues it kind of gets lost. I'm just wondering if we're missing a very important component in blood safety by doing that.

DR. BRECHER: Well, I suppose that we have to be cautious about what we say about pathogen inactivation since it's not available in this country as yet, other than to encourage research in that area, but until it's a reality, I don't know there's a whole lot we can say.

DR. SANDLER: How much of what we saw this morning was reality?

DR. BRECHER: But not in this country.

DR. SANDLER: I mean zeptograms, I mean,

where's reality?

DR. BRECHER: Reality is when we have a product that we can use in this country, at least to my mind.

Mark?

DR. SKINNER: I really can't fully read what's up there, so I'm not sure that I followed all of it. But from what I heard was being read, my concern is that there's a reference here to the blood community, and I'm not quite certain how that is defined. There's one reference in the whereases to the end users. It seems to me what we earned between last meeting and this meeting is transparency in the process, risk communication, getting real-time data out so decisions could be made. And I think what's missing here is the integration of the end users into this process. I'm particularly troubled by the last section where the patient population seems to be completely left out of the task force. I think for this to be successful and meet the needs that I've heard articulated, we need to go back and weave into this

process the involvement of the end users in each of these points, and emphasize the need for real-time risk communication and real-time availability of the data for those that are dependent upon the products that we're talking about.

DR. BRECHER: Sue, did you want to?

DR. ROSEFF: I'm sorry. I was going back to another point so I'm not sure where to put this in, but I again endorse extending this to cellular therapies, especially in light of the new FDA licensure that's going on in just I guess a week with cellular therapies and some of the differences and requirements for testing versus blood donors.

DR. BRECHER: Jeanne?

DR. LINDEN: I want to echo Mark's comment. I was a little troubled by the specificity of who is to be on this task force, and I would prefer to be a little bit more generic and just say, basically the stakeholders, you know, anyone with an interest, including but limited to, and give examples.

Also, going back to this issue about

organs, tissues, cellular therapies, et cetera, I do agree that our primary focus is blood, and I think we really get bogged down in wording if we add all these products in unless you want to have a definition that, you know, biological product includes this, that and the other thing. And one option would be to focus on blood all the way through, and then just add a statement at the end we noted that many of the issues here also apply to organs, tissues and cellular therapies, and urge that consideration of those be included in these discussions or something along that line, but focus on the blood, which is really what we primarily heard about, although there was mention of organs and tissues in some of the presentations.

DR. BRECHER: Yes. Maybe a more all-encompassing term might be human-derived cells, tissues and biologicals. That would I think grab everything. And then rather than spelling out which organizations, we can just say regulatory agencies and stakeholders. Does that sound reasonable? Okay, governmental agencies, how's

that? Governmental agencies and stakeholders.

DR. BIANCO: I like the word "end user" or some other way. There isn't hemovigilance or biovigilance without a patient there.

DR. BRECHER: So do you want to say end user or recipients?

DR. BIANCO: Or recipients.

DR. BRECHER: Sounds a little friendlier.

Harvey?

DR. KLEIN: I didn't see anything in the statement about additional support for research in the hemovigilance/biovigilance initiative, and it seems to much of what we heard today is either research being applied to or thinking of being applied to, or a need for a research entity. So I think somewhere in there that needs to be included even though we're talking about practical application.

DR. BRECHER: Art?

DR. BRACEY: It's a detail, but under No. 2--I think we've discussed this before--it sort of suggests that the tools that are needed are already

present and operative, but they really aren't in the U.S. So really, rather than integrate, it would be either develop or create, because the tools really aren't existent.

DR. BRECHER: Well, let's try wordsmithing a little. Let's go forward a little bit. How about let's start with where it says, No. 2, "National surveillance of human-derived cells, tissues, and biologics." "Human-derived, cells, tissues and biologics." And get rid of "the blood supply."

And now when we're talking about agencies, I think it's in No. 3 if we go down a little bit. "Additional resources among and within governmental agencies," and should we say recipients or recipient groups? What would be the preference?

Okay. "Governmental agencies, recipients and recipient groups," or do you want to say "advocacy groups," or "other stakeholders?"

PARTICIPANT: How about end users instead of recipients?

DR. BRECHER: That would be okay. Is that

fine? End users rather than recipients. "Governmental agencies and end users." Go up two lines and get rid of the PHS because we don't want to limit it to just federal. No, no, that's fine as it is right now. Is that right?

Go up to No. 2. Derive is spelled wrong, it needs an "E". It's d-e-r-i-v-e-d.

Mark?

DR. SKINNER: I have a comment on No. 1 if we can scroll back up to it, please. It says, "Implement a plan to coordinate public health response during routine situations and emergencies." There's two themes that I think are missing from that sentence. The first is it's coordinate. I don't know that there is--I don't see in any of the 1, 2, 3, communication, and I don't know whether it's a plan to coordinate and communicate. It might be helpful.

The other thing that seems to be missing is we talk about routine situations and we talk about emergencies. We don't mention emerging issues which I think are different than

emergencies, so that there's something that's more of a real-time kind of information, as opposed to you wait for a situation. So I would suggest adding two words, the word "communication," work it into the sentence, and the word "emerging."

DR. BRECHER: So at the bottom there it would be "routine situations, emergencies and emerging issues."

DR. SKINNER: And I would suggest to plan, and that means coordinate and communicate or--

DR. BRECHER: Let's finish the bottom part of the sentence first, "situations, emergencies and emerging issues."

Let's go up to the top of the sentence. So what would you suggest at the top--coordinate and communicate?

DR. SKINNER: The need to develop a communication plan, a risk communication plan, I mean ensuring there's transparency in what's occurring so that people know that this is more than just an internal process, that it's--

DR. BRECHER: Right. That it's open to

the public, everybody knows what's going on.

Okay. "To coordinate and communicate."

DR. KUEHNERT: So is the public response what's being communicated or is it to implement a plan to coordinate public health communication and response? So it's you're coordinating the communication or is that not right?

DR. SKINNER: It could be "coordinate public health response and communication." Maybe the "communication" needs to go after "response."

DR. KUEHNERT: Right, because you're trying to coordinate the communication.

DR. SKINNER: Right, I agree.

DR. BRECHER: Maybe a better word would be "disseminate" rather than "communicate." Think that would do the job?

DR. SKINNER: Risk communication is the theme that we talked about, and dissemination to me means that you're going to publish a report--

DR. KUEHNERT: Because dissemination is a one-way street.

DR. SKINNER: Communication to me is a

dialogue.

DR. KLEIN: I think the one suggestion was "to coordinate public health response and communication," right? Coordinate? Communication, and then go up a line and eliminate the "and."

DR. BRECHER: Jay.

DR. EPSTEIN: I suppose it's implicit that I read this and I wonder what plan are we implementing. Perhaps it's develop and implement?

DR. BRECHER: So start the sentence with "develop and implement a plan."

Jerry.

DR. SANDLER: In No. 1 do we intend more than blood-borne infections? In other words, public health response can be an extraordinarily wide thing. Are we only talking about blood-borne infections? That's all we talked about this morning.

DR. BRECHER: I think the sense of the Committee was that we wanted to generalize this to include cells and organs and other tissues as well.

DR. SANDLER: Yes, affecting those, but is

there anything more than--I mean are we talking about radioactivity? Are we talking about anything beyond infections that are spread by blood?

DR. BRECHER: No. I think our focus is on human tissues and biologics, not on other human-made disasters.

DR. BRACEY: I thought we were also considering just in essence outcomes, whether it be infection, whether it be TRALI, which is non-infectious. We need to know--if we're going to have a biovigilance program, we shouldn't restrict it to infections.

DR. BRECHER: I'm open to suggestions on how we would modify No. 1 to reflect that thought, broaden it.

MS. LIPTON: Would it be possible--I know this is awkward at this point--but to try to get copies so we're reading it all together? I mean scrolling up and down is almost--I think if we could, you know, look at it and then see where we think some pieces are missing.

DR. BRECHER: Well, one option is we could

take our lunch break now, and come back and have our copies after lunch, and resume for another half an hour to finalize this. Would that be acceptable to everyone?

MS. LIPTON: I just think we would be operating in a more coherent fashion. Right now it's so hard because each one of us are triggering to a paragraph before. Until we see the whole thing it's hard to do.

DR. BRECHER: We are adjourned for a one-hour lunch, and we will come back and finish this.

[Whereupon, at 12:35 p.m., there was a luncheon recess.]

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

Committee Discussion and Recommendations

CHAIRMAN BRECHER: If everyone will take their seats and maybe take one minute to review the printed copy and then we'll start discussing this for the next 20 to 30 minutes Maybe we can get it back on screen as well.

I know several people have already come up to me with a couple of comments/suggestions, so let's start by maybe recognizing Jay first. You had a couple comments about what we were including and not including.

DR. EPSTEIN: Well, my concern was that--excuse me, the paradigm of cells, tissues, and biologics didn't really represent the way things are classified either within regulatory bodies or really the industry.

And I think that, you know, a more conventional way is organs, cells, tissues, and blood, or if we want to be broader than that, it would just be human derived biological materials, such as.

But I think, you know, there's a deeper issue that we really need to discuss, which is whether this committee as presently constituted in

its charter ought to be commenting outside the domain of blood. Personally, I would lean that we narrow it to our blood concerns and we can comment that these concerns may be relevant to other areas of biologic safety. But I don't think our recommendation per se ought to go outside the domain of our charter.

CHAIRMAN BRECHER: Yes, I agree.

DR. KLEIN: Jay, an idea that's been percolating on this side of the table is to sort of add a final paragraph saying that while these issues involve hemo vigilance or blood issues that the concept of collaborating with organs, tissues, and cellular components, to have a true bio vigilant system would be very desirable and perhaps where we say at the bottom here that the first step is to form the task force. Perhaps an additional step might be to expand this to other biologics.

DR. EPSTEIN: Or that the task force

itself should establish close communication with groups working in the other areas.

CHAIRMAN BRECHER: Well, let's do that. Let's do it. Let's go to the bottom. Let's write a bottom paragraph, and we'll come up and clean number two in a second.

So we're going to say--well, Harvey, you were framing this additional paragraph. How would you like us to state it?

DR. KLEIN: I like Jay's idea that perhaps we ought to be saying the task force should form alliances with--and we can go on from there. I haven't written it out yet.

CHAIRMAN BRECHER: Okay. All right. Let's move on.

MS. LIPTON: Can I ask--

CHAIRMAN BRECHER: New paragraph at the bottom, Rich. The task force should provide--should create alliances?

DR. KUEHNERT: Can I suggest this--we strongly recommend integration of blood safety initiatives with other--I'm not sure that this is

right--but with other activities--well, other activities--try other activities--related to--and then I think we need to fill in the blank about other--is it other biologics? Let's just put other biologics.

CHAIRMAN BRECHER: Human-derived biologics.

DR. KUEHNERT: Human-derived biologics. Okay.

CHAIRMAN BRECHER: And then you can go parentheses e.g.--

DR. KUEHNERT: Yeah. That's good. With other human--what would be other?

CHAIRMAN BRECHER: Human-derived--

DR. KUEHNERT: 'Cause blood is included.

CHAIRMAN BRECHER: Derived biologics. Parentheses.

DR. EPSTEIN: Well, again, medical materials, because you see again, legally tissues aren't biologic products--

CHAIRMAN BRECHER: Okay.

DR. EPSTEIN: --necessarily, even though

they sometimes are--

CHAIRMAN BRECHER: Okay. So human-derived medical materials?

DR. EPSTEIN: Medical biological materials.

CHAIRMAN BRECHER: Or medical and biological materials?

DR. EPSTEIN: Right.

CHAIRMAN BRECHER: And biologic--biological materials. Medical and biologic materials. Put an "and" in there. And then we'll go to a parentheses e.g. tissues, organs, and self-therapies.

DR. KUEHNERT: Yeah. I think that e.g. is important. I don't think a surgeon thinks of themselves as implanting or transplanting medical materials.

CHAIRMAN BRECHER: Well, put the--as a blood banker, you generally think of yourself as transplanting red cells, but you are.

DR. KUEHNERT: And so to--let's see. To allow--

MS. LIPTON: You can say to that end, we recommend the task force, you know.

I said to that end, we recommend that the

task force and then did you have some language about communicate with other--

DR. KUEHNERT: Well, I was going to end with something like to allow for a comprehensive approach to bio vigilance.

CHAIRMAN BRECHER: Yeah. So new sentence. This--what do you say. This would optimize a--

DR. KUEHNERT: This would optimize a comprehensive approach--

CHAIRMAN BRECHER: To bio vigilance.

DR. KUEHNERT: To bio vigilance. Now, if we're going to use that word, we sort of have to define it above. So that's the only caveat I would have on that. Looks good to me.

CHAIRMAN BRECHER: Okay. Period. Go up to the parenthetical statement, Rich, where you have tissues and make it a small "T." Okay. Let's go up to number two. We need to back out the other tissues from number two. Surveillance of blood and

blood products instead of human-derived cells, tissue biologics. Say national surveillance of blood and blood products.

DR. EPSTEIN: Well, again, is it just human, because we do have animal blood derived products?

CHAIRMAN BRECHER: No, I would say blood and blood products, not human. Surveillance of blood and blood products.

DR. SANDLER: Hey, while we're on two, can we take out adequate, because it's not that we're going to sanitize inadequate tools.

CHAIRMAN BRECHER: Get rid of human derived cells, tissues of biologics. Leave the including. Okay. Delete. And--so get rid of the word adequate on the first line there on number two.

DR. SANDLER: And then maybe in C, get rid of representative.

DR. KUEHNERT: I think maybe there was some interest in including--why would we get rid of representative?

DR. SANDLER: Only I mean for the same reason. We're not going to monitor unrepresentative transfusion-related--

DR. KUEHNERT: Right. Right. But I think the idea is that if you say monitoring of transfusion-related adverse events, it implies that we're going to try to monitor every single one--

DR. SANDLER: Oh, I see.

DR. KUEHNERT: But I don't know. Maybe there's a better way to word it. But I think even monitoring you can take a statistical sample, and that's still monitoring. So I think you can get rid of representative.

CHAIRMAN BRECHER: All right. Jerry, you had something to say about the preamble?

DR. SANDLER: Well, I want to get back to the point I tried to communicate earlier about what is the scope of the threat. And if you look at paragraph one. The Institute of Medicine in its report on microbial threats to health, and to me if you're going to have your first sentence defining the issue, and you're going to call it microbial

threats to health, then this whole issue all the way through it seems to me is blood borne infections. Okay?

Now, having said that, I recommend we take the first two paragraphs out. I don't think that they add much to it. I would begin with the third paragraph of the whole document by saying recognizing the need for a comprehensive national program for surveillance of blood and blood products in the United States and then go into the next paragraph, 'cause I'm not sure what all of that IOM stuff in two paragraphs adds to a document. I think it just detracts from the focus.

CHAIRMAN BRECHER: Okay. First two--

DR. SAYERS: And, and then you want part of that access of abbreviation, too.

CHAIRMAN BRECHER: So the first two paragraphs out?

DR. SANDLER: And then begin the third one with recognizing the need for a comprehensive national program for surveillance of blood and blood products, i.e., hemo vigilance in the United

States. And, you know, and just go on.

CHAIRMAN BRECHER: Mark?

MR. SKINNER: I don't think you can delete the first two paragraphs. At least you've got to go back and put in the definition of blood community, or we have to rewrite the last--the next to last whereas, 'cause blood community is where we got the definition that it included--it was included in the notion of blood community at the end.

So the second paragraph to me is critical to stay in at least to the extent that it says blood community that includes collections, transfusion services, front line conditions and representatives themselves. Otherwise, I'm not comfortable that the last--the reference to formation of a task force with representatives of blood community is defined anywhere.

CHAIRMAN BRECHER: All right. Let's define the community right there.

MS. LIPTON: So, Mark, just add that to the second paragraph then--the next one.

CHAIRMAN BRECHER: Yeah. Let's define it right where we say blood community.

MS. LIPTON: Right.

CHAIRMAN BRECHER: Just put a parenthetical.

DR. EPSTEIN: I favor dropping the first paragraph. I think the second paragraph is important to retain.

DR. BIANCO: Actually, I know several people don't like it, but this would fit so well if we have whereas--whereas.

CHAIRMAN BRECHER: But let's try not to, though.

Okay. Dump that first paragraph. And we were going to say right--and dump the second paragraph? No?

DR. KUEHNERT: If we're going to keep it, then there were some things that Mark pointed out that, I mean, do we want to say blood and plasma community and also say including but not limited to collection centers, transfusion services, et cetera? I think you had pointed out those out, so.

CHAIRMAN BRECHER: Now, how would you like us to change that, Mark?

MR. SKINNER: I just--we used the term blood community in the last paragraph, so I think we don't have to restate it down there as long as there's a clear definition of what blood community

means, and that really is those that are dependent on blood and plasma products, plasma derivatives, as well as everybody else, you know, in the process up to that to the end user point. So I think it probably is an including but not limited to or for example or whatever. I mean an e.g., and I'm happy to say blood and plasma, and we know what we mean by blood, but external readers may not. So overly defining may be better than leaving it to interpretation that somebody gets left out.

CHAIRMAN BRECHER: So then blood and plasma community? Okay. Jay?

DR. EPSTEIN: I think if you say blood product safety, that's all encompassing; that's transfusion products. That's also derivatives. Also I think there's a noteworthy absence of

product manufacturers. We're not solely focused on blood components, so when you talk about collection centers, you're talking about components, but if you talk about manufacturers, then you've encompassed derivatives. So I would say blood product safety is a shared responsibility, and after including collection centers, I might add or collection centers, transfusion service, plasma fractionators.

DR. BRACEY: There's one group that traditionally gets left out, and that's the group that actually is most involved in the transfusion process, the nurses. When we say front line clinicians, you know, we speak of the clinician, the M.D. who's attending the patient, and, you know, if there's a safety element, we really need to get that group that is the nurse involved.

CHAIRMAN BRECHER: Health care providers instead of?

DR. BRACEY: Health care providers. Yes. Generalize it.

CHAIRMAN BRECHER: Health care providers

instead of clinicians. Jeanne?

DR. LINDEN: One of the issues that were discussed is the absence of industry--and by industry I mean the true manufacturers, the device makers and the test developers and so forth--if something is not really financially going to have a good market that some of these things may not be developed. So I think one of the things that came up during discussions perhaps including that piece of the pie as well, although getting representatives could be difficult, but I don't think that--

CHAIRMAN BRECHER: We could just say industry.

DR. LINDEN: Generically.

CHAIRMAN BRECHER: Yeah. Plasma fractionators comma industry. Jerry?

DR. SANDLER: I'd like to scroll down to the last paragraph, first sentence. And I'm going to recommend formation of an HHS task force, and I think we want to name the task force to bring a focus. In other words, if you're going to say form

a task force, I would say formation of an HHS task force on surveillance of blood and blood products, which is what we've said earlier.

DR. HOLMBERG: I have a--I have some difficulty with that, and I don't know whether--I'm not a lawyer, so I don't know whether we're going over a fine line.

When several people get together by direction of the government that constitutes an advisory committee, and so we can have subcommittees, but for a specified period of time and for a specified scope. So I don't think that within the government we can have a standing task force.

Now, there's some ways that we may work around this. It--you know, we may give that charge back to ourselves, which then may become the responsibility of my office. But I'm just afraid that what we're doing is we're creating another advisory committee.

DR. BIANCO: I think that's what Jerry just said is very important, because one thing that

is troubling me--and I don't want to throw a monkey wrench--but what is the difference between this proposed task force and us? Would that be our role, and, if so, how would we carry it?

CHAIRMAN BRECHER: You look like you want to say something.

DR. EPSTEIN: Well, I'm also concerned that there's a cart and horse problem here, because I think that if we're unsure what to propose that really what we're proposing is this effort to come up with ideas. I think the lead issue is a task group to develop strategies and objectives to accomplish one, two, and three. And I see some redundancy here between what's presently the third paragraph. We believe that PHS agencies, including CDC et cetera, the blood and plasma community to work together to develop a coherent framework--well, isn't that the task force thing? So I think we've said the same thing twice, and I think we've failed to make it the key recommendation.

Now, I think I endorse Jerry's point

specifying that it should be a task force is problematic because you can't just create a government and external work group to, you know, deliver government work. But I think that our chief message is that a study should be established or a study group should be established and maybe we can be a little bit softer on the details of it.

It would be fine to say a study group should be established I think.

CHAIRMAN BRECHER: Let's--okay. Let's get rid of the redundancy of those two paragraphs first. I think we can dump the second one. I think it pretty much states it in the first one. No, not that. No, the paragraph above. Right. Yeah.

Let's delete that for a second. Now, go up and let's read the paragraph above.

DR. EPSTEIN: And so what I would suggest is that we take what's currently the third paragraph and make it the first recommendation in the recommendations. We have to wordsmith it a little bit.

CHAIRMAN BRECHER: So let's make--let's go down to number three. Get rid of the number three so that it becomes a recommendation. We recommend

to development--you got development.

DR. EPSTEIN: You're adding it at a different place than I was suggesting.

CHAIRMAN BRECHER: Where would you like it?

DR. EPSTEIN: I was suggesting that current paragraph three, which says we believe that PHS agencies, including CDC, FDA, CMS, and HRQ, et cetera, that we should make that our first recommendation.

CHAIRMAN BRECHER: Oh. So move that down to the bottom? Right toward.

DR. EPSTEIN: Well, just take the text, the following actions to achieve these objectives are recommended and elevate them above the prior paragraph.

CHAIRMAN BRECHER: That becomes number one. Jeanne?

DR. LINDEN: I have a completely separate

question, which is Jerry I think did a good job putting together this list of things that we identified in this question, and I'm not clear how that fits in with this resolution. Is this list still going to be one record as a separate item?

DR. HOLMBERG: This is what we discussed last meeting.

CHAIRMAN BRECHER: Oh. Oh.

DR. LINDEN: I think there's some detail in there that doesn't come across in the resolution. I'm just not clear what the status of that list is.

MR. SKINNER: And that was the point I was trying to make earlier; that because some of the items on this list were not uncovered in the original proposal, and that's why I mentioned risk communication and, you know, transparency--that we need to pick up the themes from here and make sure they're all incorporated into our recommendations, 'cause we've identified the key areas, and I mentioned two off of that list, but I think I agree there's others that are missing as well.

CHAIRMAN BRECHER: All right. Let's incorporate them. Let's talk about transparency and decision making first. Let's put that one in

first somewhere.

MR. SKINNER: My attempt to including transparency in decision making was talking about, you know, kind of real time communication so that it implies that it's an open, ongoing process, but there may be a better way to say it than just by--or I had suggested before lunch just adding the word communication. But that was the point that I was trying to get to when I talked about transparency.

CHAIRMAN BRECHER: Well, does it fall under number one, Mark, that such a framework needs to be transparent or?

DR. KUEHNERT: Well, I thought that it's sort of originally followed this because the first was about public health coordination and then maybe it should have had some of these sub points here. The second was about surveillance, and the third was about prioritization of potential risk and risk

communication. And so--that's--it was actually--it actually followed this but maybe didn't have all the elements, but now it has sort of gotten changed around so that was the intent.

CHAIRMAN BRECHER: Let's take one second. Corey has been standing there patiently, and clearly he wants to same something. So.

MR. DUBIN: Corey Dubin, Committee of Ten Thousand. Two things: one where you list communities, I'd like to see the word end user to reinforce what Mark said. But then there's a larger comment.

As someone who worked for many years to get the IOM study and then see the establishment of this committee, I want to be sure that in this process of trying to do something, we don't abrogate what the committee is already here to do and what this group should be doing, which is that coordination. You got FDA at the table. You've got CNS at the table. You've got CDC at the table, and it seems to me I like where you're going other than when you hit that point where it looks like

you're about to establish something else. This something else is right here already. And those of us that work to see this level of coordination certainly don't want to see it undercut in the name of starting something new. I think that's a really important point that needs to be remembered. So that's one point.

And the other point is end users show there in whatever you do. Thank you very much.

CHAIRMAN BRECHER: Jay. We'll get back to end users in a second. Jay?

DR. EPSTEIN: Well, I think that point one should be talking to the Department, in other words, DHHS should establish a mechanism in cooperation with blood and plasma community to develop a coherent framework.

And I appreciate Corey's point, which I accept. But I think what we're talking about is something a little bit more nuts and bolts now. We're not talking about recognizing the need to talk to each other and cooperate, which we do and which this committee I think does fulfill, but

we're talking about establishing the necessary institutional mechanisms, you know, linkages, databases, and so forth. I think this harks back to the presentations that we heard from state and local public health departments about the extent to which they both do and don't interface with the blood system and how there really are some gaps.

So I think the two things are not mutually exclusive. You know, we have a role to play, but there is a job to be done in terms of building a public health infrastructure that fosters blood safety and availability better than we now do.

DR. KLEIN: Well, I think this is a mechanism is what you're talking about, Jay, at least that's the way I feel, a mechanism of hemo vigilance, hemo surveillance, whatever you want to call it that really is an on the ground what most European nations do with a national program. We don't have a national program, and, therefore, we don't have national hemo vigilance. But we have--or we should have--cooperation from a variety of groups, perhaps led by HHS to develop such

surveillance mechanism.

CHAIRMAN BRECHER: Is that better, Jay?

DR. EPSTEIN: Well, it's already recommended. The following actions are recommended.

CHAIRMAN BRECHER: Oh, okay.

DR. EPSTEIN: I think it just should say DHHS should establish a mechanism, and I would say in cooperation with the blood and plasma community. So a mechanism in cooperation with, and then just strike to work together--just strike to work together and just say to develop. It's the same idea. It's just another wording.

CHAIRMAN BRECHER: It's sort of like play nice.

MR. SKINNER: Can we see the upper paragraph where blood and plasma community are defined in--are those parallel structure? So up at the top, where we talk about PHS agencies and the blood community, shouldn't they be parallel, and it be blood and plasma community, including collection centers or--I mean when we talk about blood and

plasma community one place, we talk about the blood community the other. We're not consistent.

And then that definition of blood and plasma community that's used in the first paragraph would apply wherever we use that phrase throughout the document?

CHAIRMAN BRECHER: Also we have recipients in that sentence. Is where we would put end users?

MR. SKINNER: Yeah. We use recipients and end users two different places, so we should be consistent. And end users--and I think end user is actually a hyphenated word.

CHAIRMAN BRECHER: Jerry?

DR. HOLMBERG: Yeah. I think one of the messages that we got loud and clear, not only yesterday but also back in January, from the presenter for the IOM report was that we have a critical problem with our public health infrastructure at the grassroots level. And I think yesterday we heard some great presentations from local and state and city public health. And I think what we--I would like to see here or

throughout the committee what do you feel about that. Do you think that this should--that first paragraph should include that public health sector, that grassroots public health sector?

DR. LINDEN: Yeah, I think that's a very good point that there are other players. If you just said public health agencies, that would be very generic.

DR. LINDEN: Could I just offer one in the second paragraph, where it starts comprehensive. Could we just say--could you align for delete existing and just say although fragmented elements for such a potential program now exist? And exists should be singular. And then--the word exist should not.

CHAIRMAN BRECHER: Jay?

DR. EPSTEIN: I think that the essence of this second paragraph is a finding by the committee. It would make sense to me if we said the committee finds that a comprehensive national program is lacking, because what it would do here--we've reviewed all the elements, did we not?

And our finding is that they exist, but it's fragmented and that there's a larger need.

DR. SANDLER: Anyone interested in taking out fragmented, and I don't think the elements that we heard about themselves were fragmented. They were pretty whole. It's just that they are lacking in some areas, and they haven't been brought together.

CHAIRMAN BRECHER: I think we can take out fragmented. I agree. Although elements of such a potential program now exist. Okay. We have--who else would like the fragmented to stay?

DR. EPSTEIN: I would prefer to delete it. I agree with Merlyn's argument. The elements are the thing fragmented; that you have the elements, and the system as a whole is fragmented into these elements.

CHAIRMAN BRECHER: Well, and the same thing could be true--can be said of the whole blood community. It's fragmented. Let's not go there. Why don't we read this from the top down? Okay. Let's go to the top. For the record. Jay?

DR. EPSTEIN: There was a suggestion to make it blood product safety that didn't get counted.

CHAIRMAN BRECHER: Okay. All right. Blood product safety is a shared responsibility among the public health agencies and the blood and plasma community, including collection centers, transfusion services, plasma fractionators, industry, front line health care providers and end users. The committee finds that a comprehensive national program for surveillance of blood and blood products, i.e., hemo vigilance is lacking in the United States, although elements for such a potential program now exist. The following actions to achieve these objectives are recommended:

One, DHHS should establish a mechanism in cooperation with the blood and plasma community to develop a coherent framework for hemo vigilance, including communication, surveillance, and risk assessment to respond to known and unknown threats to transfusion recipient health.

Two, develop and implement a plan to

coordinate public health response and communication during routine situations, emergencies, and emerging issues.

Three, integrate and standardize tools for national surveillance of blood and blood products, including a) monitoring and analysis of data on currently screened pathogens, b) use of repositories for pathogen and disease discovery, and c) monitoring of transfusion-related adverse events.

We recommend development of a decisional process for prioritization of blood safety activities and additional resources among and within government agencies and end users, including epidemiologic and laboratory response.

We strongly recommend integration of blood safety initiatives with other activities related to other human-derived medical and biological materials, e.g., tissues, organs, and cell therapies. This would optimize a comprehensive approach to bio vigilance. I think we can bring that last sentence up into the last paragraph.

Jeanne?

DR. LINDEN: I hate to keep pressing the same point, but red--based on--my question is

presentation does seem to be pretty flexible and is addressing some of these things, but certainly the lack of adequate funding for research was a major issue that came up that I don't see in there. And also the issue that many of these pathogens may really emerge in other countries and it's a global issue, and we need liaisons with international health organizations, as well, and you know, those--you know mention PAHO and the WHO and so forth--but just another comment along the same line.

CHAIRMAN BRECHER: Well, how would you recommend we reword this to include a global outlook? Monitor the globals and monitor the global situation?

DR. KLEIN: You could put that down at the bottom with the tissues, organs, and cell therapies.

MS. LIPTON: You mean you could say other

activities within the U.S. and within and outside the United States related to other--then you can kind of pull in.

CHAIRMAN BRECHER: Yeah. Let's do that.

MS. LIPTON: We also dropped a word, by the way, that Art mentioned in number three. He said to develop tools not just--it was develop, integrate, and standardize.

CHAIRMAN BRECHER: At the beginning of number three. Develop comma integrate. Oh, okay. All right. In the paragraph under number three, we recommend development of a transparent decisional process. Can we get the transparent in there, as opposed to opaque? Jay?

DR. EPSTEIN: I have to say that this paragraph troubles me because we have not actually discussed that subject. The subject of what would constitute an appropriate decisional process for the blood system or really any public health issue is a subject in itself. You know, transparency, participation of stakeholders, use of objective analysis tools, risk communication, and risk

analysis. You know, tools of communication. What have we done in the last two meetings focusing on decisional process? I don't think that's what we've been talking about.

CHAIRMAN BRECHER: Well--

DR. EPSTEIN: Now, I agree that it's a very important element of anything that might come out of this, but are we prepared to know what our committee really thinks in that domain?

CHAIRMAN BRECHER: So you are recommending that we essentially drop that paragraph and let the chips fall as they would when they go through this?

Would it stand alone if we dropped that paragraph?

DR. EPSTEIN: Well, maybe instead of trying to characterize that, we say a suitable process? See the problem here is when you start giving it its attributes, then you're approaching that subject very naively.

CHAIRMAN BRECHER: Okay. A suitable process. And we'll imply that it's a transparent suitable process. Suitable process.

MS. LIPTON: Well, I thought a little bit of what Jay was saying was that all of this is sort of the prelude then that we would hope this would

be used in the development of, but we didn't talk about the elements of that, and I was just commenting we heard about a lot of things today that if you put it on a priority, I wouldn't put it on a high priority in terms of what were concerned--not the highest priority of other issues we've talked about.

DR. KUEHNERT: Just as a clarification on that paragraph. I think for it to make sense grammatically, it would be prioritization of blood safety activities and--'cause otherwise, it says prioritization of additional resources. Maybe it's and provision of additional resources? So for prioritization of blood safety activities and provision of additional resources.

CHAIRMAN BRECHER: Why can't we just shorten this whole paragraph to we recommend development of a suitable process for achieving these ends? And we've listed them as one, two,

three? Paul?

DR. HAAS: Excuse me. I like the shortening of it, but I'm--somewhere--I don't know if it's with this or some other document. If we don't say something about the need for getting additional resources, they're going to look at this and say, you know, that's wonderful. What else do you want? It's a huge request, and I think we need to get it out there, but I think we also need to say you've got to bring extra resources to the table. I don't know how to do that, but I think we've got to get it there.

CHAIRMAN BRECHER: Well, so then we recommend identification of resources to achieve these ends?

DR. HAAS: Yes, I think it should be a standalone statement, so it's clear.

CHAIRMAN BRECHER: Okay. We recommend identification of resources to achieve these ends. And I know--well, Jerry is not at the table. I thought he was going to say new resources. We'll say it for him. Resources. R-e-s instead of

r-e-c. To achieve these ends.

Now, do we need the rest of that paragraph?

DR. EPSTEIN: Well, I think we're getting away from the core idea, which was the decisional process. It's true that there's a need to call for resources, but I think we have two ideas working and now one is subsuming the other.

DR. HAAS: I don't like it in that paragraph.

CHAIRMAN BRECHER: New paragraph? Okay. Put a--yeah. Now, let's work on this paragraph.

DR. HOLMBERG: Let me just as you're thinking about this, does the committee--do I hear the committee telling me that at one of our next meetings we need to go through what the developmental process is and spend more time on that?

MS. LIPTON: I definitely think we really haven't talked about how we prioritize and what we think are the appropriate elements, and so I think the answer is yes. We all think that we should use

this to do that, but we've always kind of danced around that.

DR. HEATON: The fact is concerned about a major focus on additional resources. We as a committee can't make every recommendation contingent upon more and more and more resources unless you want to see the health care tax bill rise quite dramatically. So there is a linkage here between prioritization and appropriate use of your existing resources.

CHAIRMAN BRECHER: Well, we just said additional resources. We didn't say they had to be new. They could be recycled resources. The question then is do we want to move ahead with this recommendation. We can come back and flesh this out in another meeting or do we want to just table this and wait until the next meeting? I don't think we want to wait three months to get this out there. So I would--

DR. EPSTEIN: This is already the second meeting on this topic.

CHAIRMAN BRECHER: That's right. Jay?

DR. EPSTEIN: I think that items two and three are really subsets of one because I'm confused that we're adding recommendations that are

unnumbered. And so what are the recommendations? See I think two and three are sort of the things being developed under number one, and indeed the call for resources could be a subset of that as well. And then I think you have number two, which is that you recommend the development of a suitable decision making process.

CHAIRMAN BRECHER: Okay. I think that's correct.

DR. EPSTEIN: Yeah. Make that indent A.

CHAIRMAN BRECHER: So get rid of two and three.

DR. EPSTEIN: Yeah.

CHAIRMAN BRECHER: And go down to three and that becomes B.

DR. EPSTEIN: And then that's three and then there's four--I'm sorry. That's two and then there's three.

CHAIRMAN BRECHER: Can we go up a little

bit? So get rid of the we recommend in two and three, because we've already said recommend as the preamble. And then go down to below that. Would this be our fourth recommendation then? I think so. So this will be number four, and get rid of we strongly recommend.

DR. KLEIN: I would like to see under B that that be maybe a D under B that would say provision of resources for additional research in this area.

CHAIRMAN BRECHER: Well, that's a little redundant with number two, though. In other words--

DR. LINDEN: Yeah. Could I just come at--

CHAIRMAN BRECHER: --additional resources to achieve this end. These say research occurred--

DR. LINDEN: My point I think even the new D can be combined. I mean I actually don' think we would use the repositories for disease discovery for one thing, and I think it's more than discovery. It's characterization and so forth, but that's just one example.

CHAIRMAN BRECHER: Well, why don't we just say pathogen and disease research?

DR. LINDEN: Yeah. I think we need more

generic. In fact, my thought on A, B, and C is they're all too specific. I think there might want to be other things. There are--these are examples. I'd rather say something like including such things as this, this, and this, but it's not going to be limited to. Otherwise, the thing that comes across is very specific. I mean I'd personally delete it, but there are at least examples.

CHAIRMAN BRECHER: All right. Let's go to B and why don't we. Use of repositories for research. Get rid of pathogen and disease discovery. I mean it could be other.

DR. LINDEN: Well, and other--it's not just repositories.

DR. KLEIN: And repositories aren't just for research. They're used for surveillance. I mean it's--

DR. KUEHNERT: That's what it's saying at least. It's talking about surveillance, and maybe

doesn't--'cause it's talking about developing and integrating the standardized tools for--

CHAIRMAN BRECHER: All right. Let's just make it use of repositories. I almost said suppositories. Use of repositories, and get rid of pathogen and disease discovery. It's getting late.

Yeah, Jay.

DR. EPSTEIN: Yes. Under item B, I would like to see us add the word further develop, integrate, and standardize, because, after all, as you go through this list, we do many of these things; right? You know, we do have repositories. We do have FDA reporting systems. We do have CDC surveillance studies. But it's the further development and integration.

CHAIRMAN BRECHER: All right. Are we approaching happiness? Mark?

MR. SKINNER: I just--I want to come back to one thing that we said before. I mean I recognize we all have day jobs, and that we do this, and I mean we talk about additional resources. I mean we would be doing this but for a

function of time, and I'm still troubled a little if there's some way to indicate that this needs to work in collaboration with the work of this committee or not-that we're not subservient to it or removed from the process when the Secretary is doing it. And I don't know if there's a way for the committee to stay integrated and not lose our focus, because these are a lot of things that we continue to talk about as well. And if it alls goes internal or to somebody else, I'm troubled.

CHAIRMAN BRECHER: So in concert with the advisor committee?

MR. SKINNER: I don't know whether there's a way to suggest that it be done in collaboration with or in coordination with or report back to on the plans for further discussion of this committee or somehow that we keep a nexus in this committee of these responsibilities.

CHAIRMAN BRECHER: Scroll down. Let's see at the end. See if we can fit it in the end?

Go up. To the--not right now. I think that's certainly implied. So we could say in Part

B that it could be another D that such activities would be coordinated with the Advisory Committee on Blood Safety and Availability? And write ourselves in. Should be coordinated with the HHS Advisory Committee on Blood Safety and Availability. Should be. Conditional. Should, not shall. Shall is like must. Jay?

DR. EPSTEIN: I have trouble with this point. This is an advisory group, and I see us talking about creating, you know, the effector mechanisms, the actual databases, the actual data gathering tools, and that's just not the work of the advisory committee. Now, you know, coordinate is a pretty broad word, and you could coordinate most anything with anything I suppose, but we're really not part of the end product that we're calling to be created, so I'm not sure what we're asking ourselves to do when we're saying it should be coordinated.

CHAIRMAN BRECHER: Wait. We're an advisory group. We're not really a coordination group.

DR. BIANCO: But we can request reports from these groups.

CHAIRMAN BRECHER: Let's drop it. Take it

out. Take it out. Okay. Karen?

MS. LIPTON: One of the things that--I mean I know that we said that the language of this is in there, the language that we had all passed out to us, but this is talking about a strategic plan, and surveillance is really one part of a strategic plan, so I think what's troubling me is getting--I mean we use these words, but we've taken the idea of a strategic plan, which does include decision processes and priorities. But now we're leading just with surveillance as if that's the strategic plan. And I guess I'm just a little troubled by the order in which it has evolved.

And so this is part of it, but I don't think this is the plan, because where is the research and stuff we talked about? Where is the greater role on communication, not just of issues that come out of hemo vigilance, but communication on all sorts of issues to-so. I mean I don't know

what to do with it at this point, but I almost feel we kind of got off on a tangent, but we didn't define the whole plan first.

CHAIRMAN BRECHER: We could just say that this would be part of a larger strategic plan for blood safety and availability. Just so they know that there's more of it than just this piece. No? Jeanne?

DR. LINDEN: Yeah. I continue to really think that this document does not get across all of the things that we have identified. And I think it's way too detailed, and I think those details are something that comes down the line by this task force or whoever. I mean getting into, you know, that there's repositories that--I mean I just think the old original two doesn't belong there, and, you know, Karen and I agree. I don't think really the structure of this entire thing gets across really what we want to say, but I don't think we can have, you know, 25 people sit here and write a comprehensive document either. And I understand the urgency.

CHAIRMAN BRECHER: Jerry, do we have any other options for doing a recommendation from the committee? Can we do a teleconference to finish

this? Jay?

DR. EPSTEIN: I'm going to make things worse, because I think that we leaped to crafting the recommendations without actually having had our discussion about what we thought was right and wrong about the systems and what the candidate fixes might be.

So I mean I'm not uncomfortable with what we're calling for, because I happen to agree with it, but I think that the document lacks the set of findings about where we think the gaps are. I mean what's the problem we're trying to solve?

DR. ROSEFF: I agree. We listen to people speak for a day, and we've never discussed what they spoke about. So I agree. That's why we're being general, then we're specific, then we're general, because I don't think we've come to consensus about what we think about what we heard.

CHAIRMAN BRECHER: Well, we're at the

problem now that we are about out of time for this. So we have a couple possibilities. We could go ahead and bless this recommendation with the caveat that we need to have further discussions, which we may be able to do offline, on a teleconference, but probably not--'cause it's not public. It's not public.

Or we could go on to our next area and have maybe a subgroup try to draft something to be seen at the end of the day, which still doesn't give us the discussion of these points.

MS. LIPTON: I mean I think yeah to Sue's point, I think it's the discussion piece where we didn't come to consensus. We think we've come to consensus on this, but we certainly haven't come to consensus on a lot of other issues, and I don't know I'm thinking maybe that we ought to have a subcommittee put this together and actually look at it in June instead and have the pieces of it and say here's what we think we found or didn't, and have that serve as the basis for a discussion. I mean I think we talked about this before. I think

we have so man presentations and just not enough time to have the committee do the work of the committee. I think we need time for the committee to operate as a committee.

CHAIRMAN BRECHER: Jerry?

DR. HOLMBERG: Well, what I hear you saying and one of the ways that we can get out of this is we can have a subcommittee work on this document. We can come back in September and present what the subcommittee has found and have discussion on that, on the findings, the transcripts, review the transcripts from January, from this meeting, assign different people within the subcommittee to really draw the points out, and then discuss this as a committee. But once again, everything that is discussed in a subcommittee has to be brought back to the full committee, once again because we have to be transparent and open about everything.

CHAIRMAN BRECHER: Okay. So what--the recommendation is that we table this discussion for now, bring it back--we'll designate a subcommittee

to review this. We can look for volunteers now. Who would like to serve on this subcommittee?

Mark, Karen, Sue, Celso, Julie, Andy.

DR. LINDEN: Jim, now was asking if we should send it back to the same--

CHAIRMAN BRECHER: Yeah. I guess that's true. We would be the emerging protections group, so it would be Jeanne's subcommittee. That's true.

DR. HOLMBERG: But then one person that is not designated on that committee that is new, Dr. Roseff, wanted to participate. We can put her on to that committee.

DR. LINDEN: And Karen volunteered also, and I think her input would be valuable.

CHAIRMAN BRECHER: Okay. So Karen and Sue in addition.

DR. LINDEN: Right. Okay. Never mind.

DR. HOLMBERG: And, Art, did you want to participate?

DR. BRACEY: I'll participate.

CHAIRMAN BRECHER: All right. So we're going to close this discussion for now. We're not

going to vote on that recommendation because it's going to be part of the bigger picture, and we're going to move on to our next topic. As I have in the past, because of many of my research interests in bacteria and platelets, I'm going to step down as chair and Mark Skinner will chair this next session.

DR. KLEIN: Before you do that, Mark, can I just ask if this subcommittee going to provide the entire committee with some written document before the next meeting so that we can come prepared to discuss it? Is that the idea?

DR. HOLMBERG: That is the idea. Also in the spirit of openness and disclosure, I also wanted to inform the committee that one of the companies that will be presenting today is Haemonetics, and I was formerly employed by Haemonetics, and I do own stock in Haemonetics. So.

MR. SKINNER [presiding]: We're going to turn out attention now to bacterial detection methods for release platelet concentrates, and our

first presentation this afternoon will be from Larry Dumont with Gambro BCT. Larry?

Update from Gambro BCT

DR. DUMONT: Mr. Chairman, ladies and gentlemen, thanks for this opportunity to present to you today on--there we go. Technical challenges.

I will apologize. I don't have any gels to show you today, so we'll try to keep your attention.

I want to give you an update on seven-day platelet storage collected with the COBE systems and release test to do with the bacterial microbial detection systems.

The objectives are to number one, provide a brief history of the 510(k) clearance that we just recently obtained; highlight issues related to two-bottle testing, also known as the infamous anaerobic bottle; and to present an overview of the Gambro BCT post marketing surveillance study.

As background, there is actually two clearances that are associated with this project.

The first was obtained in September 2003, where we obtained clearance for the platelet function itself and our particular bag. And this is where seven-day shelf life in the platelet collection ELP bag, when coupled with a 100 percent screening for bacterial contamination, was used prior to transfusion. The kicker on this was it had to be a marketed clear device, which didn't exist.

Subsequent to that, we submitted and tried to tighten that up a bit, and actually linked our detection or our collection system with the bio Merieux bacterial alert detection system. So other than that, the wording is the same.

I do want to point out one thing, though, for this committee has actually been very helpful. Those that know the code of 510(k)s, on this first one you'll notice that was actually submitted in 2003 or 2001. It was cleared in 2003. And then there was quite a hiatus from 2003 to 2005, but what's actually quite remarkable and actually speaks to this committee and also the work of FDA was that it was submitted in the end of 2004. In

fact, this is 91 calendar days, including holidays, which is quite remarkable.

I just want to give you a little update on people that we collaborated with on this project. They'll be shown here. Actually, we didn't. We didn't collaborate with anybody. And there's been a lot of confusion about that. So I want to make it clear that we did this solo. And we didn't talk to even a lot of people in our own company about this project.

Just as a brief overview of the release test is that sampling has taken the platelet sample 24 to 36 hours post apheresis collection. Four ml aliquots of the single donor platelets are put into one aerobic and anaerobic culture bottle. And then those may be released into inventory if there's no growth indicated after 24 hours on tests.

And then the culture bottles remain on test until they either turn positive or the expiration date of the product.

And any microbiology that's indicated or clinical follow up is basically we refer to

standard practices in the industry for directions on that.

Now, the basis of the approval. First of all people, can reference the FDA web site, and we hope that they have the correct summary of safety and effectiveness up now. But please reference that.

As evidence for this release test, we submitted data that we compiled with several people from the U.S. blood industry. They cooperated with us under a non-disclosure agreement. And we submitted results of over 405 cultures of single-donor platelets in the one bottle aerobic test. And you can see that the true confirmed positives were 178 per million. You can see the confidence intervals there.

And if we included those products that were indeterminate in outcome--these were ones where they basically could not go back and do a confirmatory test--we estimate 291 per million single-donor platelet products.

We had one center that was doing

two-bottle tests, in both the aerobic and the anaerobic, of which we had 6,600 single-donor platelets that had both of those tests done at 24 hours, and so the estimate there is a little bit higher number, 606 per million of true confirmed positives.

So this was a major part of the basis of approval that FDA reviewed. So I warn you this is now a marketing slide. But it actually has some good data in it. So at the top here are key milestones. These are activities. You're going to see several of these come up. These are activities that are executed by Gambro BCT, and then these are actually things that the blood center has to do. And we're going to see what this roll out plan looks like.

After we have received approval, of course, we did all our press release and Q&As and informed our people of what was going on also.

Through work with FDA and also other groups, new product codes were established for seven-day stored single-donor platelets. And once

these were established then, this actually allows the blood centers to, as of right now, they can start changing their computer systems to put in these new codes.

And actually quite a surprise to me, this is a really major undertaking for the blood centers to do that. Some of them, it takes a long time.

We've also come out with our instructions for use for seven-day platelets and how to do the release test, and then with that information, the blood center can start doing PAS application and approval and also start modifying their own SOPs so that they could implement this.

We're right now in the throes of getting a final post marketing surveillance protocol submitted, and reviewed. FDA has that for review. And we think that this will all be completed and we should be able to have our complete information package ready first of June, which isn't very far away. And at that point, blood centers should have everything they need to begin implementation of the seven-day platelets, and we believe this is going

to happen this summer.

So I want to talk a moment on the two-bottle test system. I do want to point out that the original submission was made by Gambro suggesting a one-bottle, the aerobic test system only. However, when FDA reviewed the data, they concluded that the evidence did not support exclusion of the anaerobic bottle at this time. And, so, therefore, we amended our submission and then we have a two-bottle test system.

In addition, once should note that there are data to suggest that the two-bottle system is a superior test.

These are the data that were actually in the submission from the surveillance results in the year 2004 from one center. Sixty-six hundred products. And these are the specifics. There were four true positives, two of those, the top two, you can see the anaerobic bottle listed here on the right probably didn't help at all. This is hours to detection. However, in these two coagulation negative staphs, you can see that the aerobic

bottle was negative in both of those. Those were detected in the anaerobic bottle, and those products were interdicted before they were transfused. There were two other instances that were actually on follow shown to be false positives, and then there was a few P. acnes as we would expect that became positive at greater than three days of culture, and those had been transfused. There was no clinical problems associated with those transfusions.

So the question is is it one bottle or two bottle, what should we do?

Well, those that would support only the aerobic bottle, these are some of my ideas and what I've heard and what people would suggest would be the reason for that. Number one, you waste fewer numbers of platelets. Number two, the cost of supplies is less, because you only buy one bottle. And in our setting, with platelets that are stored aerobically, only aerobic organisms are important.

And the clinical case incidents that had been reported do not support--yes, that had been

reported--do not support the need to detect obligate anaerobes.

Action needed to follow up on positive products, you're not going to have to do that for those later bloomers. And then finally the time to detection is too long to make detection in an anaerobic bottle meaningful. So these are some of the arguments for only one bottle.

On the flip side, though, there's the argument that increasing volume increases the sensitivity of the overall test; number two, that obligate anaerobes are clinically important in platelets; and number three the anaerobic bottle is not just a bottle that doesn't have oxygen in it. It has actually got enhanced media and especially with facultative anaerobes, they may like that media mix better and they may come up faster. And that's what that last point is that we may able to detect those interdict sooner.

So those are some of the arguments, and there's a list. I won't read them. You can read them of facultative anaerobes that this might apply

to.

So we thought of in our development of a post-market surveillance study that there may be three major areas where you might be able to make a case for using the anaerobic bottle. And the first, of course, is that obligate anaerobes will only grow and be detected in that bottle. And then we have listed some likely observations that we might see in our study if that would be the case. There would be discordance between the aerobic and anaerobic bottle, with a situation that would be in favor of the anaerobic bottle for the release test. And indeed the bugs would be identified as obligate anaerobes.

So there is some literature to support that. We've seen these two references a lot, one from McDonald and one from Blajchman on some clostridium cases, one with pool platelets that resulted in the fatality, and one with red cells that did not result in a fatality.

A second area would be the facultative anaerobic organisms grow better or more rapidly in

anaerobic bottles. And again, we would expect to see a discordance between the two bottle results. And the literature it's kind of out there on that. These are some data from Holland, from my friend Dirk De Korte. I'm actually wearing a tie in honor of Amsterdam today. But--the--you can see the results from the year 2001, and, in fact, there is a significant discordance between the aerobic bottle and the anaerobic bottle results for them. On--we're not even talking about P. acnes, which are lumped down here, but we got coagulation negative staph and bacillus, and you can see that for the number of totals that were seen, there were just a few that were positive in both bottles.

So there's some suggestion that may be the case in the United States. Of course, it's difficult to generalize from Europe, 'cause things are actually very different in their products.

And finally, increasing single-donor platelet test volume may meaningfully increase the release test sensitivity, and again we would expect to see a discordance between the two products, but

it would indicate that indeed that it's just a matter of volume, and we would expect that to be a pretty even split. And there are data that would support that. The bioMerieux K on pool platelets, these are spiking studies with three different organisms. And ten different sets of bottles. This indicates that with the Klebsciela, it spiked at cfu per mL, and the aerobic bottle listed on the left and the anaerobic bottle listed on the right. The aerobic bottles detected all of the Klebsciela. There were two missing, so two negatives in the anaerobic bottle.

But when we move to the other bugs, you can see that there's kind of an even split where we kind of toggle back and forth where the positives are. So data like this on a larger scale might suggest it's simply a volume issue, and maybe there's other ways to deal with that.

So the two-bottle test system. Does the two-bottle test system provide a clinically important, as well as practical improvement in the safety of platelet products? Well, actually my

conclusion at this point is that we're probably in a condition of equipoise, and we'll probably hear more today some strong opinions on either side. And actually that's probably a good indicator when you have really strong opinions on each side maybe this is where we are.

So when you're there, what do you do? Well, I suggest what you do is you listen to the data, and so that's what we're proposing to do here in the next few months and a couple of years.

So as just an overview of our post-market surveillance study, and I will point out that FDA is still reviewing the final protocol. We have not locked in full agreement with them on this yet, so it should be coming soon, though.

Basically, we are proposing that we will have a two-tier structure. In the first tier, everybody that would implement day-seven platelets with our system, between 24 and 36 hours, they would take a sample, inoculate the two bottles. They would incubate that and see what the results are.

This is called the release test and everybody would do that. For these people, we would ask them to--we would actually require them

to submit all their data on all their testing results, all their microbiology, any relevant clinical follow up that would occur because of transfusion of a contaminated product or clinical event.

And you might appreciate that some of the customers that would be in this tier one are very small, and it would be very difficult to enroll them in anything more intense than that. It would take a tremendous amount of resource. The burden would be very high.

Then as the product is stored, there will be some products that will outdate, we anticipate. And there will be large blood centers that we enroll in a tier two system, and in the tier two system, samples from outdated products will be recultured, using the two bottle test system. And we're targeting for approximately 50,000. I know you've seen that number before. And we'll compare

those results to the initial test, and that's how we'll make a determination of the results.

I'm not going to go into anymore exact detail on that, 'cause we view that as proprietary right now. But I do want to list the hypotheses. I think these are important.

The primary hypothesis that we'll be testing is actually came from FDA words at this meeting, August last year. Seven-day single-donor platelets when tested using the BacT/Alert device and methods as described will not present a greater risk of a detectable bacterially contaminated platelet unit than five-day single-donor platelet untested for bacterial contamination. So that's our primary hypothesis.

A secondary hypothesis we're essentially going to ask the same question, specifically just for the aerobic bottle, and we will also make use of both of those culture results to make a determination if the anaerobic culture actually makes a meaningful contribution. And part of that meaningful contribution, of course, is not only

detecting a bacteria, but also be able to detect it in time to interdict it. It doesn't help you very much if you don't pick it up 'til day seven. So that will be some of our outcomes, secondary outcomes.

Specific aims will be to determine the specificity, sensitivity, negative predicted value and positive predicted value of the two-bottle release test and determine the prevalence of bacterial contamination for untested and for two-bottle BacT/Alert tested single donor platelets, and then also again looking at the performance contribution of the anaerobic bottle.

With that, I thank you, and we'll take questions.

MR. SKINNER: Thank you. To make certain we have time to hear all of our speakers, I'd like the committee to keep their questions just to a minimum. Celso?

DR. BIANCO: Thank you, Larry. It's just one quick question. Oh, congratulations for being the first.

I don't know if you are addressing the conflict between aerobic and both bottles exactly in your protocol with your secondary hypothesis. I

think that the only way to answer the--this is my opinion--the question about volume would be if you would run in parallel one aerobic bottle inoculated with eight mL instead of four mL, that would give you the true response. Here you have half of the inoculate that you have in the two bottles.

DR. DUMONT: Sure. Now, that's a good point. This is one of those difficult things that there's probably 50 good designs, and we really had to target to verify our release test.

However, I do know that in Ireland, they're running eight to ten mLs. in an aerobic bottle. The Welsh Blood Service is running both bottles with eight to 10 mLs. So data like that will be coming out.

DR. HEATON: Larry, I note that you collect sample 24 to 36 hours after collection. When do you read the result?

DR. DUMONT: The results are read every 10

minutes.

DR. HEATON: I know, but let me--

DR. DUMONT: Until you take it off.

DR. HEATON: --I mean at what is it declared negative and available for release? Twenty-four hours after the--

DR. DUMONT: Twenty-four hours after it's been on test.

DR. HEATON: After it's been on the test.

DR. DUMONT: So that means collection; 24 hours later take the sample, inoculate the bottle, put it on test; 24 hours later you could declare it releasable.

DR. HEATON: Okay.

MR. SKINNER: Other questions. Thank you, Dr. Dumont.

DR. DUMONT: Thanks.

MR. SKINNER: Our next speaker will be Dr. Stein Holme, with Pall BioMedical, who will update us on their activities.

Update from Pall

DR. HOLME: Okay. First, I'd like to

thank you for the invitation and the opportunity to give an update, where we are with the Pall Bacterial Detection System, eBDS, and seven days of storage, as well as where we are with the Pall Pre-Storage Pooling System for random donor PC, both five and seven days of storage.

First eBDS and the seven-day storage update. The eBDs was approved February 2002 for QC use. And a 510(k) submission for seven days of storage with the eBDs was submitted to the FDA March 2005, where we provided field data with testing conducted under actual routine use conditions, as well as a post-marketing protocol. And this submission is intended for release use indication allowing for seven-day storage of all single product, apheresis and random donor platelets already improved in terms of storage quality.

And the random donor platelets that are approved for storage is the product from all the RCPL system with CLX as the storage container.

Here's the field data that was submitted

to the FDA. This was the results of 114,828 tests performed at 23 blood centers in the U.S., March to November 2004. We had all these 103 fail results where 23 was confirmed true positive. It was the presence of bacteria in the eBDS pouch. And we had 43 false positives, where there was no present bacteria in the eBDS pouch by culture.

We also had one missed detection, with sepsis with confirmed presence of bacteria in the mother bag by culture. Therefore, it was that--we later was able to look at these particular organisms and were finding out that it was able to detect by our eBDS system, so we think there was a case where the level was too low so the bag was missed just by a sampling error.

Okay. This is supposed to be a table here. I guess it didn't come up. Basically, the table shows that the true positive rate was about one per 5,000, which is similar to what has been found with other culture methods. You can see here this is the results that was presented at the last advisory committee meeting. The results were from

the Task Force Surveys on Bacterial Contamination that showed a true positive rate of one per 4,023, which was very similar to what we found with the eBDS system, which was one per 4,990. And most of the data was with BacT/Alert was 27 or 30 blood centers that used BacT/Alert testing in this study. Eighty-five is aerobic bottle only, and there was 15 percent that used both aerobic as well as anaerobic bottle.

Regarding--Larry mentioned the issue about the anaerobes, and I just have a few comments about this. Anaerobic bacteria are rarely implicated in sepsis reactions. They have been reported in Japan, less so in Europe, and as far as I know not reported at all in the U.S. for the FDA. And that at least was based on search of the literature with the latest information as was from 2001.

More recent report from the hemo vigilance studies in U.K. This is from the SHOT Report for 2003. And that shows that all the reported bacterial transmissions in the past eight years, from 1995 to 2003, have been from aerobic

organisms.

Going to pre-storage pooling, I will give you an update. Here's the system for pre-storage luekoreduced pool platelet product. We see the leads that connect to the individual PC to the left on the figure, which is connected then to a storage bag, which has the eBDS in line.

This is a system with--for non-leukoreduced PC, and you can see here the leads which connect to the individual PC. It goes first into a pooling bag here, and the content of the pooling bag or the non-leukoreduced product is then leukoreduced by filter ending up in a platelet storage bag, which again has the bacterial detection system in line.

The studies with pooling set number two is just about to start and should be complete--we expect it to be completed approximately at the same--in May next year.

Coming back for system number two, which is for the already leukoreduced pre-storage pool platelet product. This system is meant to be

suitable for pool leukoreduced PC from whole blood collection with the Pall Leukotrap RC-PL system using CP2D as an anticoagulant.

It should be able to store pools of four to six units with a volume of the individual yield that's ranging from 40 to 65 ml in plasma for five days with total yields ranging from 2.2 to 5.8 times 10 to the 11th.

And it's supposed to be used with an approved bacterial detection system, the eBDS.

What's the concern and challenges with pre-storage pooling of random donor PC? There's a risk of elevated bacterial levels after storage. We have--it's about lymphocyte activation and generation of harmful levels of cytokines, complemented and clotting factors, and also issues about platelet storage quality. The bag needs be able to handle four to six random PC which have a large variability in both yields and volume. And that's probably the last is the bigger challenge.

We now have conducted completed studies, which demonstrate satisfactory in vitro and in vivo

quality and also lymphocyte activation levels at five days of storage with pooled random donor platelets stored in the CLX-HP bag.

This line shows the recent platelet capacity studies we have done with the CLX-HP storage bag. We see here on the Y-axis, this is the pH after five days of storage. On the X-axis, there are various yields that have been used in the container. And we can see that the yields range from two to six that are still able to maintain a satisfactory pH above levels of 6.6. The six units you see here was obtained by having a very highly concentrated platelet product as obtained using a 40 mL PC volume of the PC. Excuse me.

We also just complete detection of bacteria in the pool platelet product, with the eBDS. The studies were done in Albuquerque, New Mexico, and also in Covina about Pall Medical. This abstract has been submitted to this year's AMEB meeting.

Briefly, the study design was constructed to simulate the actual use conditions as closely as

possible, in which a PC was inoculated with a dose of one to 15 CFU per mL of various bacteria. It was then stored for 24 hours at room temperature and after that the PC was mixed with five other non-inoculated stored PC and samples were then taken for culture and eBDS testing immediate after pooling. And the eBDS samples were then incubated for 24 hours at 35 degrees Celsius for measurement of oxygen to determine pass/fail.

Unfortunately, the tables do not come up. And I have no idea. The tables basically show we had 134 tests and out of those 134 tests, there were 37 that had levels below five CFU at the time of sampling. So what I'm saying is that 30 percent of the units that were tested after pooling had levels below five CFU per mL, and basically we--out of 134 tests that were made, we only missed one case. This was a case where staph epi had a level of 1 CFU per mL at the individual PC, so it was clearly a statistical sampling error that we didn't detect in this case.

So just to summarize where we are in terms

of the pre-storage pool platelet product, and the FDA submission. We have completed the lymphocyte plasma activation studies, in vitro, in vivo storage quality have been completed. Capacity studies that were requested by the FDA with low and high yield and low and high volumes have been completed and found satisfactory and finally we also have now completed studies on eBDS testing of bacteria with pool platelet products. That has been completed and found also to be satisfactory. We're basically getting very much the same sensitivity level of detection, down to one CFU, as we've seen with standard single donor platelet product.

We have--are probably going to submit to the FDA either this week or next week on this pooling system.

So in summary, what are the advantages in terms of blood safety and availability? You have a pre-storage pooling with an inline sensitive bacterial detection system. Basically, we have one bacterial test for four to six random donor PC,

which enables continuous use of random donor platelets as an important source of platelets, and also enables improved bacterial detection compared to the current practice.

We heard at the last VSAC meeting in January that there were problems with the current practice of bacterial detecting using random donor platelets, using a dipstick, pH, glucose and so forth, that these were tests that were not insensitive enough for detection of bacteria.

Thank you.

MR. SKINNER: Questions for Dr. Holme. Celso?

DR. BIANCO: Just one question, Dr. Holme. We just heard from Dr. Dumont from Gambro that when they presented their materials to FDA, FDA sent them back home and said we don't want just aerobic. We want both, aerobic and anaerobic. What are you going to do if they do the same to you?

DR. HOLME: Well, I really don't want to argue because--I don't want to argue about that. There's--the other argument is going to be that

there is no--as far as I know, there is nothing out in the literature that shows there's any clinical relevance to--

DR. BIANCO: Oh, I happen to agree with you. I'm very skeptical about the need to--

DR. HOLME: So I don't know--

DR. BIANCO: But they just did that. Do you have an alternative?

DR. HOLME: Well, the alternative could be as--as you mentioned also that there could be an issue about sampling size. So by having a larger volume taken for bacterial testing--instead of having two bottles, you can have with our pouch, they can have--if you test a larger sample, you could potentially accomplish very much the same thing as having a one aerobic and anaerobic bottle.

MR. SKINNER: Mark?

CHAIRMAN BRECHER: So, Stein, are you going to have an economy-sized eBDS for the pools, of a larger volume?

MR. HOLME: I beg your pardon?

CHAIRMAN BRECHER: Are you going to have

an eBDs with a larger volume than the current?

DR. HOLME: It depends on what kind of outcome we'll have in our negotiations with the FDA. What we will be planning to do. But, you know, potentially that's--that could be done.

MR. SKINNER: Thank you. Our next speaker this afternoon will be Dr. Sherrill Slichter, the Vice President of Research for the Puget Sound Blood Center, who's going to update us on the work of Haemonetics. She actually is going to make two presentations and then followed--we'll also receive an update on whole blood-derived platelets.

Update on Haemonetics and WBDP

DR. SLICHTER: Well, thank you for inviting me. I would just tell the committee that I have no association with any of the products that I am about to discuss. Haemonetics was invited to come before the committee, and Leslie Rose from Haemonetics asked me to give the presentation because Ed Snyder and I did this Haemonetics studies on extended stored platelets. They supported those studies, and they've also supported

my trip here. Once they see how much I'm going to discuss their data, I may have to walk home. But I really want to kind of share with you, if I can, studies that we've been doing with apheresis platelets as well as random donor concentrates and kind of go through the data with you.

Some of the last slides in your packet are labeled confidential because this data has not been published, but I certainly did want to share the information with this committee.

So as a background basically for about two years, both platelet concentrates and apheresis platelets were approved for seven-day storage at room temperature. However, the storage time is currently limited to five days because of concerns about bacterial overgrowth during 22 degree storage, so even though we were licensed for seven days, we have been short dated for many years now. But with the availability of pre-release bacterial testing or pathogen reduction, the FDA, in fact, has indicated they will consider extending platelet storage time as long as platelet quality is

maintained. And this is the operative word here, which is still under discussion about what the quality has to be at the end of storage.

Now, I would just share with you that there is basically a sequential method to assess platelet quality that are usually done by people like myself who are interested in the issues related to platelet storage. The first step, which is basically required of any platelet product is a series of in vitro assays of platelet metabolism and function, and I'm not going to discuss those further.

The second step is basically paired in vivo radio labeled platelet recovery and survival measurements of autologous platelets prepared as test or control, so there's a comparison between the experimental platelets and whatever you're using as control platelets, and again the control platelet has been in evolution as to what that should be. Because we have two labels that can be used for platelets, we can do simultaneous or sequential transfusions of two autologous products

given back to their same normal volunteer.

Now, in discussions that the FDA has given at scientific meetings, they have basically said that step two needs to be done for any new platelet storage bag, for any new method of platelet collection, and for extended storage of platelets in plasma.

Step three, which is a whole other ball game, is transfusion experiments in thrombocytopenic patients to evaluate platelet viability and function, and by viability we are talking about post-transfusion increments and intervals between transfusion; and by function, we're talking about ability to provide hemostasis. And examples of when this would be required would be storage in solutions other than plasma, with or without extended storage, pathogen activated platelets, with or without extended storage, platelet substitute or chemically modified platelets, and I think you heard Larry Corash talk this morning. The first study that I'm aware of that really had hemostasis in thrombocytopenic

patients as an endpoint was the Ceres-Baxter pathogen inactivated product and he showed you that that was equally efficacious compared to the control platelets.

Now, what I'm going to concentrate on is basically I'm not going to talk to you about step one. I'm not going to talk to you about step three. I'm going to concentrate my information on step two.

So the criteria for platelet viability after extended storage, first of all, the committee should know that there's no established criteria in the U.S. as there are for red cells. So for red cells, it's an absolute criteria that you have to have 75 percent of the red cells circulating 24 hours after transfusion, with a 9 percent plus or minus standard deviation and a lower confidence interval of 70 percent.

Now, prior studies that have led to the licensing of platelets have usually compared the extended stored platelets with platelets at the end of the current bathing period. So, for example, we

compared five-day platelets to three-day platelets or we compared seven-day platelets to five-day platelets. That obviously promotes a downward creep in the platelet quality because the extended stored, whatever that was, had to be within 20 percent of the currently licensed product and so, as you can see, that just was on a slippery slope.

So about two years ago now, Dr. Scott Murphy, who's one of the pioneers in platelet storage studies, as all of you know, proposed a criteria which would be that platelet recovery of extended stored platelets should be two-thirds of fresh and platelet survival should be half of fresh. And by fresh, that basically meant product collected from the same autologous donor and a ratio was done of the fresh--of the stored compared to the fresh.

The FDA is interested in this criteria, but they basically have said that the fresh--the stored compared to fresh should be two-thirds for both recovery and survival, and I was told that that was because Dr. Epstein would sleep better at

night and so I've brought some sleeping pills for him. So hopefully, we can get beyond this particular point.

One issue that I would bring up for the committee to consider is that whenever you talk about a ratio measurement, biostatisticians basically pass out and go to sleep and they're not--they dislike this, and I'm going to show you some examples in the data that I'm going to present to you that points up some problems actually with this ratio measurement and my last slide is going to be a discussion of whether we should, in fact, consider a different criteria than what either of these has been proposed on this slide.

Now, why does Dr. Murphy think that the survival only has to be half of fresh and the FDA has two-thirds, and let me just share with you some data that we published actually some time ago how thrombocytopenic patients respond to platelet transfusions compared to normal individuals.

And so as all of you know, only about two-thirds of the platelets transfused actually

circulate. The additional third are pooled in the spleen. Interestingly, in thrombocytopenic patients, they do almost as well for platelet recovery as do normals, but they're clearly not as good at platelet survival as normals, and this is the reason why Dr. Murphy thinks that we don't have to have a 66 percent of fresh for survival, but only 50 percent because that's all the patient really needs.

And what we know about loss of platelets from circulation is that there's two mechanisms of platelet removal. First is the senescence. Like other blood cells, the maximum life span is about 10.3 days, but in addition to that, there is a random loss of platelets, about 7,000 platelets per micro liter per day that we think is involved in maintaining hemostasis.

Now, if you've got a normal platelet count of 250,000, you can't see that 7,000 random loss, so your platelet survival is not affected.

However, if you are thrombocytopenic to a level that we would actually consider transfusing

you, there's a direct relationship between platelet count and platelet survival, and you'll notice that this is a very steep relationship here, and I would also remind this audience that prophylactic platelet transfusions, which are about 80 percent of the transfusions that we give in the U.S., are given at platelet counts in the past of about 20,000. They're now down to 10,000. In addition, there's an ongoing NHLBI-sponsored clinical trial trying to look at platelet dose so that we are reducing both the prophylactic trigger and potentially even the dose, which will further reduce the level of circulating platelets and, therefore, also directly affect the time between transfusions.

And if you look at days to next transfusion in thrombocytopenic patients, what I present here is data from a very large transfusion study that was conducted in the United States to look at prevention of alloimmunization. There were 533 patients with AML undergoing induction chemotherapy. They received over 6,000 platelet

transfusions.

In this study, the prophylactic trigger was 20. I have now told you it's down to 10. With that prophylactic trigger, the days to next transfusion was 1.8 plus or minus 1.3.

So in my opinion, anyway, we need to make sure that our platelets have at least two days of survival, but and with some margin of safety. But it does not need to be the 10 days that you find in normal volunteers.

Now, these studies that I'm going to start with here are studies that were actually supported by the NHLBI via grant to my laboratory to look at extended storage of apheresis platelets. And what we did in this study is we collected from the normal volunteer an apheresis collection. We used two different machines, either Haemonetics or Cobe. We had two bags of platelets, one of which was the control and one of which was the test. And what we started with as our control was the currently licensed five-day product because these--that was what we thought the criteria was going to be.

Because now the criteria is changing to fresh, we then did some studies with apheresis platelets that were collected the day before. They were radiolabeled and transfused so they were less than or equal to one-day old, and then the stored platelets were seven, eight, or nine days, all in plasma, so each bag was stored for a selected number of days. The isotopes were rotated between the control and test so that at the end of a series of experiments we had the same number of platelets labeled with each isotope. The autologous platelets were reinfused at the end of their selected storage and then we collected samples to determine platelet recovery and survival.

So what I show you here and I'm going to show you on a series of slides is this is platelet recovery as a percent. This then is storage time in days on this axis. In this slide, the Cobe data is in blue. The Haemonetics data is in pink. And the number of observations, the N is reported with each data point, and then in addition, for each data point is reported the percent of fresh of the

stored platelets and I've used the respective fresh data because these were not always paired with fresh, but some of them were actually five-day storage data.

So this is generic data, in other words, this fresh data was divided into each of these stored products to give the percent recovery.

And what you can see is there's a bit of better recovery for the Cobe platelets than the Haemonetics platelets. These are put on the slide as standard error and at least my biostatistician tells me if the error bars don't overlap, there's not a statistically significant difference. If they do, there is.

So I would point out to you here that the absolute, for example, absolutes recovery of the Haemonetics and the Cobe platelets at day seven is the same. But this is now 86 percent. This is 69 percent, so just because of the ratio measurement and where the fresh started out, this appears that Cobe is worse than Haemonetics, but that I would suggest is artifact of how the measurements are

being done.

This is now survival data, so this is platelet survival compared to storage time in days. And again, I think I get a gold star for getting everything piled right on top of each other and what you can see here is that at seven days this is 65 percent I think, and this is like about 58 percent. So at seven days, we meet for both the Cobe and the Haemonetics platelets, the FDA criteria for end of storage. At eight days, we meet Dr. Murphy's criteria, but not the FDA criteria.

Now, what I've shown you here is the actual data. So this is observations Haemonetics code number of things. This is Haemonetics recovery data at various time periods here. This is survival, and then this is the average of the data from all of the studies.

So what I would share with you is that basically, there's not really any difference between Haemonetics and Cobe, that at the end of storage, we're still at 4.6 days, which is almost

2.3 times what we really need to have for transfusion in thrombocytopenic patients. Nine days there are only three observations listed here. That's because two out of the five studies that we did, one Haemonetics, one Cobe, had pH's at the end of storage that were at an unacceptable level, and they had non-viable platelets.

So basically, I think the bottom line is we can go to eight days, but we cannot go to nine days.

Now, then because I had been going to all of these things where the FDA was talking about what we should be doing, along with other scientists. We did do an actual direct comparison of apheresis platelets store for eight days, compared to the other bag, which was stored for less than or equal to 24 hours, and so what I've shown you here is the data for--we actually did 10 studies with each machine, but interestingly two Haemonetics and one Cobe study were excluded because the fresh platelet survivals were I think reduced at six days, so comparing the storage

survivals, we had fresh compared to stored of 148 percent, 137 percent, 106 percent, and I spared you from putting that data in here, which, in my opinion, would have substantially skewed the results and which is why I think it's important that we reexamine whether we really want to use a ratio measure rather than some absolute criteria for the quality of the platelets at the end of storage. But what you can see here is that when we did a direct fresh compared to stored, they made the criteria of both Dr. Murphy and the FDA for recovery, but neither one of them made it for the FDA survival, but would have met Dr. Murphy's criteria.

Now, this now is again another iteration, another evolution of what the FDA wants to see in terms of data to allow extended storage. And so what I shared with you is data that we had done at our blood center comparing platelets in two different apheresis bags. It has now been decided that the fresh platelets should truly be an aliquot of blood obtained from the donor at the time the

stored platelets are to be infused. The fresh platelets are radiolabeled with either Indium or Chromium and reinjected into the donor at the same time as the stored platelets. So this has an advantage of having the vessel, i.e., the donor, be the same in the same condition for the fresh and stored, and I think that that vessel may make a difference if you're doing transfusion studies in patients, but I suspect eight days later, most of us look the same then as we did before, at least I would hope so.

So this is the first study that's been reported using what the FDA is now suggesting should be the appropriate experimental design for extended stored platelets. So on day zero, ten normal volunteers collected in each of two sites, and it was Dr. Snyder and myself who were the two sites, and then at the end of eight days of storage of the apheresis platelets, we collected whole blood from each donor, radiolabeled the two products, simultaneously reinfused and did post transfusion recoveries and survivals.

What I show you here is all 20 observations, because there was no difference based on the isotope used or based on the trial site, and

so this is the data that was achieved for the results of the Haemonetics supported eight-day apheresis study, and this is fresh and stored. Eighty-one percent of fresh compared to stored, and in this study we were able to meet the FDA criteria of being 66 percent of fresh for both recovery and survival.

Now, what I want to show you on this slide is a comparison of the Haemonetics eight-day data that I just showed you. That's on the first line. Our blood center apheresis storage, which was either Haemonetics or Cobe, and then the recently published data from Cobe on their seven-day product. So there is eight-day data here; seven-day data here. This is then control which would have been the sample drawn at the same time as reinfusion. In our studies, it was drawn at the time of the collection of apheresis platelets. This is then the stored and the stored as a percent

of fresh.

And what I would share with you here is that whether you store for eight-days or seven days, basically everybody is getting the same answer here. And then if you look at the survival of the stored platelets, basically it's anywhere from about 4.6 to 5.6. It's really interesting that it's relatively consistent data.

Now, I want to shift gears and start to show you now data on platelet concentrates and leave apheresis platelets, because I'm interested that the title of this committee is safety and availability, because one of the concerns that some of us have is that we have to be able in my opinion to have random donor platelet concentrates as well as apheresis platelets available for transfusion because at least at our blood center we cannot meet all of the patients' needs with apheresis.

In addition, if the pooled random donor concentrates are still less costly than apheresis. In addition, you'd have to consider that if you don't make platelet concentrates from your whole

blood collection, then the cost of recruiting the donor, testing the donor has to go to plasma and red cells so the cost of those products will also increase.

And so I think it's extremely important that we have random donor concentrates. So this is a similar experimental design as to the Haemonetics study I just showed you. We stored the platelets for either six, seven or eight days, and then we collected a fresh sample of blood from the donor, rotated the labels, reinfused and collected samples.

And this is now that data, again, in the same format. So this is platelet recovery, storage time. These percent of fresh are with their respective fresh data, so each donor had fresh data so this is the respective data from that donor.

What you can see here is that eight days of storage, we only did three, because the data was so poor. But we're only at 41 percent of fresh recovery. We're down into an absolute about 30 percent recovery for stored.

At eight days, we're at 74, 78, 83, and this is a basically a relatively gentle slope so that at seven days of storage, we're at 74 percent,

which meets everyone's criteria.

And what I did here, since we hadn't done five-day storage data yet, we started with eight because that's what we had been able to do with apheresis. We went to seven. We went to six, and this is the projected five-day data.

Now, this is the similar data on survival, and so what you can see here is that we're at survivals that are about two days here for eight-day. We're at about four days here. We're at about 47 percent of fresh recovery, so we're close to meeting the Murphy criteria; don't meet the FDA criteria until we get to where we currently store a platelet. So if we use the FDA criteria, we're going to have at best five-day platelets and again I would say to the committee we used seven-day platelets for years. Nobody died and went to heaven that I'm aware of with seven-day platelets, and so that really brings into question

I think kind of the, if you will, arbitrary nature of how these criteria are being selected and rather than use arbitrary criteria, I think we should look at the actual data that we get on the products and then set the guideline based on what we, in fact, can achieve.

So this again is the table that shows observation number. This is six-day, seven-day, eight-day. This is fresh for the donors used in that study. This is stored, and what you can see here is that this is very poor recovery and also very poor survival. At seven days, we're at--meet everybody's criteria. At seven days, we are close to meeting Murphy criteria, not at even six days to meet the FDA criteria, and so where we are in my opinion is that we can store apheresis platelets for eight days. And with the survival of 4.6 days in our studies at the blood center, and 5.6 days in the most recent Haemonetics study, we are still 2.8 times what the patient actually needs or 3.1 times what the patient needs.

Now, I have been told that at a meeting of

the AABB, Dr. Saline Haddad from the FDA said, look, if you want to store platelets longer than seven days, you have to do transfusion studies in thrombocytopenic patients, and between you and me and the gatepost, I think there is no need in my opinion to do thrombocytopenic studies, and I think we ought to allow licensing of the apheresis platelets for eight days. We just heard the bacterial people tell us that it takes 24 hours for them to put the things in culture; another 24 hours to read it before they can release. So anything we can do to extend the storage time of platelets is going to increase the availability of platelets and reduce the outdating, which is a substantial cost.

Now in terms of platelet concentrates, I think we can only store for seven days. Again, we're at about 2.3 times what the patient's requirements may be.

So in conclusion, during storage, platelet recoveries are better maintained than platelet survivals. Acceptable storage time that meet patients' needs and maintain platelet availability

are, as I've said, eight-day for apheresis storage and seven days for platelet concentrate storage, and the final slide is whether we, in fact, should consider some alternate approach to evaluate platelet viability that does not depend on a ratio of stored to fresh, but is similar to the red cell criteria, which is this is an absolute criteria that the platelets have to meet. What I've shown here is the range of average platelet recoveries for fresh or less than or equal to one day stored, which is 63 to 72 percent. Range of average survivals is seven and a half to eight and a half for fresh products, and range for seven or eight days stored apheresis or random platelet concentrates is 48 to 56 and survival 4.1 to 5.6.

So this number and this number are the data for the platelet concentrates and to give a little wiggle room maybe we can go 10 percent less than the lower end of the stored ranges here. So absolute platelet recovery would have to be 43 percent plus or minus something with a confidence interval of plus or minus something. And platelet

survival similarly would be 3.7 days, which is still twice what the patient needs.

So I would be more than happy to share all this data with the FDA and their biostatistician to see if maybe we can come up with some kind of absolute criteria that the platelets have to meet that would also meet the patient's needs. Thank you.

MR. SKINNER: Other questions? Andy?

DR. HEATON: Yes, I have a few questions. First of all, Sherrill, you made the observation that you don't like the ratio--

DR. SLICHTER: Yes.

DR. HEATON: But yet when I look, for example, at your day one and day eight platelets--

DR. SLICHTER: Yeah.

DR. HEATON: Your Haemonetics fresh platelets, you clearly had recoveries over a 100 percent day one. So it's true a ratio measurement is a problem, but you've got to be very, very careful of your baseline.

DR. SLICHTER: Yes.

DR. HEATON: And you had enough variability in your baseline that, to some extent, I suspect that affected your ratio. And I accept

that statisticians don't like ratios--

DR. SLICHTER: Right.

DR. HEATON: But the key thing about a ratio is the accuracy and precision of the value of your denominator.

DR. SLICHTER: Well, what I would share with you is that although we had some data like that, you know, when we did a head to head comparison with my laboratory with Ed's laboratory, we basically got the same data. So as all of us know, there's some variability amongst donors and also some variability in terms of how reproducible the recovery and survival measurements are.

DR. HEATON: Yeah. But if you do the evaluations on the same day, and you use the same standards, you, in effect, eliminate that as a variable.

But moving on to a second issue is you're proposing an alternative standard here based on an

inferiority of about 10 percent. But if you look at your last set of studies with Haemonetics, with 2.9 days survivals, you're below even your proposed standard.

DR. SLICHTER: I'm sorry?

DR. HEATON: The last set of studies you did where you expressed concern that the survivals at eight days fell below the FDA's 66 percent, but at 2.9 days they're below the 3.7 that you would propose as an acceptable standard.

DR. SLICHTER: Well, but isn't that nine-day storage, and I'm not suggesting nine-day storage, Andy.

DR. HEATON: It was eight days of storage, Sherrill.

DR. SLICHTER: What number are you on?

DR. HEATON: I'm on slide 21. So, from my perspective, I continue to--

DR. SLICHTER: That's eight days storage of platelet concentrate, and I'm not suggesting that we do eight-day storage of platelet concentrates for the very reason you've suggested.

DR. HEATON: Okay. All right.

DR. SLICHTER: Okay?

DR. HEATON: But I continue to believe

that a ratio standard with the fresh product gives you the clearest reference against the current survival in the donor, because platelet survivals in donors does vary chronologically from time period to time period and that would give you the most accurate results.

DR. SLICHTER: Well, Andy, I guess having been through all this data and looked at the ratio and fiddled with the data, and we've got a fair amount of data, which we've presented here. I just sat down after I got through making all these slides and just said in my opinion we are better off than conceivably have an absolute standard that the product has to meet. We then only have to give one label to the donor. We don't have to do dual label studies. We don't have to depend on this variability, which we've seen, and it will conform, if you will, to the red cell standard, which has an absolute criteria.

MR. SKINNER: Dr. Epstein?

DR. EPSTEIN: Okay, Harvey. You've been waiting.

DR. KLEIN: Thanks. Sherrill, granted that your numbers are relatively small and all the provisions of what a platelet quality measurement

is, it still seems to me that from your data the whole blood-derived platelets in terms of survival are inferior to the apheresis platelets. Why not then label them for five days where they seem to meet virtually everybody's standard in the apheresis platelets. Surely, the logistics are not that difficult if you have a dual population of platelet collections.

DR. SLICHTER: Well, Harvey, I am suggesting that the apheresis platelets be licensed for eight days and that the PRP platelet concentrates be licensed for only seven days, but I guess I would disagree that we should remain with only five days of storage for platelet concentrates. That's going to drive the marketplace to more and more apheresis which is a

more expensive product. It's also going to reduce the ability to cover the costs of the plasma and red cells, and I think there is no reason to do that. We had seven-day platelet concentrate storage for years. I'm not aware that we had any problems. It's true that the survival is shorter, but I'm not sure that that's going to make a big difference for thrombocytopenic patients, particularly when the trigger is now 10, and the dose may be substantially less. I don't think we need platelets that survive for long periods of time.

DR. KLEIN: I guess not to push this too much further, but there are in-patients and out-patients and frequency of transfusion, which are also issues--

DR. SLICHTER: Sure. Yes. Yes.

DR. KLEIN: --but it just seemed to me that again although your data were based on relatively small numbers, I think there were 22, there seemed to be quite a break point in those whole blood-derived platelets and if that turns out

with larger numbers to be true, and if, in fact, survival is some kind of surrogate for quality, it seems to me that that's probably an inferior product.

DR. SLICHTER: Well, I don't have stock in apheresis companies. I don't know what you have.

MR. SKINNER: Jay?

DR. EPSTEIN: Yeah. I just want to make a couple of comments. But perhaps the most important one is that we respect the fact that you've generated data, and without data, there would be nothing to talk about of a meaningful nature.

DR. SLICHTER: Right.

DR. EPSTEIN: So, you know, I think the most important observation is how appreciative we are that you're doing these, you know, very laborious studies.

Now that said, I think there are a couple of arguments and I might be making them in my sleep. I don't know.

First, with respect to more than a hundred percent recovery.

DR. SLICHTER: Yeah. Well, the time zero sample is a reference preparation, and it is not always inherently like the test sample that you're

investigating. So for example, if you compare, you know, a reference sample made from whole blood under certain conditions to an apheresis platelet, you may be counting platelets, but the ratio does not inherently have to be a hundred percent or less.

So I think we shouldn't be frightened by the fact that it could be above or below a hundred because it's simply against an arbitrary reference.

DR. SLICHTER: Right.

DR. EPSTEIN: The idea of the time zero sample was both that it should be fresh and also that it should be a reference prep. The reference prep is not inherently the ideal prep. It's simply a standard prep. That's point one.

Point two about whether the standard should be based on survival in thrombocytopenic--the standard for survival should be based on the thrombocytopenic patient or the

normal patient. I think that the argument that has motivated FDA I that if you do see a fall off from what you would expect with survival of normal platelets, you are seeing some change in the platelets. What follows from that is a beltline argument. In other words, how much inferior can it be and still accept it as an equivalent product, and I think that again you've correctly framed the argument as one of clinical relevance, but I think that the point of concession really is the point that Dr. Klein just made, which is that if you are seeing less survival, you are seeing some other damage to platelets. The debate is does it matter or doesn't it matter. But using the standard of, you know, a two-thirds recovery is a better--is a more stringent measure of whether platelets have been damaged. And so I think from that point on, the debate is clinical, and, you know, these data are helpful.

And then the last point that I want to make is that FDA has not, you know, drawn a line in the sand. Our procedure is, as you know, to make

proposals to discuss them publicly. We vetted our proposed standard for the platelet at a blood products advisory committee meeting. And that will be followed with publication of a draft guidance document, and there will be ample opportunity for comment, debate, submission of data, and, you know, we all hope we land in a reasonable place.

So, you know, no one should think that we've reached closure on this issue, and, of course, as I said earlier, nothing helps more than data.

DR. SLICHTER: Well, and I appreciate and understand what you've just said, Dr. Epstein, that one of the reasons I'm here presenting the data is because I know you haven't come to closure, and what I'm trying to suggest is that to maintain availability of products, we need to be very careful that we don't have what are really arbitrary standards without data that does not allow us to have products available for transfusion to patients.

MR. SKINNER: Susan?

DR. ROSEFF: I'd like to thank you for your presentation. I'm in a market with a very poor apheresis donor supply, not poor, but actually

I guess inadequate, and we would be in great trouble if we weren't able to depend on whole blood-derived platelets and the question is what is the outcome of these patients, not how many days did the platelets survive, but how did the patients do. I know our bone marrow transplanters are always comparing their survival to other institutions and do very favorably. And they get most of our apheresis platelets but still get a lot of whole blood-derived platelets.

DR. SLICHTER: Right.

DR. ROSEFF: So I agree, and I appreciate what you're saying.

MR. SKINNER: Mark and then we need to bring this discussion to a close.

CHAIRMAN BRECHER: Just a quick comment. At the original meeting where Scott proposed his cutoffs to the FDA, I supported a finite cutoff similar to the red cells. And one of the arguments

I made was that fresh platelets are in flux, too. It depends on the method you use to make your fresh platelets, and, for example, the way you're doing it, you're making your fresh platelets from 60 mLs of whole blood and that's not an apheresis platelet, and so that's a slippery slope--that can be a slippery slope as well.

DR. SLICHTER: Well, I just put this up as the last slide because I'd heard others besides yourself actually, Mark, make a suggestion that maybe we should have an absolute criteria so we don't have to worry about ratios. We don't have to worry about whether they hiccupped or I hiccupped or whatever. So I just think it's something that we need to consider as an alternate standard rather than a ratio measurement, and that's why I bring it up.

Thank you very much.

MR. SKINNER: Thank you. And our next presentation is going to be from Dr. Jaroslav Vostal, who is a Senior Medical Officer in the Division of Hematology at CBER.

Comments from FDA

DR. VOSTAL: Okay. Thank you very much for an opportunity to present to you our current

approach to approval or clearance of bacterial detection devices for platelet release test indication and also the extension of platelet dating.

So let me start off by summarizing what our current approach is to validation of a release test indication for bacterial detection device.

The current approach is to obtain data on performance of the FDA-cleared devices when these are used to meet the AABB detection standard that was in place since last March.

We're going to use the data as a basis of approval of seven-day platelets provided there is a commitment to perform a post-marketing study. The post-marketing study will consist of an additional culture on outdated products, and this would be at day seven, to confirm the day one negative culture readings. The goal of such studies is to demonstrate a point estimate of risk at day seven

to be less than one per 10,000, with a 95 percent upper confidence limit that the risk is less than one per 5,000.

And such study based on the statistics and current estimates of contamination is approximately 50,000 platelet units.

Now, here's a schematic that shows the kind of data we can obtain from a dataset obtained for QC as opposed to a dataset obtained from the clinical use and post-market data. Here you have under current routine conditions platelet units are tested by bacterial detection device, and you get either a positive or a negative result.

If you get a positive result, these units are taken out of clinical circulation and they are then tested again to determine if it was a true positive or a false positive. This is the basis of the QC data that we're looking at for approval of some of these devices.

If the result here is negative, the unit then goes into clinical use and if it survives to outdate, it's then again tested for bacterial

contamination and if the result is negative it's declared a true negative, and if the result is positive, then it's declared--the initial negative is declared a false negative.

So our current post-market study design consists of a first sample collected early in storage, and right now we're--our design includes both aerobic and anaerobic bottles. And this is followed by a second sample collected at outdate, after day seven, and this is also an aerobic and anaerobic bottle.

The type of data we expect to see as a result of the post-market study, we anticipate we'll be able to determine the residual risk of bacterial contamination for day seven platelet, platelets tested early in storage by a bacterial detection device. The acceptable risk for aerobic and anaerobic bacteria is as I've already described earlier. It's the contamination rate should be less than one per 10,000, with a 95 percent upper confidence limit that it's less than one per 5,000.

We anticipate we'll be able to compare

detection rates for aerobic and anaerobic bacteria in the aerobic and anaerobic bottles or other types of detection.

Now, if the anaerobic detection method is capable of reducing the residual risk of anaerobic bacteria, as defined above, then it may be possible that the anaerobic method will not be required for release of platelets in the future.

Now, that's our current design. However, we're open to alternative post-market study designs and if you come to us with an alternative study design, we'd like you to keep in mind the following criteria.

Now, a sponsor may chose not to test the early storage sample by an anaerobic method, and so the testing up front will be only aerobic if the test sensitivity is enhanced in other ways--and one that could be done is by increasing the volume of the initial sample. Now, due to the undefined risk of anaerobic bacteria, this type of study design will need to be performed under an IND if the platelets are transfused after day five storage.

In addition, the final sample of this alternate post-market study will also have to be tested with both aerobic and anaerobic method. And

if there's a finding of a clinical significant anaerobe and outdate, it would require a change in the study protocol to culture for anaerobes early in storage. So basically, as a study is proceeding, if there is--if it's discovered that the initial aerobic culture missed an anaerobe, we would then request that the study protocol be redesigned that the anaerobic bottle and or anaerobic culture be done up front as well to increase the safety of the study.

Now, finally if the study data is collected and compiled, it can be used to seek clearance of the device's release test.

Now, a lot of this depends--a lot of the study design and our decision designs on the estimate of risk from anaerobic bacteria that are contaminating platelet products.

Now, the true risk for anaerobic bacteria in platelet products in the U.S. has not been

defined. There are published studies and reports to the FDA that indicate that the risk exists, although it's most likely small.

Now, we searched the FDA database that is made up of adverse events reported due to transfusion transmitted bacteria, and we found three cases of mortalities from a transfusion transmitted product that contained anaerobic bacteria. In year 2000, it was a clostridium contaminating red cells. In 2001, it was a clostridium that contaminated platelets. And very recently, we've had a case of Eubacterium limosum that also contaminated platelets. And all these were associated with fatalities.

Now, a search of the literature revealed two studies that were already discussed by Dr. Dumont. In 1998, it was a report of a clostridium contaminated platelet product that turned out to be a fatal transfusion. In 2001, it was a clostridium contaminated red cell product that was associated with a sepsis.

Now, the next three studies I'm going to

talk about summarize data that was being collected in Northern Europe. And in Europe, they've been collecting data with the bacterium--they've been routinely culturing platelet products with the BacT/Alert device for several years now. And the interesting thing in comparison of these three studies is that they pretty much have the same type of platelet product that they're testing. They have very similar approaches, but the major difference is that one of the studies is not using the anaerobic bottle up front.

So here's a study that was reported in Transfusion in 2005. The data was collected from the Sanguin Blood Bank in the Southwest region of the Netherlands. And this study looked at or sampled approximately 28,000 pooled platelet concentrates, and they used both the aerobic and the anaerobic bottle up front, and each was inoculated with five to ten mL per bottle.

With the results from the study, the overall contamination rate was about one per 140 for bacteria. For anaerobic Gram positive cocci,

the contamination rate was about one per 3,000 and for Proprioibacterium, or P. acnes, the contamination rate was also about one per 3,000.

The next study is done by--it was presented by Dr. Pieters at the FDA BPAC Committee in March 2003. This is the--the data comes from the Sanguin Blood Bank Northwest region in the Netherlands. They sample approximately 9,000 platelet concentrates. They also use the BacT/Alert bottles, both aerobic and anaerobic, and again used five to 10 mL per bottle.

Here the overall contamination rate was also one per a couple hundred. Here's 120. They did observe a lot of Propionibacterium. The rate was there one per 237, and they did identify three obligate anaerobes peptostreptococcus, and the contamination rate was there--was one per 3,000.

Now, this is contrast to the study that was done in Denmark, published in Transfusion in 2004. These investigators sampled about 22,000 platelet concentrates. The major difference here is that they've only used anaerobic bottle to

sample up front, and the sample volume was 10 mL per bottle.

The overall contamination rate was similar to the previous studies. Here it's one per 300. They did observe a lot of P. acnes. The contamination was one per 2,000, but the major difference is that they only identified one obligate anaerobe, which is clostridium perfringens, for a contamination rate of one per 20,000.

So I think looking at these three studies, it suggests that anaerobic bacteria are present in these types of blood products, and if you're not looking for them with an anaerobic bottle, you're likely to miss these--you're likely to miss them and you may be transfusing contaminated blood products.

Now, a lot of discussion has been going on at this meeting and other meetings about whether P. acnes is really a risk to transfusion recipients. Most people consider P. acnes as a harmless contaminant, but I searched the literature and

found a number of reports that indicate that P. acnes could be associated with severe clinical situations.

Here's a study reported by Jakab in 1996. And these authors admitted that P. acnes is a frequent contaminant of blood, and they went on to describe a situation where P. acnes has been associated with clinical syndromes of endocarditis, post craniotomy infections, arthritis, spondylodiscitis, endopthamlitis and pansinusitis--all relatively serious clinical conditions.

Their pre-dominant, pre-disposing conditions in these patients were surgery preceding infection two weeks to four years before and also implantation of foreign bodies.

So in certain situations, P. acnes can be a pathogen in certain patients. And the question remains can P. acnes cause disease in some patients receiving contaminated blood.

To address this issue, there was another report in Transfusion in 2001. This comes from the

French BACTHEM case control study, and these authors followed patients who were actually transfused with contaminated units of transfusion products. They had clinical presentations of 16 cases. None of these were fatal. Three out of 16 of these cases were positive for P. acnes. And here's the brief summary of the clinical presentation of what happened to these patients when they were transfused. The initial one was a male, 41 years old. He suffered from fever and urticaria. However, he did not develop shock or sepsis as a result of that transfusion. The second patient was not as fortunate. He suffered a fever, fatigue, and consciousness disorders, and he did develop severe sepsis as a result of the transfusion.

Finally, a male of 71 years old suffered fever, chills, anxiety and erythematous rash, and he also did not have shock or sepsis.

So these are cases where people have been transfused with P. acnes and did develop some significant clinical episodes.

So I would just like to summarize, finish with a summary of submission--of the submission process for bacterial detection devices to release

platelet products and extent platelet shelf life to seven days. So if someone comes to us with a new bacterial detection device, we would advise them to define the analytical sensitivity of that device and this is usually done with spiking studies. Based on this, we would b able to clear the device for quality control use for platelet products, and with that clearance, they could collect QC data to determine the true and false positive rate in a clinical setting. The use of the QC data could then be--the QC data can then be used to support a submission of a release test indication, and this could be cleared as a release test with the commitment to do a post-market study. Such a post-market study would require an aerobic and anaerobic detection from an early sampling, confirmed with an aerobic and anaerobic detection at outdate. This is our current study design. However, we are open to alternate designs in the

post-market studies. However, some of these designs may have to be done under an IND. Thank you very much.

MR. SKINNER: Are there questions? Seeing none. Thank you.

DR. BIANCO: A quick one. You know, one of the problems with P. acnes is that it shows up very late. And so it's not just the clinical significance in terms of--but sensitivity in terms of hours to get it and find it before it's transfused is also very low. And so I wonder if you can really call this very clinically significant. The other ones, no doubt. But you need a definition of the clinically significant, too.

DR. VOSTAL: Right. I think we're going to be debating this for some time. I mean it's not a clear cut decision whether it's a pathogen or not, and I think you'll be best to see how the data comes out from some of these post-market studies.

DR. KLEIN: I think that's a very important point. I mean I commend you for finding

that reference because very few of us get the Yale Journal's Biological Research. And I frankly am very suspicious that all of those cases of endocarditis and infections--fairly severe infections--were, in fact, due to that particular organism. I'm not saying they weren't, but that is two standard deviations from the mean if anybody else has experience.

So I think we need to take that with a little grain of salt, continue to be sensitive that this may be an issue, but not to bias all of our regulatory requirements based on that particular publication.

MR. SKINNER: Mark?

CHAIRMAN BRECHER: Jaroslav, correct me if I'm wrong, but the French BACTHEM cases, one of them had severe sepsis. But as I recall, they isolated some other bacteria in that patient as well.

DR. VOSTAL: I actually don't remember that point. So.

MR. SKINNER: Thank you. We've completed

the formal presentations. We're now going to move into public comment. And we've had one request for public comment, Dr. James AuBuchon, who is the Medical Director at Dartmouth Hitchcock Medical Center.

Public Comments

DR. AUBUCHON: Thank you very much. I would first like to commend the committee for their perseverance, and I would like to note that there were two types of conversations which I have heard at recent scientific meetings, which prompted me to ask for this opportunity to speak before the committee, for which I'm grateful.

The first comments from a number of large academic medical centers that are well respected in this country. Thank you. That are fully committed to the continued use of whole blood-derived platelets. They do this for a number of reasons, including availability, as Dr. Slichter noted, but also restraint of resources, which we know is becoming more and more of a problem, particularly in academic medical centers.

And the second is a series of comments which I've heard at a number of scientific meetings, including I heard earlier today, that

apheresis platelets are better than whole blood-derived platelets. And I thought that we had some data that might bear on that question, because I think it's one that's still open for consideration.

Whoops. This isn't the right presentation. My apologies.

Thank you. For a moment there, I thought I was going to have to play like a lawyer and talk without slides.

We are very grateful in the field of transfusion medicine to be seeing the problem of bacterial contamination of platelets being addressed. At the same time, as we are eliminating or reducing the possibility of bacterial contamination by culture and potentially by other means in the future, we are hopeful to take advantage of this increased safety in order to extend the storage of platelets to seven days for a

variety of logistic and economic reasons.

However, at the same time, questions have been raised, as you've heard, about the capabilities of the current collection and storage systems to provide an efficacious platelet product at seven days and also whether or not the various means of collecting and producing these different platelet products are equivalent.

With acknowledgement of the number of blood bankers sitting around the table, I'm not going to go into a long discussion of how components are produced, but just for the record note that with every whole blood donation in this country, some 14 million a year, there is the potential for creating a platelet concentrate that is infrequently utilized today. The usual method for producing platelet concentrates in this country is via a PRP method, where the unit of whole blood undergoes a soft spin to separate the red cells and the platelet rich plasma is subsequently subjected to a hard spin, which pellets the platelets and enough plasma is left on those platelets in order

to maintain pH balance during storage.

The alternative, which is being used increasingly frequently is collection via apheresis, producing what is colloquially known as a single donor platelet product.

Now, the data shown here were kindly provided by the Chairman of this committee and some other work that he had done, and he has compiled the proportion of platelet transfusions reported in this country as coming from apheresis. And as Mark has noted, this is a straight line function, interestingly, which unlike some of our common thought has not been accelerated by the introduction of bacterial detection in the last couple of years.

If one were to project out in this similar straight line fashion, one would project that in May of 2010, we would transfuse our last whole blood-derived platelet in this country.

But that would require a change of heart of many medical centers, who would like to maintain the capability of doing that. And is that a wise

choice?

Well, there are certainly plusses and minuses on both sides of the equation here. The cost of producing a whole blood-derived platelet is much lower than the apheresis platelet. It is essentially a byproduct of a whole blood donation, which is usually driven by the need for the red cells. The cost of the hospital for a whole blood-derived platelet product, even when one has to use five or six units and pool them together is lower than an apheresis product as usually priced by a substantial proportion.

The ease of bacterial testing, however, is much lower in whole blood-derived platelet products, because for no other reason, one has to test five or six units rather than just a single unit. So the ease is better with apheresis products as is the ease of leukoreduction, for the same reason. One only has to apply a filter once if any filter is to be applied at all because most of these products are leukoreduced at the time of collection.

The hospital preparation costs are much higher, therefore, for whole blood-derived platelet products because of the technologist's time in

pooling, preparing the units for transfusion.

The number of donor exposures is also higher, and that has prompted some people to switch to apheresis products, although the blood supply is incredibly safe. Every donor exposure carries with it some finite risk.

HLA matching for alloimmunized recipients is not possible with whole blood-derived platelet products, but certainly is available with apheresis units.

The content of an apheresis unit is also known. It can only be approximated with whole blood-derived platelet unit because the platelet count is not known in each bag; therefore, platelet specific dosing can be accomplished with apheresis products if one wishes to do that. It's more difficult or less precise to accomplish with whole blood-derived platelet where one would merely change the number of units in the pool.

We've been involved in two different studies looking at the capability of both apheresis products and whole blood-derived products at different time intervals. The first was a study conducted at the Red Cross in Norfolk, Virginia, as well as our center, under the auspices and contract

of Gambro BCT, which Larry Dumont headed up.

In this study, we collected units of apheresis platelets on the TRIMA or the spectra, stored them for five days or seven days--I should say five days and seven days--and then reinfused aliquots at both of those times.

There certainly were differences in the biochemical and hematologic parameters of these units as they were stored for additional times. Changes in pH, for example, were noted, but all the pHs remained well within the acceptable range of above 6.2. We did see changes in morphology score, presentation of antigens, which reflected activation of platelets became greater during the storage time, as would be expected. And there were some changes, relatively smaller in this case, of

the functionality of the platelets.

The proof of the pudding is, of course, the radiolabeled recovery and survival, and the comparison between day five and day seven recovery that showed statistically significant difference, as did the survival.

Looking at this in tabular form, in comparison to data that were published in 1983, when seven-day platelets were first licensed, you can see that there was indeed a difference in both recovery and survival, although apparently these parameters were better now than they were 20 years ago, with the different storage and collection systems.

We have had the opportunity to use platelets beyond five-day outdate because of our location, far from any external supply where we are occasionally short of platelets and when we have done that, we have found CCIs, indicating that indeed seven-day platelets appear to provide clinical beneficial effects, as has been seen also in Europe as they have introduced seven-day

platelets after bacterial detection.

Subsequent to that, we are involved in a study sponsored by Pall Medical, with the same laboratory in Virginia, in which we were performing a similar study, looking at whole blood-derived platelet.

Here, we started with 26 subjects, two of whom had to be discontinued. We ended up with 24 whole blood collections, which were turned into platelet rich plasma and subsequently filtered to produce a leukoreduced platelet product in CLX which was stored for seven days. That red cell unit was then reinfused to the subject. Two days later, that became day zero for the second collection, which was also turned into PRP and produced another platelet unit, so we had two platelet units from the same donor two days apart. These became the day seven and day five storage units. They were tested on the first day of storage and the last prior to radiolabeling and reinfusion to determine recovery and survival.

At the same time, types of chemical and

functional analysis were performed. Again, one can see a difference from pH over the storage time and a difference between day five and day seven pHs, but those were all acceptable and changes in morphology and changes in presentation of P selectant on the surface of platelets, and some change in hypotonic shock response, although not great or not great change in the extent of shape change either.

Again, recovery and survivals were determined by radiolabel techniques, and there were differences between day five and day seven.

Now, it's shown on this slide. There is comparison between the data looking at whole blood-derived platelets that I just showed you and the previous data I showed you on apheresis platelets--a direct comparison using the same methods in the same two laboratories for recovery and survival. Now, are there really differences between those two? Although one could conduct a statistical test between them, I would submit that there are not large clinically significant

differences between the recoveries and the survivals in those two studies.

So I would just offer the commentary that both whole blood-derived and apheresis platelets have been found to be clinically efficacious for multiple decades. The preparation methods are important when looking at whole blood-derived platelets because in the second hard spin, the platelets are pushed to the bottom of the bag, and if the spin is not conducted in a standard and appropriate manner, one can unduly activate the platelets and end up with an inferior platelet product. So the preparation methods are important, but when they are carefully produced, via standard method, and direct comparisons are conducted, there are not large differences, as in clinically significant differences between the results with these two methods of producing platelets.

So I thank you for the opportunity to present these data and make the case that we need to understand better how best to produce platelets and to keep our options open until we have clear

evidence that there's one way that is better than another. Thank you.

MR. SKINNER: Questions? Dr. Epstein?

DR. EPSTEIN: Jim, thank you for these comments. Have you looked at buffy coat platelets in comparison at all?

DR. AUBUCHON: We have not, but I'm sure Scott Murphy would be happy to talk with you.

MR. SKINNER: Other questions, comments?

DR. HEATON: What were your relative recoveries in the manually prepared pheresis? Did you do relative recoveries?

DR. AUBUCHON: No. These studies were conducted before Scott proposed comparing them against fresh. However, I would note that both of the studies yielded recoveries and survivals that met the minimum that Sherrill was suggesting as appropriate.

MR. SKINNER: Celso?

DR. BIANCO: Are you going to go back to use whole blood-derived platelets in your service?

DR. AUBUCHON: We have been using solely

apheresis products for over a decade, primarily for the other reasons listed as advantages of apheresis products. Given our size and the fact that we have committed to try to collect as much of our own components as possible and because we would not be able to support our platelet needs based on our red cell needs, we will probably stay with apheresis products. But there are different situations in different medical centers.

MR. SKINNER: Thank you. Are there others that wanted to comment during the open public comment period?

If not then, I would just inform the committee that we don't have a quorum, so we can't--we've just lost our quorum, so we can't officially transact any business at this point, nor make any recommendations, but if there's any comments or discussion of the committee, I think we're free to do that and perhaps then carry forward to our next meeting. Art?

Committee Discussion/Recommendations

DR. BRACEY: I thought that one of the

points of discussion was the question regarding the quality of the whole blood-derived platelets today as contrasted to the apheresis platelets. I think it's important to discuss that because as published in a letter from our Chairman, the techniques that are used currently to screen the whole blood-derived platelets are inferior, so in effect until we begin to culture the whole blood-derived platelets as well as the apheresis platelets, we, in fact, do have two levels of care, and that is a point of great concern for me. And I would simply encourage us to move with great haste to eliminate the disparity.

MR. SKINNER: Celso?

DR. BIANCO: Well, I certainly have to agree 100 percent with what was said. However, I think that we are missing, Art, some data. I don't know what the impact so far of all the things that we have implemented. It's--and maybe the fraction that is the random donor platelet is much smaller. And we really don't know. They may be transfused earlier with less chance for bacterial growth and

lots of things like that. So I'd like to see that data. But definitely, I think that we have to be able to have a practical way of testing the whole blood-derived platelet for bacterial contamination.

MR. SKINNER: Harvey?

DR. KLEIN: No, I agree with you, and I think the solution is eventually going to be pooling and storing with some kind of testing, whether it's culture or some other test. If it turns out--and I think we don't have sufficient data right now and we may be a while in getting it--that the whole blood-derived platelets by some technique are less good, however you measure that, whether it's survival or some other combination of measurements. I still no reason why you couldn't store it for a period when the quality would be as good, whether that's five days or six days or whatever.

Maybe you could charge five-sevenths and still not drive the whole blood-derived platelets out of the marketplace. I think there will be a place for these, and I just think that we ought to

make sure that we have the quality as good as we can, recognizing that the tests that we use today really are surrogates, and we don't have good measurements for real platelet quality.

MR. SKINNER: Are there other--is there any other discussion or comments from the committee at this point?

DR. SLICHTER: I wonder if--

MR. SKINNER: Yes.

DR. SLICHTER: Mr. Chairman, if I could just make a comment and that is--

MR. SKINNER: Sure.

DR. SLICHTER: --that is that the studies that we did with the platelet concentrates were done prior to expecting to go to pooled random donor platelet studies, and so what we wanted to do was to determine how long we thought we could store them and then do pooled studies and so, I would hope that the FDA would help those of us who are interested in pre-storage pool platelet concentrates to facilitate the entry of that particular product, which will then solve the

bacterial testing business. So.

MR. SKINNER: Thank you. Any other questions or comments? Dr. Holmberg. Dr. Brecher, anything before we close? Then the committee is adjourned. Thank you.

[Whereupon, at 4:35 p.m., the committee was adjourned.]

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