DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

BLOOD PRODUCTS ADVISORY COMMITTEE

60TH MEETING

Volume I

Thursday, September 17, 1998

8:00 a.m.

Doubletree Hotel
1750 Rockville Pike
Rockville, Maryland 20852

MILLER REPORTING COMPANY, INC.
507 C Street, N.E.
Washington, D.C. 20002

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Donald H. Buchholz, M.D.

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John D. Hamilton, M.D.
Paul McCurdy, M.D.
Simon Ogamdi, Ph.D.
Carmelita U. Tuazon, M.D.

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

DR. SMALLWOOD: Welcome to the 60th Meeting of the Blood Products Advisory Committee. I am Linda Smallwood, the Executive Secretary. At this time, I will read to you the conflict of interest statement that pertains to this meeting.

Statement of Conflict of Interest

This announcement is made a part of the record at this meeting of the Blood Products Advisory Committee on September 17th and 18th, 1998.

Pursuant to the authority granted under the Committee charter, the Director of the FDA, Center for Biologics Evaluation and Research, has appointed Dr. Paul McCurdy as a Temporary Voting Member for all committee deliberations. In addition, the Acting Commissioner of FDA has appointed as Temporary Voting Members: Dr. John Hamilton for the discussions on Topics IV and V in your agendas; Dr. Carmelita Tuazon for the discussions on Topic V; and Dr. Simon Ogamdi for the discussions on Topic VI.

Based on the agenda made available and on relevant data reported by participating members and consultants, it has been determined that all financial interests in firms regulated by the Center for Biologics Evaluation and Research that may be affected by the Committee's discussions have been considered. No waivers under Title 18, United

States Code 208, Section B3, were necessary.

In addition, Mr. Corey Dubin disclosed a potential conflict of interest which has been deemed by FDA as not requiring a waiver, but does suggest an appearance of a conflict of interest. On March 1998, the Agency approved a written appearance determination under Title 5, Code of Federal Regulations, Part 2635.502, on the status of ethical conduct for this appearance.

The determination is relevant for this meeting and Mr. Dubin is permitted to participate and vote on all committee discussions.

In addition, we would like to disclose for the record that Dr. Donald Buchholz' employer has a patent for bacterial removal from blood products using a leukodepletion filter. Because of Dr. Buchholz' role as a non-voting industry representative, it is not subject to Section 208, which means there is not a conflict, and he may participate fully in the discussions of leukoreduction.

In regards to FDA's invited guests, the Agency has determined that the services of these guests are essential. There are reported interests which are being made public to allow meeting participants to objectively evaluate any presentation and/or comments made by the participants.

The interests are as follows: For Topic VI, the discussions of leukoreduction, Dr. Ronald Gilcher reported

that he consulted with Haemonetics, Dr. Edward Snyder reported that he is the principal investigator on a contract supported by Baxter on leukoreduction studies. He is also the principal investigator on grants supported by Baxter, Paul, and Terimo on leukoreduction. He also has received speaker fees from Baxter and Paul. In addition, three to four years ago he performed studies on leukoreduction filters for Terimo.

Dr. Loran Williamson reported that she has a contract and a grant with Baxter and a grant with Paul. In addition, she is associated with the National Blood Service which purchases filters from Baxter, Paul, and Haemonetics.

Drs. Juan Alonso-Echanove, John Freedman, and Mark Popovsky have no financial interests to report for the discussion for leukoreduction filters.

In the event that discussions involve specific products or firms not on the agenda for which FDA participants have a financial interest, the participants are aware of the need to exclude themselves from such involvement, and their exclusion will be noted for the public record.

Screens were conducted to prevent any appearance, real or apparent, of conflict of interest in the committee discussions. A copy of the appearance determination addressed in this announcement is available by written

request under the Freedom of Information Act.

With respect to all other meeting participants, we ask in the interest of fairness that they address any current or previous financial involvement with any firm whose products they wish to comment upon.

At this time, I will ask if there are any declarations from any of the committee members that have not been addressed.

[No response.]

DR. SMALLWOOD: Hearing none, I would like to introduce to you the members of the Blood Products Advisory Committee.

Dr. Blaine Hollinger, the Chairman. Dr. Jerry Holmberg. Dr. Joel Verter. Dr. Richard Kagan. Dr. Paul McCurdy.

Dr. Carmelita Tuazon, who is a Temporary Voting Member with us today. Dr. Hamilton, who is also a Temporary Voting Member with us today. Dr. Ogamdi, a Temporary Voting Member with us.

Ms. Katherine Knowles. Dr. Buchholz. Dr. Jeanne Linden. Dr. Ohene-Frempong. Dr. John Boyle. Dr. Mark Mitchell. Dr. Marion Koerper. Mr. Corey Dubin. Dr. Rima Khabbaz. Dr. Norig Ellison.

Thank you.

We have a very full agenda today. I would hope

that all of the participants will adhere to the time frames that have been allotted. We would like for everyone to please cooperate with our procedures here and we can get through this.

At this time, I would like to turn over the proceedings of the meeting to our chairman, Dr. Hollinger.

Welcome and Opening Remarks

DR. HOLLINGER: Thank you, Dr. Smallwood.

We do have a very full day today and tomorrow and I think a very interesting session, as we always I think have in this committee.

We are going to begin today with again some committee updates as we always do to start with. We will then have some discussion on three workshops which have already been held: Stem Cell Workshop, Granulocyte Workshop, and an HCV PCR Workshop.

I have asked Dr. Smallwood if she would arrange to have us discuss today another very interesting virus which has gotten a lot of play, called the TT virus, and there will be a lot of discussion on that which I think you will find interesting.

This afternoon there will be a session on the Abbott Laboratories PRISM detection assay system.

With that as a background, we will start with our Committee Updates. First, on HCV nucleic acid testing, Dr.

Leonard Wilson.

COMMITTEE UPDATES

HCV Nucleic Acid Testing

DR. WILSON: The testing of blood donations for markers of bloodborne pathogens has a history spanning nearly three decades. Using hepatitis B as a model pathway, the virus was discovered in 1965, and several years later, immunoassays were developed to detect an envelope protein hepatitis B surface antigen or HBsAg, as it is commonly known today.

Since 1971, when FDA began regulating such tests as biologic devices under the Public Health Service Act, test kit performance, that is, sensitivity, specificity, sensitivity and specificity steadily improved.

These improvements were based on changes in technology both in the method of detection and in the nature and quality of active components. In regard to detection methods, the evolution of methods began with agar gel diffusion followed by agglutination assays, followed by radioimmune assays, followed by enzyme immunoassays.

In regard to the nature and quality of active components, native viral antigens and conventionally prepared immune serum were starting points. For example, an improvement in viral antigen raw material technology was the development of the recombinant hepatitis B core antigen

which enabled the development of the antibody to hepatitis B core test, which is in use today.

Another example is the improvement in immunoassay sensitivity and specificity provided by the development of monoclonal antibodies. The development and ownership via patent rights, by both raw material suppliers and test kit manufacturers of such recombinant DNA technologies or viral or antibody products based on these technologies are strewn throughout the last two decades.

[Slide.]

The granting of U.S. patents by the U.S. Patent and Trademark Office has its roots in the Constitution, Article 1, Section 8, and has been executed by the laws passed by Congress beginning in 1790 to promote the progress of science and useful arts by securing for limited times to authors and inventors the exclusive right to their respective writings and discoveries.

Today is the 211th anniversary of that signing of the Constitution.

Recent developments in the technologies that detect the presence of viral nucleic acids and also determine the viral nucleic acid sequence have led to the logical next step, the development of assays to detect viral nucleic acid which can be significantly more sensitive in certain circumstances than current immunoassays.

Accordingly, many of these technologies and sequences have been granted patents by the U.S. Patent and Trademark Office and are thereby granted exclusivity for a time.

FDA does not delay the licensure of a test kit based on a sole source situation, however when sole source supplier test kit submissions are reviewed by FDA, product availability is part of the discussion.

The history of such situations has been that when a test kit is licensed, manufacturers have prepared an adequate supply to cover the needs of the U.S. blood supply. FDA does not regulate patents.

The only patent activity that FDA engages in is in relation to the approval process for generic drugs where FDA requests statements in a generic drug application regarding the freedom of infringement of patent rights.

It has come to the attention of FDA that Chiron Corporation, which holds certain patent rights to the HCV genome, has explored various business options to develop nucleic acid assays. FDA wishes to simply state that such arrangements are business in nature and business exclusivity is not within FDA's jurisdiction.

FDA has been advising firms who wish to use patented technologies for donor testing that they should contact the owners of such products to understand test

availability options. If the firm does not wish to use a patented product, the usual option is to invent or acquire an alternative product which does not infringe on the patented product.

FDA expects that all participants in the development and manufacturing of test kits used to protect the blood supply will continue to act responsibly to ensure the protection of public health.

Thank you.

DR. HOLLINGER: The next update on the Year 2000 Computer Software, Mary Gustafson.

Year 2000 Computer Software

MS. GUSTAFSON: Thank you, Chairman, and the Committee.

We have brought to this committee issues related to computerized systems used in blood processing and transfusion in the past. These discussions have centered primarily on the regulation of stand-alone blood establishment computer software.

Today's update will address more generally all computerized devices used in collection, processing, and transfusion of blood and blood products. I will share with you FDA's activities in addressing the year 2000 problems as they relate to medical devices.

For those who may not be aware of the Y2K problem,

it is the potential crisis that may occur on January 1, 2000, in virtually every aspect of our life that involves computers. This includes health care, banking, transportation, wholesale/retail sales, just about everything that we do anymore.

As with the systems used in all other aspects of our lives, medical devices that could be affected by the Year 2000 include those two-digit year format for date representation. Devices affected include those incorporating dates in algorithms, calculations, or recordkeeping.

FDA and the Department of Health and Human Services have taken numerous actions to ensure that medical device manufacturers are aware of their responsibility to ensure that their products are Y2K compliant.

[Slide.]

FDA's outreach effort began with a June 25th, 1997 letter to device manufacturers from the Director of the Center for Devices and Radiological Health. This letter stated that computer systems and software applications currently used in medical devices may experience problems beginning January 1, 2000, due to the use of the two-digit fields per date representation.

On January 8th, 1998, this center issued guidance for industry entitled "Year 2000 Date Change for Computer

Systems and Software Applications Used in the Manufacture of Blood Products."

This document outlines the Y2K responsibilities for the manufacturers of devices and also addresses user responsibilities.

[Slide.]

On January 21st of this year, the Department of Health and Human Services issued a letter to manufacturers of medical devices registered with the Food and Drug Administration, and this includes all devices regulated by both CDRH and CBER, approximately 13,000 manufacturers, and requested that they voluntarily provide information on the status of all of their products relative to the Year 2000 date problem.

Responses to this request, responses have been published on the Center for Devices and Radiological Health web page. The response, however, has been incomplete. I think to date, around 30 percent of medical device manufacturers have responded to this request.

On May 15th, 1998, the Center for Devices and Radiological Health issued a quite comprehensive guidance for industry entitled, "FDA's Expectations of Medical Device Manufacturers Concerning the Year 2000 Date Problem."

On September 2nd, 1998, CDRH issued another letter to those manufacturers who had not responded to FDA with the

Year 2000 status information for the medical devices that they market. This September 2nd letter announces FDA's intention to publicly identify those device manufacturers who have not provided the information. Up until this time, only the positive responders had been listed on the web page. Now it will be a complete inventory of the registered device manufacturers including both positive and negative responses.

What does this actually mean? It all boils down to the fact that both manufacturers of medical devices used in the blood industry and the users of those devices have responsibilities to ensure that patient care is not adversely affected by the device's performance on January 1st, 2000.

Medical device manufacturers' responsibilities include assessing the device's performance affected by the Y2K date change. Voluntarily reporting the Y2K compliance status of each device manufactured to the CDRH database at the CDRH web site listed, and that is www.fda.gov/CDRH/year2000/year2000.html.

They also need to provide the users of their devices with a list of functionalities impacted by the Y2K design changes and correct the Y2K design problems with an updated device or modification of the current device in the field, and also assist the customers who have purchased the

device.

The Agency has stated that generally, a submission to the FDA will not be necessary. That would be either a 510(k) or a PMA supplement provided that the modifications of the device do not change the safety and effectiveness of that device.

[Slide.]

The user's responsibilities include identifying and inventorying those devices that they use that may be affected by the Y2K problem, contacting the manufacturer, and the first thing that can be done is to go to the CDRH web page, which was noted in the previous overhead, to check and see whether or not their manufacturers have voluntarily reported.

If they haven't or if the information is incomplete or if they have questions, then, they need to contact the manufacturer to determine the functionalities impacted by the Y2K design changes. Once the manufacturers have incorporated those changes in the device, then, they need to validate the functionalities in their own workplace.

Thank you.

DR. HOLLINGER: In some cases, these pictures are relatively simple with proper software, but in many cases they are quite complex, and it is important to initiate this.

The next update is on the recent review of albumin clinical trials by John Finlayson.

Recent Review of Albumin Clinical Trials

DR. FINLAYSON: Good morning. The late George Bernard Shaw used to delight in saying, "I often quote myself, it adds spice to my conversation."

With that modest beginning, I would like to give a little background for the update by quoting from a review that was published in 1980. Obviously, modesty forbids my mentioning the author of that review.

This review was talking about a review which had been published three years previous to that, and I quote.

[Slide.]

"Tullis has classified uses of albumin into four categories: appropriate, occasional, unjustified, and requiring additional data. The difficulties presented by this classification are simply finding the exact uses to place in the first category and finding any that can be excluded from the last."

Now, what is perhaps most remarkable is that albumin was first licensed in 1941, making it the oldest living licensed human plasma derivative, a status to which I can relate.

What is equally impressive is that it has continued to command our attention here at the FDA so

forcefully, for so long. In fact, the review by Dr. Tullis grew out of a workshop that CBER's predecessor cosponsored with what was then called the National Heart and Lung Institute, which workshop was held in 1975.

Now, at the time of its publication in 1977, the Tullis review largely set the tone for what was considered to be the appropriate use of albumin in the clinic and also what was considered to be appropriate labeled indications for albumin.

However, within just a few years after Dr. Tullis' review, things began to change. In the late 1970s, various groups reported at least equal effectiveness of albumin and crystalloid solutions in supporting surgical and trauma patients.

Furthermore, and contrary to earlier assumptions, such results were reported both for younger patients, that is, in the 20's age group, and older patients in the 50's, 60's, and older age groups. Moreover, at this time, some of the old shibboleths began to have fewer and fewer believers.

[Slide.]

One had heard for a long time, statements such as those made by Dr. Surgenor, for the patient who receives albumin inappropriately there will almost never be a penalty. Well, this no longer held up.

At the end of the '70s and the early '80s, Lucas

and coworkers showed convincingly that large volumes of albumin solutions administered to trauma patients who had already been rehydrated could have serious consequences including death.

At a workshop sponsored jointly by CBER and NHLBI in 1991, it became clear that many clinical conditions which had previously been thought to require albumin could be treated successfully with crystalloid solutions, and this trend of thinking continued.

Other groups, for example, the University Hospital Consortium, which is a nonprofit alliance of American medical centers, examined in the '90s the evidence for the benefit of albumin, non-protein colloids, and crystalloids in a wide variety of clinical settings.

In many of these, which I have summarized for the committee in the table that you have, the evidence supported, not simply that crystalloid solutions were equally effective as albumin, but actually that crystalloids were the treatment of choice.

With this background, an article that appeared in the July 25th issue of the British Medical Journal this year assumes considerable interest. In this issue, the Cochrane Injuries Group, which is affiliated with the Department of Epidemiology and Public Health Institute of Child Health in London, reported the results of a meta-analysis of

essentially all available existing randomized controlled trials in which albumin or albumin plus crystalloid was compared with no albumin or usually with crystalloid alone.

The endpoint used was mortality. This was the only endpoint used. When published mortality data were incomplete, the Cochrane Group sought them directly from the authors of the original papers.

As a result, 30 trials involving 1,419 patients were stratified into three sets representing three of the major indications for albumin, namely, hypovolemia, burns, and hypoproteinemia, sometimes referred to as hypoalbuminemia.

Of these studies, there were 24 in which at least one death occurred. The results of these 24 studies, representing 1,204 patients, are presented graphically in the next overhead.

[Slide.]

You may not be able to see the numbers from the back of the room. That's all right. Do as Alex Guinness in the film, "The Horse's Mouth," told someone to whom he was explaining how to appreciate art, he said, "Don't look at the picture, just feel it with your eyes."

The points here, these individual points, are on a logarithmic scale that is a horizontal scale with 1 on this scale being where the vertical line is shown. These points

represent the relative risk of death. The horizontal lines represent the extent of the 95 percent confidence interval.

A ratio greater than 1, that is, with a point over on the righthand side of this vertical line, indicates greater mortality in the albumin-treated group. A ratio of less than 1, that is, over on the left side of the vertical line, represents greater mortality in the controls, that is usually crystalloid alone.

Visual examination reveals that in hypovolemia, which is this first grouping here, that is, hypovolemia in trauma and surgical settings, there is some wavering of the points. Nonetheless, when weighted mean ratios were computed, the mean for hypovolemia was 1.46, with the lower extreme of the confidence intervals just barely dipping below 1. As a matter of fact, it was 0.97.

Similarly, the ratios for burn patients were 2.4 and for hypoproteinemic patients, 1.69. This figure that says 10.2 here should actually read 40.2, I think like the Pakistani rug weavers who always make one deliberate mistake, the Cochrane Review Group put that in just so I would have something to find.

When one combines all of these studies, the overall ratio is 1.68. Now, the first question that is obviously raised in a meta-analysis like this, is are all studies, even though they were all randomized and all

controlled, are all studies created equal, and the answer, which was supplied by the Cochrane Group itself, is no.

First, some studies were relatively small. The studies did not all have equal numbers of patients. Furthermore, not all the studies followed the patients for equal length of times, and most importantly, in the assignment of patients to one group or the other, there was a difference among studies in the blinding, which the authors of the Cochrane report called "concealment."

Therefore, they grouped the concealment into three categories, namely, inadequate, unclear, and adequate. There were 13 trials representing 811 patients in which concealment was deemed to be adequate.

When only these, that is, the 13 studies in which concealment was deemed to be adequate, were treated in the same way as the entire group here, these weighted risk averages, as well as the total weighted risk average, were virtually identical.

But these are ratios. What do they mean in absolute terms? Well, specifically, in absolute terms, the risk of death was 6 percent greater in the albumin-treated patients, meaning 1 additional death per 16 or 17 patients treated.

The next question that could be asked is how well did the Cochrane statisticians do those statistics. Dr.

Lockenbrook, Chief of CBER's Biostatistics Branch, kindly analyzed the data in this paper, and Dr. Lockenbrook is here if you have any statistical questions you wish to direct at him.

Using a somewhat different approach, he computed odds ratios as well as fitted probabilities of death. The overall odds ratio -- I should say ratios because he used a variety of approaches that he obtained ranged around 1.9, in other words, slightly greater even than the overall risk ratio computed by the Cochrane Group -- when probabilities were broken down by clinical indication and adequacy of concealment, using the criteria that the Cochrane Group had, the probability of death in the albumin-treated group was found by Dr. Lockenbrook to be higher in 6 out of 7 cases.

Interestingly enough, the Medicines Control Agency, the MCA, which I will casually refer to as the FDA of the UK, also examined these studies. They looked at the 30 original studies, considered that 29 of them were evaluable, and then went through them very carefully, both from a statistical and from a clinical point of view.

They concluded that although there were some studies in which the randomization led to an imbalance which could have biased the results against albumin, such results were not prevalent or large enough to affect the overall conclusion.

So, what did the Cochrane Group and its consultants conclude, and what is FDA's perspective? The initial statement in the abstract of the British Medical Journal article, which the committee has in its package, was to say the least eye-catching. I quote, "These data suggest that use of human albumin in critically ill patients should be urgently reviewed and that it should not be used outside the context of rigorously conducted, randomized controlled trials" -- not exactly mincing words.

Somewhat less emphatic statements have been issued subsequently, however, we are still left with the overall conclusion that I again quote from that same abstract, "There is no evidence that albumin administration reduces mortality in critically ill patients with hypovolemia, burns, or hypoalbuminemia."

Therefore, on August 31st of this year, FDA issued a Dear Doctor letter, which you also have in your packet and which the audience can get on the FDA web site, urging physicians to exercise discretion in the use of albumin or plasma protein fraction based on their own assessment of the data presented by the Cochrane Group.

In addition, it appears prudent for the FDA to work with manufacturers and examine any clinical data that manufacturers may have generated, to examine current labeling to determine whether indications and/or other parts

of the labeling should be revised, and to work with organizations, such as NHLBI, to determine if there are additional clinical trials that should be funded and conducted, and, of course, it was deemed appropriate to update the committee on this, and this concludes my update.

Open Committee Discussion

DR. HOLLINGER: Stay up there just a minute, John, would you, please.

DR. SMALLWOOD: Excuse me. I have been asked that all speakers please speak directly into the microphones. That would include our committee members, as well as our presenters. Thank you.

DR. HOLLINGER: Put up the data on the Cochrane, if you would, please. You spent some time on this, John, but, first of all, the subtotal in the bottom there with hypoalbuminemia should not be 10.2, it should be 40.2 instead of 10.2 if you add them up.

DR. FINLAYSON: That is what I mentioned, yes.

DR. HOLLINGER: In addition, if you look at all the relative risks up there, the 95 percent confidence intervals, they are all below 1 except when you do add them all up and get a subtotal with these groups, meaning that there probably is not a lot of differences at least in every one of the individual studies that was looked at.

Finally, just to make things, I think put it on an

even keel, there is clearly, at least from the hepatology standpoint, use of albumin for patients with large amounts of acidic fluid, and so on, to reduce that with albumin has been very successful, and is very useful, and in addition, as you know, the Barcelona Group, as distinct from the group in California, feels that the addition of albumin in patients who have had a paracentesis, who do not have any peripheral edema, may be very useful and beneficial in reducing changes in the kidney status or in the renal status.

So, there is still a lot of controversies here, and I think I would not want to leave this, after looking at all this data which is compiled here, I do think albumin is way overly used, no question about it, but I do think there are some indications for albumin despite this.

Yes.

DR. FINLAYSON: Could I make the comment, yes, I would like to underline what Dr. Hollinger said. In the University Hospital Consortium's list of indications, which is in the paper in Archives of Internal Medicine, which you also have in your package, in that table, paracentesis where more than 4 liters of fluid was removed was indeed one of the indications where they felt use of albumin was appropriate and well supported by evidence.

DR. HOLLINGER: Dr. Buchholz.

DR. BUCHHOLZ: I do clinical trials as part of what I do in my every-day work, and I would like to address a question to FDA regarding this albumin matter which concerns me as a person who does clinical trials.

Leaving the idea of the concept of meta-analysis out of it, I mean there is a substantial amount of this presentation that has been a discussion of the meta-analysis, and I am not a statistician and I am not going to even attempt to try and understand that, but from a clinical trial point, if I came to FDA with a new product that I had done a clinical trial involving 1,400 patients in 30 centers with a wide diversity of diseases, that I retrospectively grouped into groups after the study was over, that had variable blinding, that had no common protocol, that is, the modes of administration of the various drugs were not the same, that had multiple different control groups -- and I believe critique of this study has indicated that there is something like 14 separate controls groups, that is, different groups in the 30 studies reported, there undoubtedly were protocol violations. There are protocol violations in every study that is done if people will admit to it.

There was an ignoring of the study purpose and study design, and to me, very importantly, an ignoring of the study endpoint, and then a new study endpoint was

retrospectively decided upon after the study was complete.

Now, my question to FDA is if I came to you with a regulatory submission with those kinds of flaws, my suspicion is you would send me packing. Perhaps I should take the message home that if I did a meta-analysis, I could make this better, but it seems to me that there are basic flaws in the way these studies were performed, and certainly just taking into account the studies span of 20-year period, there are some real issues I think with how medicine has changed during that period of time.

So, my question here is really this article has caused a lot of controversy, and it seems to me that there steps being taken or actions proposed that are outside the science that underlies the basic study.

I would like to ask FDA if I came to you with a study, such as what I have just described, what do you all think the chances would be of getting it cleared or approved.

DR. HOLLINGER: John, do you want to respond to that?

DR. FINLAYSON: The first thing I would answer is, of course, FDA does not hypothesize, you bring the study, we will do the analysis. That is the official answer. That is the correct answer.

Now I am going to give my answer. If you were to

bring albumin to us as a new product today, and we said you have to do an efficacy trial, what clinical setting or settings would you propose and what endpoints would you seek.

I say this not all facetiously, because albumin, as I indicated, was first licensed in 1941. It is difficult to know exactly on what clinical basis it was licensed other than the fact that Charles Janeway, Sr., had collected patients who were successfully maintained with albumin.

Certainly, subsequent to licensure, we had an event that is fixed in the minds of all of us over a certain age, which is Pearl Harbor. At that time, Isadore Ravdin put some bottles of 25 percent albumin, which was the only kind of albumin that was available at that time, in fact, was the only kind of albumin that was available for many years, into his pockets, got on an airplane, flew to Pearl Harbor, and came back with glowing testimonials. As they say in the literary world, the rest is history.

We have, to my knowledge, since that time, not required for licensure of albumin anything that would approach the efficacy data that we would require for any product today. Furthermore, as far as safety was concerned, safety usually meant when a new albumin product or a new albumin manufacturer came along, safety in the acute sense, did one get allergic reactions, did one get hyper- or

hypotensive reactions in the short run over the first 24 or 48 hours.

So, the sorts of long-term analyses that we are seeing here, with all of the problems that Dr. Buchholz pointed out, have simply not been, to my knowledge, done in anything representing a prospective way from the FDA's point of view. This is not to say that individual studies, reasonably well conducted, have not been performed.

DR. HOLLINGER: Joel, do you have a comment?

DR. VERTER: In addition to agreeing with what Dr. Buchholz said, I would like to make one or two other comments. First of all, and I am probably in the fringe on this, because meta-analysis is like a virus, it has really taken over a lot, I would like to step back from that term a bit.

I think these type of overviews -- and I have a great admiration for the Cochrane Group, I think they do a very good job up to a point -- it is the bottom line that makes me nervous. When they summarize in an overall odds ratio a relative risk, whatever it is, I think that is when we can have a problem.

Up until that point, they presented a good overview, a summary of all the clinical work that has been done. I think they do that very well. However, if you just notice in just the first part, the hypovolemia group, of the

13 studies I think 8 had 2 or fewer patients in each group or events, I am sorry.

That is hardly something that I would be secure about in making inferences, in other words, one more event in either group changes it dramatically. A zero or a 1 in one group, and a zero or a 1 or 2 in the other group, I think we should view with great caution in trying to view inferences from.

The next point I would like to make is also what Dr. Buchholz said, is that if all of those control groups are the same, in other words, if they all received placebo or if they all received some other standard, that would be one thing. If they are different, I find it hard to understand how you could combine them into one overall outcome.

Finally, if you look at the event rate in the control group, if we make the obviously incorrect assumption that those are all the same control groups, the event rate is 10 percent. If you came to me as a clinical trial designer and asked me to design a trial in which the "placebo," quote, unquote, rate is 10 percent, and you are looking for a 20 percent or so reduction, it would take far more than 250 patients.

So, the studies individually are clearly underpowered for any reasonable alternative hypothesis, and

even as a, quote, unquote, "meta-analysis," it is quite underpowered to find something. So, I think caution is needed here.

DR. HOLLINGER: Dr. McCurdy.

DR. McCURDY: Following on what Joel said, I asked our Biostatistical Department to give me an estimate of sample size necessary in order to do a randomized, controlled trial. It seems likely that all three of those groups up there should be treated separately, certainly, the one with the very low mortality would have to be treated separately, and we came up with a figure of somewhere between 2,500 and 3,000 patients in each of the major subgroups.

This would be a rather difficult and expensive trial to do. The NHLBI, of course, is a reasonable place where such a trial might be handled. At the present time, I don't see it being a real high priority, but we certainly would be willing to talk with FDA, industry, other groups that might have an interest in doing such a trial.

I think I am a bit older than John, so I have a little bit more history behind me. Wasn't the initially developed albumin considerably more salty than the later one, and by the late '40s, I remember people were talking about salt-poor albumin, which really wasn't very poor in salt.

DR. FINLAYSON: First, this is in the conflict of interest that Dr. Smallwood mentioned. Dr. McCurdy and I did not rehearse this, but it is the perfect straight line, because he has couched his question or his comment in my minuscule area of knowledge.

At the time that albumin was being developed by Professor Cohn and his group, those again of us of a certain age remember that we were fighting a war in North Africa, and the saying was albumin holds up in Boston, but what will it do in a tank in Tobruk.

So, there was an undertaking of a series of, in retrospect, very ingenious stability studies. What they used was called the cloud point as an endpoint, where the solution became cloudy. Dr. Schrake in our group has now used much more sophisticated analyses, but found that, by and large, all the conclusions drawn by the Boston Group were absolutely correct.

They began to look for what could be used to stabilize albumin. What they found was, remarkably enough, good, old sodium chloride, if used in sufficient concentration, was pretty good at stabilizing albumin, and so whereas, the very first albumin that had been made was in just plain water, which of course was something that didn't last very long because the first thing people noticed was that the solution then came out at the isoelectric point of

albumin, which was about 4.8, and then began thinking, well, once you put it in physiological saline, possibly with a little buffer, but when these stability studies were done, it was decided that one should put albumin into three-tenths molar sodium chloride.

That became what was known as the standard Army and Navy package. Now, that, of course, was what was distributed, but people didn't stop. They began to look for better stabilizers, and in a very short time, through the work of a number of workers, Boyer, Lum, Luck, and others, they came up with two stabilizers that are still used today, namely, sodium acetyltryptophanate and sodium caprylate or sodium octanoate if you prefer the more chemical name.

When they found that these stabilizers were so effective and that they were able to use them at much lower molar concentrations, they could prepare a solution of albumin which had a considerably lower sodium content, but now you had a second kind of albumin, and so you had to name this new one something, so unfortunately, they named it salt-poor even though sometimes the sodium concentration was of the order of, let's say, 150 milliequivalents per liter, you know, right in the physiological range, and even when it was low, it was still probably above 100 milliequivalents per liter.

The so-called high-salt material disappeared

within about a year because the Army and Navy contracts no longer requested it, they wanted the new stuff with these new, wonderful stabilizers, but the name salt-poor albumin persisted until a predecessor of this group was able to recommend to us that we remove it, and it disappeared with the publication of the additional standards for albumin on May 31st, 1977.

DR. BOYLE: I would like to ask a non-statistical question. In the Letter to the Doctors, there is a line, "On the basis of their analysis, the authors conclude that albumin should not be given to critically ill patients outside of rigorously conducted randomized, controlled trials." Later, it states, "The FDA urges treating physicians to exercise discretion in the use of albumin and PPF based on their own assessment of the data."

What is your estimate of what this is going to do to the demand for albumin?

DR. FINLAYSON: Dr. Epstein and I are exchanging knowing glances. Probably increase it.

[Laughter.]

DR. FINLAYSON: I mean I base that on very good scientific evidence, but with a n of 1, namely, in 1984, there was a consensus development workshop on the use of fresh frozen plasma, and the panel, even though they were very, very generous to fresh frozen plasma, came up with the

concept that the indications for fresh frozen plasma were probably about one-tenth of what had previously been used, and if you got these august persons out in the hall, they said it was probably more like 1 1/100th of that.

What was the result of NIH's reporting of the consensus statement? The use of fresh frozen plasma went up.

DR. HOLLINGER: Jay, we are going to bring this to a close, but, Jay, do you have any comment? Dr. Epstein.

DR. EPSTEIN: I just wanted to be sure that the take-home messages are clear. FDA doesn't know the right answer either. Our point of view is that we reviewed these studies. We found on the face of them that they were not methodologically flawed.

They certainly suffer from serious limitations as outlined by Drs. Buchholz and Verter. We felt, however, that the finding was of such potential significance that physicians should be made aware and that they should be exercising discretion. Particularly, there is a domain of overuse of albumin which ought to be curtailed based on the knowledge that random use is not benign.

We fully believe that there may be patient subsets that continue to benefit, and we think work lies ahead to reclarify which studies in the past have been adequate and which have not, and which need to be repeated.

DR. HOLLINGER: Thank you, Jay. Thank you, John. Stay around. We need people like you to help us understand these things along with Paul.

We are going to move on then to the summary of the workshops, and we will start with the first one, the Summary of the Stem Cell Workshop, Dr. Harvath.

Summary of Stem Cell Workshop

DR. HARVATH: Good morning.

[Slide.]

One week ago today the FDA and Center for Biologics cosponsored in cooperation with the National Heart, Lung, and Blood Institute a workshop, which really was the fourth in a series of workshops since 1995 dedicated to the topic of hematopoietic stem and progenitor cell biology, as well as the development of these products.

The workshop was held on the NIH campus and it was open to the public.

[Slide.]

The workshop objectives were to overview a Federal Register Notice that we published in January of this year entitled, "The Request for Proposed Standards for Unrelated Allogeneic Peripheral and Placental/Umbilical Cord Blood, Hematopoietic Stem/Progenitor Cell Products; Request for Comments." I will talk a little bit about that document, just reviewing it in the next slide.

The second objective was to discuss issues regarding the administration of cytokines to normal donors for mobilization of peripheral blood hematopoietic stem cells; the third to discuss the current status of related and unrelated allogeneic peripheral blood stem/progenitor cell collection and transplantation, to discuss the current status of unrelated allogeneic placental/umbilical cord blood banking and transplantation, and then the last session of the day was to discuss the status of professional voluntary standards development.

In this 20-minute presentation, I will attempt to summarize as briefly as possible the highlights of that meeting. It is our intention to make available on our web site the entire unedited transcript of this meeting. We are going to give that a try and we hope then all of you will be able to view it.

[Slide.]

I would like to just take this opportunity to acknowledge the participants in this workshop and to identify those speakers.

Dr. Mary Horowitz, the scientific director of the International Bone Marrow Transplant Registry, Autologous Bone Marrow Transplant Registry, talked about transplant registry data and some of the statistical consideration.

Paolo Anderlini, a physician from M.D. Anderson

cancer study, presented data on normal donors and cytokine administration, the M.D. Anderson experience.

Dr. Richard Champlin spoke about the M.D. Anderson experience regarding related allogeneic peripheral blood stem cell transplants, and Dr. John DePesio discussed the Washington University experience in the related allogeneic peripheral blood cell transplant setting.

Dr. Dennis Confer, Medical Director of the National Marrow Donor Program, spoke about their trial for unrelated allogeneic peripheral blood stem transplants.

[Slide.]

The third session was dedicated to the topic of cord blood banking and transplantation. Dr. Mitch Cairo, who is currently at Georgetown University, presented the overview of the Heart, Lung, and Blood Institute multicenter cord blood banking and transplantation study.

Dr. Pablo Rubenstein, from the New York Blood Center, presented their experience in banking placental blood, and Dr. Joanne Kurtzberg discussed the unrelated cord blood transplant experience in the United States.

We had a brief presentation from Dr. Donna Wall from the St. Louis cord blood bank.

The last session, we invited participants who are involved in the development of professional standards. Dr. Rebecca Haley presented for the American Association of

Blood Banks, development of their progenitor cell standards.

Three individuals presented, representing the Foundation for Accreditation of Hematopoietic Cell Therapies, Dr. Elizabeth Shpall, the president of that organization, Dr. Scott Rowley, president of the International Society for Hematotherapy and Graft Engineering, and then Dr. Fred LaMaistre spoke on behalf of the American Society for Blood and Marrow Transplantation.

[Slide.]

As I mentioned, this January 20th, FDA published in the Federal Register a request for data, inviting all interested parties from the public to send to the Agency their comments and data to support the development of standards for products in the unrelated allogeneic setting that are derived from placental/umbilical cord blood or from peripheral blood.

This specifically deals with minimally manipulated unrelated allogeneic peripheral and placental/umbilical cord blood, progenitor cells, stem/progenitor cells that are intended for hematopoietic reconstitution, and as outlined in that document, it is stated that it may be possible to develop product standards, establishment controls and processing controls from existing scientific and clinical data, and, if so, then to issue guidance for establishment controls, processing controls, and product standards with

the ultimate goal to grant licensure for products certified as meeting issued standards.

As outlined in that document, if FDA determines that data are available to support the development of standards, then, the Agency intends to publicly announce such standards and licensure may be granted for products certified as meeting the promulgated standards.

However, if sufficient data are not available to develop standards, then, after a specified period of time, unrelated allogeneic stem cell transplants would be subject to IND and marketing application requirements.

This proposal is a follow-up of what we had outlined in the February 28th, 1997, proposed approach to the regulation of cellular and tissue-based products, but this specifically deals with hematopoietic stem and progenitor products.

[Slide.]

So, to summarize some of the findings, I will start first with what we heard regarding peripheral blood stem and progenitor cell products, and rather than continuing to say peripheral blood hematopoietic stem and progenitor cells, I am just going to say peripheral blood stem cells or umbilical cord blood.

The issues here are that normal donors who are providing the product to the recipient are treated for a

period of five to six days with cytokines that are given daily. These may be either granulocyte colony-stimulating factor or granulocyte macrophage colony-stimulating factor, and then they are apheresed, and they may undergo an apheresis procedure once or twice or possibly more times than that.

Most of the peripheral blood experience in collecting these products thus far has occurred with HLA identical sibling donor recipient pairs, and we heard from those two centers that are doing some of that work that in the HLA identical sibling donor recipient pairs, the reported advantages to peripheral blood stem cells when they were contrasted to HLA identical sibling bone marrow donor recipients, during the first 100 days post-transplant, appear to be a decreased time to myeloid recovery with an absolute neutrophil count greater than or equal to 500 neutrophils per microliter.

This appears to be four to five days quicker than what has been seen with bone marrow transplants, however, I would like to point out that these have not been randomized trials, they have not been controlled studies, but basically looking at groups of patients receiving either peripheral blood or bone marrow at different institutions.

There is a decrease in patient stay, hospital pharmacy costs, and blood product support. So, these are

the reported advantages.

Graft-versus-host disease is a major consideration for these type of transplants, and it was reported that thus far the incidence of acute graft-versus-host disease does not appear to be different, however, a surprising outcome is appearing in the instance of chronic graft-versus-host disease in that it appears to be increased in the recipients of these HLA identical grafts.

The bone marrow transplant registry data that have taken analysis up to one year post-transplant indicate a trend to 75 percent incidence in peripheral blood stem cell transplants when they are compared to controlled groups of 45 percent in the bone marrow recipients.

One group Washington University has followed their recipients out to two years post-transplant and are finding a 90 percent actuarial incidence in peripheral blood stem cell transplant reported graft-versus-host disease, chronic GVHD, which is compared to 40 to 60 percent. So, clearly, this is an area that is going to need more follow-up.

[Slide.]

In the related allogeneic peripheral blood donor setting, when the grafts are mismatched at one antigen, that is, they vary by one of the six major HLA antigens, reported data at this conference indicated that this can result in a 100 percent incidence of chronic graft-versus-host disease,

and that 40 percent of this graft-versus-host disease is severe, at grade 3 to 4.

It is believed that the increased incidence of chronic GVHD is associated with a high CD34-positive cell, as well as lymphocyte doses of these grafts, and these investigators are pursuing studies to quantify the effects of lower cell dose and different types of conditioning regimens on the incidence of chronic graft-versus-host disease, and this was an area that a number of investigators identified as an area in need of further research.

Now, all of what I just spoke about was in the related setting. In the unrelated allogeneic peripheral blood setting, there really are very few data available, and the National Marrow Donor Program has undertaken an investigational protocol to collect prospectively information monitoring normal donor safety, as well as the outcome data for recipients of unrelated allogeneic peripheral blood stem cell transplants that are given in a second transplant setting.

What this means is that their donors, their normal marrow donors who have already given bone marrow to a recipient may then be called back because that recipient has done poorly, has relapsed.

They may be called back for a second donation, and in this protocol they are then having an option to address

the use of peripheral blood stem cells in this setting, and they reported that they received 119 requests.

They started their protocol in February of 1997, and as of the beginning of August of '98, they had given 34 normal donors the granulocyte colony-stimulating factor to mobilize stem cells, and presented the status that 17 donors have had one apheresis collection, 15, two apheresis collections, that one donor had been given the cytokine, but no stem cells were collected, and another donor had been given the cytokine, the stem cells were collected, but not infused.

The reasons for these two categories here were that the potential recipient of the products, the medical conditions had worsened such that a transplant was not possible at that time.

As I said, they report that donors and recipients are being extensively studied and that NMDP announced that they intend to develop a similar study for unrelated peripheral blood stem cells for first donation transplants.

[Slide.]

So, the potential disadvantages of peripheral blood stem cells that were noted involved more frequent cytomegalovirus viremia, there are unknown risks of increased chronic graft-versus-host disease, and these really need to be monitored more carefully.

It is unknown if there is a survival of the peripheral blood stem cells compared to bone marrow, and these raise new risks for the normal donor.

One of the topics that we had discussion a great deal about at this conference and the conference the following day was the topic of cytokine administration to normal donors.

Some of the reported short-term safety issues for normal donors have been bone pain, headache, fatigue, nausea, transient elevations, and alkaline phosphatase and lactate dehydrogenase.

There are infrequent episodes of chest pain, fluid retention, collection of these products in this setting can require a central venous catheter placement and complications with that, electrolyte and fluid shifts, and then a self-limiting and transient leukocytosis and thrombocytopenia. This may last for a couple of weeks, and so there has been some attention to monitoring this and discussions about the importance of following these donors.

The big question here remains the unknown in terms of long-term safety issues for normal donors, and many of these investigators feel that it would be prudent to set up a long-term follow-up registry of normal donors who are given these cytokines.

[Slide.]

Areas that were proposed by the speakers for future research in the peripheral blood stem cell area include the establishment of a normal donor registry to monitor the long-term, that is, at least out to 10 years, follow-up of normal donors, further studies of the biological and clinical effects of cytokines and apheresis procedures in normal donors, approaches to control graft-versus-host disease, studies to assess the stability of peripheral blood stem cell engraftment to assess the functional effects of T cell depletion, methods for standardizing the CD34 cell assays, and also standardization of some tumor cell assays that are currently being developed in the breast cancer and AML setting.

[Slide.]

Shifting to the afternoon session, the topic was cord blood banking and transplantation, an area which I am sure all of you have heard there has been great excitement and activity. We focused our attention at this meeting for the unrelated allogeneic banking and transplant setting, and the NHLBI multicenter cord blood banking and transplantation study was reviewed.

For any great detail on that, Dr. McCurdy who sits on the panel is the expert here in that design, but in essence, it consists of three banks - Duke, UCLA, and Georgetown, and six transplant centers - Duke, the

University of Minnesota, UCLA, Fred Hutchinson, Indiana University, and the Dana Farber.

This will be a five-year extensive study to characterize the cord blood product that is collected and banked, as well as the transplant outcome data results using uniform protocols for the collection and banking of the product, as well as the conditioning protocols for the treatment of these patients.

The New York Placental Blood Program was reviewed by Dr. Pablo Rubenstein, the director of that program. It is the first unrelated placental/umbilical cord blood bank that was established. It was established in 1992.

They have banked and characterized more than 7,700 units of placental blood that are made available for the public use. They have provided 700 units of placental blood for transplantation thus far, and Dr. Rubenstein just informed me this week that the results of the first 562 consecutive transplants, that study is in press in the New England Journal of Medicine, so I think it will be very interesting for us to keep our eyes open for that paper.

Basically, what has been observed is that the speed of myeloid engraftment appears to be associated primarily with the graft cell dose, and that the transplant related events, complicated events, are associated with the patient's underlying disease, their age, the graft cell

dose, the disparity of the HLA, and the transplant center. Differences are observed in the United States as compared to foreign transplant centers. Again, those details will be provided in the New England Journal paper.

The St. Louis cord blood bank, unlike the New York bank and the NHLBI study, I didn't mention it here, but the NHLBI study and the New York blood center have dedicated personnel to collect their cord blood units who are trained and very much dedicated for that purpose.

In contrast, the St. Louis cord blood bank, which is a community-based, unrelated cord blood bank, has decided to evaluate the role of obstetricians and nurse midwifes who perform the collections, and they reported that they have collected 10,000 units and have banked 3,000 of those units that they have found to be acceptable.

The reason for the 30 percent banking of those units has been low volume and there have been instances where they have bacterial contamination of those units.

So, we have three different approaches underway. I would like to make the point that all of these groups have publicly acknowledged that they are conducting their studies under INDs.

[Slide.]

The areas proposed by speakers for further research in the cord blood area include ex-vivo expansion of

cord blood to try and increase the cell graft dose, adoptive cellular therapies, studies using haplo-identical related cord blood transplants, exploring the use of cord blood with gene therapy, and immunological vaccine development.

[Slide.]

Now, the last session of the day, which is beginning on this slide, involved a very lively discussion of the status of professional standards in this area. There really are two major organization, there really are three groups who have standards - the National Marrow Donor Program has their own set of standards for their program which are very analogous to other professional standards.

I would like to point out that the two organizations represented in the panel discussion and presentation summaries were the American Association of Blood Banks, and many of you are very familiar with this organization, they were established in 1947, and they currently represent 8,500 individual as well as 2,200 institutional members.

They have published standards for hematopoietic progenitor cells since 1991, and their approach to developing standards has invited the participation not only of their own colleagues, but professionals from the American Society for Apheresis, the FDA, FAHCT, which represents the International Society for Hematotherapy and Graft

Engineering, ISHAGE, as well as the American Society for Blood and Marrow Transplantation, as well as members from the National Marrow Donor Program.

They are also including in their standards development two public members, one, an ethicist, and the other, a patient who has received hematopoietic progenitor cells as a therapeutic protocol.

They are currently revising their published 1996 standards to incorporate the ISO-9000 model for prospective comprehensive quality management program.

[Slide.]

Representatives speaking on behalf of the FAHCT standards described their program. They were founded in 1996 by the two parent organizations, ASBMT and ISHAGE, consisting of 900 individual members for ASBMT and 1,000 individual members for ISHAGE.

Standards are established for the purpose of high quality medical and laboratory practice, and they have developed and have implemented voluntary inspection and accreditation.

Their committee is comprised of members from their three organizations - ASBMT, ISHAGE, and FAHCT. They expressed concerns at this meeting regarding FDA's proposed rule for facility registration and product listing. Now, that was the proposed rule that we did not particularly

address at this conference, but it was another proposed rule that came out this year, in May.

Their concerns were that registration alone does not necessarily improve safety. They are concerned about sequelae to registration. They are unsure of the FDA's ultimate intentions in this area, and they feel additional regulations have the potential to impede technological advances and compromise optimal patient care.

FAHCT collection center standards include a number of things, and two of them include donor health screening including genetic diseases, as well as having a requirement for recording clinical outcome data.

FAHCT has proposed at the meeting that FDA grant them deemed status, and they acknowledge that some groups may choose not to participate in the voluntary accreditation.

[Slide.]

So, at the end of the day, I think, in the final slide, at the conclusion of this particular panel discussion, I think it was quite obvious to everyone who was there that a topic in need of further discussion is the importance of developing a single set of standards that are acceptable to all interested professionals in this field.

I will stop there and see if there are any questions, and then I will move on to reviewing the next day

workshop, which was on granulocytes for transfusion.

DR. HOLLINGER: Why don't you go ahead.

Summary of the Granulocyte Workshop

[Slide.]

DR. HARVATH: So, moving along, the next day, which was Friday, September the 11th, we had a conference on the topic of granulocytes for transfusion, research and clinical experience.

This was cosponsored by the Center for Biologics and the Heart, Lung, and Blood Institute, the National Institute of Allergy and Infectious Diseases, and the Warren Grant Magnuson NIH Clinical Center.

I would like to comment at this point that the last time FDA had even addressed the topic of granulocytes for collection or transfusion was in October of 1980, at an FDA-sponsored conference which primarily focused on donor safety in the leukapheresis setting.

[Slide.]

The workshop objectives for this conference were based really on the findings and the renewed interest in granulocytes for transfusion and that comes from the fact that the administration of these cytokines G-CSF and GM-CSF, the same two cytokines that are used to mobilize hematopoietic progenitor cells in the peripheral blood, have also been found to increase the number of circulating

granulocytes. In the normal volunteers, this results in the peripheral mobilization of high concentrations of granulocytes, and so it has renewed an interest in the collection of granulocytes for transfusion.

The purposes of the workshop were to discuss the current scientific and clinical experience with cytokine mobilized granulocyte transfusion products. Again, this area of concern, the effects of cytokine administration on normal donors, the functional properties of the transfusion product, and to identify studies that are needed to establish the safety and effectiveness of the transfusion product.

[Slide.]

This conference, since it was much smaller and much more focused, I think we had fewer speakers and I think more opportunity to go into a lot more detail on specific scientific data that were collected, and I will spare you the details of that.

However, I would like to at this point in time again acknowledge the contributors to this meeting. They included Dr. David Dale, who gave a historical perspective and a summary of clinical trial considerations. Dr. Dale is from the University of Washington at Seattle, and has been involved in this field for 30 years.

Dr. Thomas Price, Puget Sound Blood Center,

discussed cytokine administration to normal granulocyte donors. Dr. Dan Ambruso, from [Bonafice] Blood Center in Denver, Colorado, and the University of Colorado, spoke about functional properties of granulocytes from normal donors after G-CSF administration, and Dr. Susan Leitman, whom all of you know from the NIH Clinical Center, Department of Transfusion Medicine, talked about the NIH experience on cytokine mobilized granulocytes and an assessment of efficacy.

The afternoon session, which was moderated by BPAC member Dr. Dave Stroncek, focused on the product evaluation, and Dr. Douglas Adkins from Washington University in St. Louis spoke about their research in that area.

Dr. Thomas Lane from the University of San Diego spoke about storage considerations, and Dr. Conrad Liles from the University of Washington spoke about in-vitro assays predictive of product function.

[Slide.]

Issues that were discussed at this conference included topics, such as the optimal granulocyte mobilization strategies and the need to try to work in a cooperative manner to share data to get at the best strategy for mobilizing these products, quality of the cell product, normal donor willingness to participate as a donor for these products, as well as the safety experience, the recipient

benefit and risk, as well as the topic of therapeutic efficacy.

[Slide.]

Dr. Dale focused part of his discussion on randomized clinical trial design, and in that it became very clear that an area that needs to be finalized is the patient population. It is pretty much agreed that the ideal patients for this would be people who are on aggressive chemotherapy, perhaps because they are a stem cell transplant recipient, during a time when they are neutropenic and they have fungal or resistant bacterial infections which cannot be controlled by the armamentarium of the currently available antimicrobial therapies.

The primary endpoint -- and there appeared to be two that are possible -- one is the resolution of documented infections and/or alternatively, the prophylaxis of infection, and we heard discussion of the possibility of both of these as a primary endpoint and the need to make a decision in designing a clinical trial as to which one would be the most appropriate.

Secondary endpoints that were proposed included survival, safety, and a cost analysis.

[Slide.]

The normal donors are typically the community blood donors who are the pedigreed platelet apheresis donors

who are these individuals who are willing to donate granulocytes. They may also be the bone marrow or peripheral blood stem cell donor, and they may be related to the allogeneic recipient or they may be unrelated.

These normal donors may be given a single dose of G-CSF with a total dosing of 300 to 600 micrograms with or without 8 milligrams of dexamethasone, and this, either alone or in combination, is given 12 hours prior to leukapheresis.

In contrast to the collection of peripheral blood stem cells which require a five- to six-day course of daily injections of the cytokine, the granulocyte collection strategy is a single dose of the cytokine with or without dexamethasone and then the collection of the product.

However, these same donors may be multiply pheresed, so they may come back in another few weeks or monthly to donate product, so there are some concerns in following the short term as well as long term safety of these normal individuals.

[Slide.]

I would like to present a summary of just an example of data. This is data from Dr. Tom Price at Puget Sound Blood Center, and these are examples from a series of 175 normal donors that were given G-CSF with dexamethasone and looking at the neutrophil counts before they were given

the G-CSF and then right before they were apheresed and then talking about the types of yields.

The reason for the excitement in this field is that you can it is possible to achieve fairly routinely a 10-fold increase in the number of granulocytes for collection in the free circulation and yields from 81 -- well, here, the mean was 81.9 times 10^9 granulocytes total product, but they range from 23.8 to 144 times 10^9. This is a concentration that was previously just simply not achievable.

[Slide.]

Another discussion focused on the properties of mobilized granulocyte products, and what is interesting to note is that these products, a bag of granulocytes, will contain in that same bag a unit of platelets that is also collected by this process.

So, it is an interesting set of scientific issues, and Dr. Snyder, who was there from AABB, commented that he felt it was important to look at platelet activation in this setting to determine what that might do to the quality of the granulocyte product.

Indium-labeled studies presented at this conference showed that the indium-labeled granulocytes that are subsequently transfused will initially locate in the lung and then they go to spleen and ultimately to sites of

inflammation and infection.

A single transfusion unit of the granulocytes was found to restore in many patients a normal granulocyte count one hour post-transfusion, and this frequently remained for 24 hours after the transfusion.

The studies described also being able to mobilize cells to the gingival area and by simply doing a mouthwash with saline and then counting the cells that were in the mouth, they were able to ascertain that the cells that had been transfused had actually migrated there.

So, I think that there was a lot of interest in I think cooperatively looking at a way to address the best way to assess the efficacy of the product and to characterize this product.

[Slide.]

In the session on in-vitro assays of product function, the following areas were addressed, and I won't go into detail, but everyone agreed that chemotaxis is probably the most sensitive in-vitro assay predictive of product function, however in vitro assay it happens to be the most variable, that their studies looking at cell death or apoptosis and that it has been found that this is delayed in these products.

That means the cells potentially have a longer shelf life than granulocytes that have not been exposed to

the cytokine, studies of their ability to generate an oxidative metabolic burst which, as you know, is associated with the ability of the cells to kill microbes and their ability to phagocytize and kill a variety of microbes both fungi, as well as bacteria.

[Slide.]

The last slide, I would like to end the presentation here and say that the recommendations of the speakers were fairly unanimous that they felt the time is here now for a randomized Phase III clinical trial, and they also agree that normal donors for these products should be followed for long-term effects of cytokine administration and the apheresis procedures.

Thank you for your attention.

DR. HOLLINGER: Thank you.

The third summary is going to be by Ed Tabor of an HCV PCR workshop that was just completed yesterday.

Summary of HCV PCR Workshop

DR. TABOR: The Workshop on Nucleic Acid Testing for HCV and Other Viruses in Blood Donors, sponsored by the Food and Drug Administration, was held yesterday at the Parklawn Building.

Its purpose was to bring together representatives of government, industry, and other interested persons to discuss the use of nucleic acid tests, referred to as NAT,

in screening minipools of plasma to eliminate those few donates units of blood and plasma with HCV that are not now detected by currently licensed screening tests.

Such testing will be required in Europe for all plasma for fractionation by July 1, 1999, and that has been the force driving its rapid evaluation in the United States. Although some other viruses were discussed in the workshop, particularly by Dr. Peter Simmonds, my summary this morning will focus on the problem of HCV.

As pointed out by Dr. Celso Bianco, all source plasma and recovered plasma intended for fractionation is screened by sensitive tests for antibody to HCV already, and all plasma derivatives that are licensed in the United States undergo processes during manufacture that remove or inactivate HCV except for some IGIMs.

Thus, there has been no documented transmission of HCV by licensed plasma derivatives since 1987, with the sole exception of one outbreak associated with one manufacturer's IGIV in 1993, which was from a nonviral inactivated product at that time, and there has been no transmission by IGIV since 1994.

So, the additional testing for plasma would function primarily as an additional safety net. The fact that there are no similar methods to remove or inactivate HCV in whole blood at present means that NAT screening of

whole blood and cellular components for HCV RNA would significantly augment the current screening tests for anti-HCV for those products.

As described by Dr. Harvey Alter in the workshop, 85 percent of HCV infections are chronic infections; 20 percent of those will develop cirrhosis within about 20 to 30 years after onset of infection. Concurrent use of alcoholic beverages is a major risk factor for an HCV-infected person developing cirrhosis, the O.R. being 147.

An interesting clinical aspect of HCV infection was described by Dr. Betty Robertson, who pointed out that whereas about 74 percent of most persons with anti-HCV have HCV RNA detectable by NAT, among black males 95 percent of anti-HCV-positive individuals have HCV RNA. In addition, more black males have HCV genotype 1b infections than are found in other groups in the U.S. This is a more virulent form of the virus, however, further conclusions were felt not to be possible at present.

Dr. Steven Kleinman described data from the REDS study and a Red Cross study showing that donations are indeed occurring in the window period of HCV infection and are being missed. This window period is a period when the virus is present in the donor's blood, as detected by NAT tests, but when anti-HCV is not yet detectable.

Window period donations by first time donors with

HCV are 26 times more prevalent than window period donations for HIV, and this is the reason why the blood collecting community is focusing on NAT testing for HCV first.

During the workshop, 11 representatives from 11 companies presented their data from NAT testing. This was one of the most interesting parts of the workshop.

At least five of the manufacturers are involved in collaborations with one or more of the other manufacturers. Most, but not all, said they are currently doing these studies under INDs. It is noteworthy that some of the manufacturers have been able to detect as few as 10 International Units/ml of HCV RNA, which translates into approximately 20 to 50 copies per ml.

The technology has advanced sufficiently to identify six genotypes of HCV, as described earlier in the day by Dr. Jens Bukh; all of the manufacturers reported that their methods could detect all genotypes with equal sensitivity, and most had tested all six genotypes.

Dr. Indira Hewlett described FDA's view of assay validation and evaluating analytical sensitivity. Perspectives on the regulation and use of NAT tests in the Netherlands, Germany, and in the European Union in general were provided by Dr. Nico Lelie, Dr. Micha Nubling, and Dr. Guy Rautman, respectively.

Drs. John Saldanha and Dr. Mei-ying Yu described

the international and FDA reference standards that have been developed or are under development. As part of the effort to develop the WHO HCV International Standard, an effort has been made to convert from talking about RNA copies or genome equivalents to International Units with 1 International Unit being equal to between 2 and 5 genome equivalents.

In September 1996, FDA held an earlier conference on the feasibility of genetic technology to close the HIV window in donor screening. At that time, implementation was not considered practical due to limitations in technology. FDA is encouraged to see the rapid pace at which nucleic acid technologies are being developed for implementation and pooled testing under INDs. We continue to encourage technology development that would further improve the viral safety of blood and blood products.

I would like to acknowledge the work of the other members of the organizing committee - Drs. Yu, Fashid, Epstein, and Hewlett, and the participation of Drs. Dodd, Miriam Alter, Harvey Alter, Dr. Seeff, and Dr. Bianco in the panel discussion.

Thank you.

DR. HOLLINGER: Thank you, Ed. I want to thank all the members of the FDA staff for these comments on the updates and the workshops. I know it is a lot of trouble to really put these together.

We have a little bit of time here. Does the committee have any questions in regards to anything that has so far been discussed this morning, any comments that they would like to make? Yes, Corey. Mr. Dubin.

MR. DUBIN: Corey is fine, Blaine.

My comments are more for what is lacking rather than what is there, and I in part bring this to the committee because my board has brought this to my attention. I appreciate the updates this morning. I think they were very important, but I continue to have trouble understanding something.

In terms of the HCV lookback, we have just seen the guidance pulled from the web and we are told that that is being reevaluated at FDA, and we get no update there. The CJD real change, and no update there, and 50 percent of the manufacturers are under consent decree with regard to GMP problems, and yet the committee is getting no updates.

I personally, and my board, are suggesting that we are asked to come to the table and address GMP and safety questions in a climate when we have no updates about the status of the consent decrees or compliance, and when requesting the consent decrees, I, as a member of the Blood Products Advisory Committee, was told I had to go through the Freedom of Information Act, which was a little surprising.

Last time, Dr. Mitchell raised the question of HCC research and whether or not that was proper at FDA or whether or not that should be done over at the National Institutes of Health.

I think what I am saying is two things. A sense of a lack of a follow-up in terms of things we discuss and come back to, but more important, working in a vacuum, and it makes me question what our real role is, because these things are going on out there, for instance, the GAO report is about to be released, are we going to get an update from staff.

The title of the GAO report is Plasma products are safe if GMP is followed. Well, that speaks directly to this question of 50 percent of the manufacturers under consent decree and whether or not GMP standards are being followed, and for me it is hard to come to the table and do this job correctly and responsibly, feeling like this committee lives in a vacuum.

I think it is important we begin to raise these questions and ask why we don't get updates, why we don't talk about the consent decrees, why the GAO report doesn't come to the committee, and why we don't circle around.

I do think everyone would agree that pullback of the HCV lookback guidance is a big event. The Liver Foundation has sent a rather scathing letter to Secretary

Shalala about this, so I feel we come into this room and sit in a vacuum with these things kind of swirling around us, and these things directly impact what we do here.

Thank you, Blaine.

DR. HOLLINGER: Corey, I think those are good points. I think the lookback issue turned out to be one of logistics, but perhaps, Robin, would you or Jay perhaps, would you want to just comment about that briefly?

DR. EPSTEIN: Well, I agree that this committee has an interest in the entire spectrum of blood safety and availability issues that the FDA deals with, however, it is also a fact that this is not the only forum in which information is brought forward and that for the CJD issue, for example, we now have an advisory committee on transmissible spongiform encephalopathies, that we have, in fact, periodically updated that committee.

Additionally, where it has been recognized that the decisions go beyond merely a scientific discussion, and involve global public health, economic, social, legal, ethical considerations, that those issues have been brought before an advisory committee of the Department of Health and Human Services, namely, the advisory committee on Blood Safety and Availability, and I think it would be unfair to assert that we have not appropriately and periodically updated that group on these very issues, including CJD and

hepatitis C lookback.

More specifically to the point of concern regarding FDA's withdrawal of the March 20th, 1998 guidance on hepatitis C lookback, the Agency did place a notice on the CBER web site on September the 8th, withdrawing the extant guidance, however, the notice makes clear that the intent was to remove the September 20th, 1998, implementation expectation date that is contained in the March guidance, and the reason for that was made clear at this advisory committee back in June of '98, where FDA stated that it had received what it felt were significant comments to the March guidance, that they necessitated changes in the guidance that would improve, not just clarity, but the effectiveness of the lookback program, that these spoke directly to donor retesting, they spoke directly to implementation time frames, and it was stated in June '98 that we would reissue the guidance.

The reason that we withdraw the guidance on the 8th was to prevent a situation in which blood establishments would be captured by that implementation date when they knew and we knew that the guidance was going to be changed and imminently.

So, I would say stay tuned, the guidance is going to issue very, very soon, that it is not, in fact, a cessation of the lookback initiative by any stretch, that

there is no misunderstanding of this by the blood industry, indeed, the AABB put out a notice on the faxnet acknowledging the withdrawal of the policy, but advising its members to continue to proceed with the record searches and to fully expect that the notifications would begin consistent with reissued guidance.

So, I think that that, if you will, although it has the appearance of a dramatic gesture, is, in fact, a minor technical realignment consistent with our stated updates.

So, I understand what you are saying, Corey, that there is a problem of seeing issues with tunnel vision and a need and desire to have local pictures painted, but I do think that it remains a fact of life that, you know, not every issue at every time comes before this committee, but that is not the same thing as saying that the Agency is not being appropriately public or not updating its advisory committees.

DR. HOLLINGER: Thank you. Also, is Dr. Stramer here?

MR. DUBIN: Jay, I want to be real clear about what I am saying, so there is no misunderstanding absolutely at all.

We are very clear about blood safety and availability and the discussions going on there, and the

need to take economic and supply issues there because they are necessarily FDA as a focus, and that FDA has participated in that discussion, I am absolutely clear about that.

I even understand very clearly your response about the HCV guidance. I think it is helpful that that gets on the agenda and that response is made because it clears the air. The landscape issue, I also understand what you are saying about the bigger landscape, but let me try to hone this down just a little, so I can be really specific about what is coming from my board.

We are asked to sit and discuss problems and safety margin standards that relate to GMP, and 50 percent of the manufacturers are under consent decree, in part for GMP violations, and I am unclear or have not felt and seen at the meetings those discussions.

That troubles me when I am asked to make a vote, and understand, not being a doctor, being a consumer at this table conveys a certain degree of responsibility that we have, that we take quite seriously, and we obviously are on display in some way and have to do well here, and we have demonstrated we can be in this process, but there is a discomfort with what is going on out in the world, not in issues that don't belong in FDA, but GMP issues very specific, and the committee's ability to advise, which is

all we do, granted, we understand that, and not being updated on compliance, GMP, consent decree, those kind of issues which are the purview of FDA and the committee.

Therefore, that is really the focus, and I think that is very specific rather than so global that I am off in right field or kind of between committees. I think I am focused on an FDA issue.

As it is brought by my board, the question is you are voting on decisions, yet, these consent decrees exist, some on GMP, and we don't see it on the committee agenda, and you don't come back and tell us that you were updated in this way.

Thank you, Blaine.

DR. HOLLINGER: We have got just a few more minutes. Thank you, Corey.

I asked Sue if she would present just a couple of slides on an issue that had been brought up before. One of the issues that had been discussed before was whether it was necessary always to do RIBA testing in place of ratios or degrees of positivity, where there is a relationship between degrees of positivity of the EIA anti-HCV tests and the RIBA tests, therefore, whether one could get by with just doing EIA testing in most cases instead of having to supplement it with RIBA testing. It goes to some of the issues from the FDA guidance summary, as well.

She has a couple of slides. I think I have asked her to take about five minutes if she would do that. Please, Sue.

RIBA and EIA Data

DR. STRAMER: While the projector is being turned on, I will just give you some background information.

Following the last Blood Products Advisory Committee meeting where confirmatory testing were some issues that were highlighted by the FDA guidance document on HCV lookback, the question was asked how predictive signal-to-cutoff ratios are for the EIA-2 and the EIA-3.

Well, since June of '93, we have collated our EIA data relative to the confirmatory data using the Ortho-2.0 and then the Ortho-3.0 screen in tandem with the corresponding supplemental assays, either the FDA-licensed RIBA-2 assay or the unlicensed RIBA-3, which we have been using under an investigational protocol.

If the slide projector is on, that if fine, if not, I will just do this.

DR. HOLLINGER: If you will hold for just a minute, Susan, please.

John, you had a comment.

DR. BOYLE: I just wanted to agree with Mr. Dubin -- I won't call him Corey -- but just to add that we are being asked to make a lot of decisions in a context where

there are ongoing problems. We only meet four times a year. Sometimes we have updates, sometimes we don't on these issues, the GMPs being the issues, availability of product, and so on.

It would be helpful if we could have an ongoing update on those issues at each meeting. It doesn't have to take a lot of time, but for those people who don't run around to each of the different committee meetings that deal with some of this, I think it would be very helpful.

DR. HOLLINGER: Mark.

DR. MITCHELL: I also think it might be helpful to get the minutes from the other committees. Obviously, again, we know that we don't have the purview, but that we need to coordinate with some of the other committees, like the CJD committee, and so on, and I think it would be helpful for us in our decisionmaking to receive minutes from our meetings, as well as from the other committee meetings.

DR. HOLLINGER: Ms. Knowles.

MS. KNOWLES: I have been interested in this issue since actually injunctions were first leveled in 1993, and I actually sent a written request asking for an update on those last spring, I believe, to FDA.

MR. DUBIN: It would be nice to get answered, Blaine, why we have to go through FOIA to get a consent decree. It would be nice to have that answered.

DR. HOLLINGER: Okay. Why don't you go ahead and summarize.

DR. STRAMER: I will just summarize in six basic lines what the data state. These data have been provided to Paul Mied of CBER for FDA review, as well as to Blaine following the last BPAC meeting.

For HCV 2.0, including the time periods from June '93 through June '96, there have been 45,320 repeat reactive samples, donor samples that the American Red Cross -- what do you know, there they are.

[Slide.]

Of those samples, I am just going to focus on those that are repeat reactive and confirmed positive, so it is this bar up here, so what you see on this bar and whisker plot is signal-to-cutoff ratio, and in this case, this is the HCV 2.0 including 45,320 repeat reactive samples against the outcome on RIBA. These are the samples that we will focus on in advance anyway, but let me go through these.

[Slide.]

28,153 or 62 percent of the total population confirmed positive. For those centers, for example, who didn't do RIBA, I will tell you what these signal-to-cutoff ratios mean in the absence of a confirmatory test.

DR. HOLLINGER: Could you take the timer off of that thing in the back. I think that is what is on, and

maybe go back one, if you would, please.

DR. STRAMER: Okay. Hopefully, it will stay there.

[Slide.]

Of these samples listed here, 26,950 or 60 percent had signal-to-cutoffs greater than 3.5 and confirmed positive. Of those 23,599 of the total I just mentioned, with S to COs greater than 3.5, or 26,950, 87.5 percent confirmed positive.

So, the bottom line from these data on 2.0, and then I will review the same data for 3.0, is of the total population, approximately 60 percent had S to COs greater than 3.5, and 87 percent of those confirmed as positive.

So, if you took a signal-to-cutoff ratio of 3.5, 87.5 percent of those were confirmed positive even in the absence of doing a confirmatory test, so one could trigger a lookback based on the use --if that is one possibility -- one could trigger a lookback based on a signal-to-cutoff ratio of greater than 3.5 on the EIA-2.

[Slide.]

Looking at these same data, these are just the population distributions of the different confirmed outcomes, those are confirmed positive, these are indeterminates against the signal-to-cutoff ratios.

[Slide.]

These are the negative distributions.

[Slide.]

Looking at the same data for EIA-3 and the use of the unlicensed RIBA test, containing the 3.0 antigens, you can see there is a higher signal-to-cutoff ratio for the confirmed positives.

[Slide.]

Of these, the positive predictive value is even better. So, if you are using 3.0, for example, in the absence of RIBA, a total of 66.5 percent of those samples would confirm as positive.

So, in total, 15,165 had signal-to-cutoff ratios greater than 3.5, and confirmed as positive for positive predictive value of 92.3 percent. So, in contrast to the HCV 2.0, in which if you had an S to CO of greater than 3.5, 87.5 percent confirmed as positive, in this case, an S to CO greater than 3.5 had a positive predictive value of 92.3 percent of confirming as positive.

DR. HOLLINGER: Thank you very much, Susan.

We are going to take a break now, and we will reconvene at 10:30 for the TT and Transfusion Safety.

[Recess.]

DR. SMALLWOOD: We will proceed with the next agenda item. I would just like to acknowledge the previous discussion and concerns expressed by advisory committee

members.

While FDA appreciates the concerns expressed by the committee members to be updated with pertinent information on regulatory compliance, the procedure is to direct inquiries through the committee chair or through the executive secretary, by which we then can consider this to be an official request, and then we will respond within the scope of the FDA authority.

With regard to the requests for the GAO report, copies had been requested for the advisory committee members, unfortunately, they have not arrived yet, but once we have received them, they will be distributed to the committee members.

Finally, as an aside, I would just like to ask all presenters, including FDA, as well, if you have not submitted to me copies of your presentations, please do so before you leave. If not, I will continue to harass you. Thank you.

DR. HOLLINGER: Thank you, Dr. Smallwood.

This is going to be an interesting topic about a virus discovered in Japan. I think it will be useful to the committee as an update -- I mean not an update -- but really for informational purposes only, something that we may need to consider and talk about later on, but that is really why the whole session is being assembled.

We will start today. Dr. Ed Tabor is going to give us an overview of the topic.

TT Virus and Transfusion Safety

Introduction and Overview

DR. TABOR: The first slide, please.

[Slide.]

I am going to talk to you this morning about TT virus, a newly recognized virus in human blood and liver. TT virus was first reported less than a year ago, and it is really very exciting when there is a newly-recognized virus or when a virus that has been around and we haven't recognized it comes to scientific attention and we suddenly realize it has been there.

It is a little bit like being in a newly-discovered continent, a newly-discovered land, and we are seeing a lot of these newly-discovered, newly-recognized viruses nowadays, and the reason is because we are looking for them. We are looking for new viruses that might be causes of post-transfusion hepatitis.

[Slide.]

Why are we looking for those? Well, for one thing, there is a measurable percentage of post-transfusion hepatitis cases that are not due to any of the known hepatitis viruses, and there are also many cases of fulminant hepatitis and chronic liver disease that do not

have any known hepatitis virus associated with them.

[Slide.]

The TT virus was first reported at the very end of last year by Nishizawa, et al. It was isolated from the serum of a patient whose initials were TT, and Dr. Nishizawa unfortunately chose the unfortunate name of TT virus. It is unfortunate because it is ready-made for confusion with TTV, which to those of us in virology means transfusion-transmitted viruses, which was the name of a very major study run by NHLBI a number of years ago.

Nonetheless, they isolated this virus from the serum of patient TT who had a peak ALT of 180 International Units between 8 and 10 weeks post-transfusion. The way they isolated this virus was they took a 1 to 1 mixture of the serum from TT at weeks 8 and 10 and also a sample from week 2 when his ALT was normal, and they used a procedure called "representational difference analysis," and what this basically is, is a tag is put on the samples from 8 to 10 weeks, and then PCR is done on both samples together, and samples that have the tag because of the nature of the primers that are used, samples that have the tag can amplify exponentially whereas those without the tag can only amplify in a linear fashion and therefore not much of those are produced, and what you end up with is an amplification of whatever nucleic acids were present in the 8 to 10 week

sample, but were not present in the 2 week sample.

When they looked at this in sequence, they found it did not have a sequence that matched anything in the existing data banks.

[Slide.]

There are two major issues involving TT virus. These are is TT virus a TTV, that is, is it transmitted by transfusion, and secondly, does it cause disease.

What I am going to do this morning is describe for you all that has been published about this virus, but I think that at the end of the session this morning, those of you who are in this room are going to know more about TT virus than anyone in the United States because we have a series of speakers from all of the leading laboratories this morning, all the leading U.S. laboratories, who are working on this virus, and I know some of them are going to present data that have not been published previously, and also data that may change some of the concepts that I am describing.

What I am describing to you now is what is known at the beginning of this conference, and I think it is going to be a very exciting conference, and we are going to learn a lot about this virus today.

[Slide.]

First of all, is TT virus a TTV? There is some very persuasive evidence to suggest that it is transmitted

by transfusion. First of all, it was isolated from the serum of a patient with post-transfusion hepatitis.

In addition, Nishizawa reported finding viremia, as finding the virus in the serum of three to five patients with post-transfusion hepatitis that was not due to hepatitis A through G, non-A-to-G post-transfusion hepatitis.

It is very highly prevalent in hemophiliacs, and in a study published by Naoumov, which since he was in Roger Williams' group, I assume that these were patients in Roger Williams' clinic, the study did not specify, but in these patients at a British clinic, 63 percent of those with TT virus had a history of transfusion or a history of IV drug abuse.

However, as of the present time, no one has shown that a patient who receives a transfusion of TT virus-positive blood acquires TT virus infection.

[Slide.]

Dr. Peter Simmonds has conducted some very impressive studies on TT virus. We are very, very fortunate to have him here today, and he will be presenting this morning, as well. He was here for our workshop yesterday.

Dr. Simmonds is in Edinburgh, Scotland, and he reported finding TT virus in the serum of 17 out of 1,000 qualified blood donors. He examined the sequences and found

that the two reported genotypes were present, and he found evidence that there might be a third new genotype.

When he looked at lots of anti-hemophilic factor, Factor VIII and Factor IX, that had been made since viral inactivation was put in place, TT virus sequences were found in 36 percent of the lots, whereas, in those lots of AHF and Factor IX made before viral inactivation, TT virus could be found in 75 percent, and this at least suggests that the virus is partially inactivated or partially destroyed by the inactivation processes.

When he looked at the serums of hemophiliacs who had been treated only with inactivated product, either mild hemophiliacs or those who had been diagnosed since the availability of inactivated product, TT virus was present in the serum of only 5 percent, whereas, those who had been treated before the availability of inactivated product, TT virus was present in 27 percent.

He also found evidence that with increasingly severe hemophilia, the prevalence of TT virus increased, and this is, of course, presumably due to greater numbers of transfusions and exposure to product.

[Slide.]

Well, TT virus has these characteristics. It is a single-stranded DNA virus, it is non-enveloped, it has a number of genotypes that differ from each other at the

nucleotide level by at least 30 percent.

It is thought at present to consist of more than 3,700 bases, and it has at least two open reading frames.

[Slide.]

Viremia, as I have described, has been reported. It has been reported in individuals for as long as 21 weeks. The longest reported period followed, but presumably there are chronic infections because the virus is so prevalent in the population studied, and it is more common in middle-aged blood donors than in younger blood donors.

[Slide.]

TT virus has many similarities to parvoviruses, and it is very possible that in the presentations that follow, you will learn other information that may change this picture, but the published data so far shows some similarity between TT virus and parvoviruses.

First of all, it is a single-stranded DNA virus. That is one similarity. It at least in reports so far has appeared to be linear. When inverse PCR was applied, it could not be amplified. It is very rich in adenine and has many TATA sequences and polyadenylation signals.

These are sequences that are involved in RNA polymerase and transcription of the gene, and there are many similarities in the open reading frames.

[Slide.]

The one major difference that is apparent so far is that parvovirus is not affected by inactivation procedures, and as I described earlier, there is at least a little evidence that TT virus may be affected by some of the inactivation procedures.

Dr. Okamoto, et al., published the second report from the same lab that Dr. Nishizawa is from about TT virus, and they took some of the conserved regions of the virus and did PCR on a number of patients at a number of different populations, and they found the virus was present in 12 percent of asymptomatic blood donors in Japan, 12 percent of the general population.

It was also present in 68 percent of hemophiliacs, additional evidence suggesting that it could be transfusion transmitted, and they did studies on patients with liver disease that suggests that perhaps this virus could be associated with liver disease.

They found that in 46 percent of patients with chronic liver disease with no known viral cause and in 47 percent of fulminant hepatitis cases with no associated hepatitis virus. This is, as you can see, higher than in the general population in Japan.

[Slide.]

Similar studies with similar primers from the conserved regions were published by Naoumov from the UK. He

reported that the virus could be obtained from the serum of 10 percent of what he called "healthy control." These were not described clearly. We do not know what he means by "healthy controls," and we do not know whether these patients who were presumably in Roger Williams' clinic were natives of the UK or were individuals who came to Roger Williams from overseas.

He found the virus in 38 percent of those with non-B non-C liver disease, in 25 percent of those who had had liver transplantation for severe liver disease with no known viral etiology, and in 24 percent of those with post-transfusion hepatitis C virus infection.

[Slide.]

So, does TT virus cause liver disease? As you can see from the clinical data, there is a somewhat higher prevalence of the virus in those with liver disease than in the general population both in the UK and in Japan.

The virus titer has been shown to be higher, from 10 to 100 times higher in the liver than in the serum of the same patients, suggesting that it at least targets the liver.

In the Naoumov study, Dr. Naoumov focused on the number of individuals with TT virus infection who had normal ALT levels and normal liver histology in their biopsies, 58 percent with normal ALTs and 71 percent with normal liver

histology, and he concluded from that, that this probably did not cause liver disease.

I think at the present time and until we have more data, one should also focus on the other people who are not in this percentage. If there are 58 percent with normal liver function tests, then, there are 42 percent who have elevated ALTs and there are 29 percent with abnormal liver histology.

[Slide.]

So, what can we conclude from what has been published so far about this virus? There is no question the virus is present in blood, and there is strong, but not yet conclusive evidence that it is transmitted by transfusion.

It certainly appears to cause chronic infections, and it has some similarities to the parvoviruses, but there is a lot that we still do not know. We do not know whether this virus causes disease.

Thank you.

DR. HOLLINGER: Thank you, Ed.

We are now going to look at some of the clinical studies, particularly the virus in the blood in donors, and so on. I would ask the speakers as they go through their talk to remember what Dr. Tabor has shown, so we don't reduplicate much of what he has said in their presentations and really just get to the point.

The first speaker is Harvey Alter from the Clinical Center, who is going to talk on clinical studies.

Clinical Studies

Harvey Alter, M.D.

DR. ALTER: Thank you, Blaine.

[Slide.]

Well, I have been coming to BPAC meetings for a long time and the intensity and the nature of these meetings has changed dramatically over the years. I particularly enjoyed these meetings back in the late sixties, as shown here. It was a much more relaxed, less intense situation.

[Laughter.]

[Slide.]

DR. ALTER: Actually, I was in attendance at the very first BPAC meeting as shown here. At that time we were addressing the issue of whether we should ask donors if they had ever been cut by a starched ruffled collar that had been shared by another individual. We never did resolve that issue.

I didn't realize that Ed Tabor was going to give such an extensive background. In my narcissistic viewpoint, I thought he was just here to introduce me.

[Laughter.]

DR. ALTER: So, I will skip over my first 12 slides.

[Slide.]

Actually, a lot of the impetus to look for new agents came out of our post-transfusion studies where we showed that 12 percent of people followed prospectively had what appeared to be a non-A-B-C hepatitis, and then the CDC, in their community-acquired studies, showed that 18 percent were classified as non-A-B-C, and this was actually the starting point for looking for new viruses which wound up with the hepatitis G virus.

I cut out my slides on G, I won't go over that again, but just to reiterate that from our standpoint, hepatitis G did not appear to be -- this is still controversial -- but from our standpoint, hepatitis G did not appear to be the agent of non-A-B-C because we found basically the same frequency of G infection in those who did and those who did not get hepatitis.

We found a dissociation between levels of G and levels of ALT, and we found that the rate of G in the donors was inconsistent with the diminishing rate of hepatitis in recipients, so that we sort of then went into trying to do the same type of analysis with TTV.

[Slide.]

Now, this was just the one thing I can show you that Ed didn't have is a pretty slide of the actual first description of the three cases. They found three out of

five patients with post-transfusion hepatitis.

This is from the Japanese Mushahwar study where the ALT is shown here in the dashed line, and the RNA levels in the yellow line, and what you can see is that all of these cases were very mild hepatitis. Here is the peak ALT is under 200 in all the cases, and that was true of all the non-A-B-C cases that we have had. They have all been mild with enzymes less than 200.

But also this slide shows some association between the level of the virus and the appearance of ALT, particularly in this first case, but also some dissociation is shown in this case where the virus is still going up while the ALT level is coming down, and that is what we had found in the G situation fairly routinely.

I am going to skip over the slides that Ed has already covered and get into our own data.

[Slide.]

Now, this is from our own donor population and from our prospective study of transfusion recipients. So, using the primer set that we have used, and this work done in my lab my Dr. Aki Matsumoto, and I am going to acknowledge him later, but we found the prevalence in donors to be 7.5 percent. So, it was 12 percent as reported in Japan, 7.5 percent in our laboratory, and this may be highly dependent on the primer set that is used. The numbers could

be even higher, as Dr. Mushahwar will show you.

But interestingly, we found virtually the same rate in blood recipients prior to the time they were transfused, so basically, this is not a transfusion occurrence, this is just the prevalence in a patient population. These are open heart surgery patients. So, this is high in everybody in the population. This is the background rate.

[Slide.]

Now, when we actually looked at the recipients, and we looked for acute, these are new infections where we took away people who had pre-existing TT virus, and looked at those who had acquired new infections after transfusion or new infections after the surgery, and what we found is that the rate of infection in the transfused population was 24.5 percent, so 25 percent of people virtually who were getting blood transfusions were getting new TTV infections, but very interestingly, here is a non-transfused population, and 6.8 percent of the non-transfused population was getting a new TTV infection while in the hospital. This is unprecedented, I think, nosocomial spread of a virus.

So, this did show that the TTV was transfusion transmitted. This difference was significant. But also a very high rate of acquisition in a hospital setting for reasons which are not clear, but one of the things that Ed

mentioned is that this virus has now been found by the Japanese to be in bile and in stool, and this may be an enterically transmitted virus, as well as a parenterally transmitted virus.

[Slide.]

This just shows you more transfusion relationship just as the number of units transfused increases, so does the acquisition of TTV infection. So, this clearly is a transfusion-transmitted disease, but the key is what happens in the recipients, what is the relationship to hepatitis in the recipients.

The numbers in this slide are a little bit smaller than the number in the next slide, but basically, this is a prevalence estimate of TTV virus in prospectively followed transfusion recipients. Here is the group of 12 patients who had non-A-B-C or non-A-to-G hepatitis.

We would love to increase this number, but that is all we have is 12, and a quarter of those patients were TTV-positive when tested post-transfusion. This is about a six- to eight-week sample post-transfusion. But so, too, were a quarter of the patients who were prospectively followed and did not develop hepatitis, patients who had perfectly normal ALT levels sequentially after transfusion.

So, too, in the prospectively followed patients who were not transfused. These numbers are not

statistically different. So, it is just again a very prevalent virus in all patients.

[Slide.]

But shown here now are the new infections again. This is actual acquisition of TTV after transfusion, so again 25 percent in the group who were classified as non-A-to-G, 32 percent of the patients who were diagnosed as hepatitis C had coexistent new infection with TTV.

Among the patients who had no hepatitis now, this number was down to 14 percent as compared to the prevalence estimate that I showed you on the previous slide, but still these numbers were not statistically different or not even close to statistically different, and here again is the non-transfused population with 6 percent having new infections.

[Slide.]

This is the relationship of the TT virus to ALT level. Among our patients who had non-A-B-C hepatitis, no difference in the level of ALT in those who were TTV-positive and those who were TTV-negative.

Similarly, in the patients who had hepatitis C, no significant difference in the peak ALT level or the number who became jaundiced between those who were not infected with TTV and those who were coinfected with TTV. This is exactly the same thing we found with hepatitis G. There was no impact of G or TTV on coexistent hepatitis C. It doesn't

make another liver virus worse.

[Slide.]

I don't know how well this will show up. I didn't have time to make a better slide on this. But basically, this is again to show the relationship of TTV DNA versus ALT level. ALT is under this pinkish block, and these are the RNA levels, and I think it shows again the dissociation. Here is the RNA up high, ALT has barely peaked, RNA coming down when ALT is going up. RNA level is level while ALT is going up again.

The same thing here. Here is a pretty level RNA level with ALT climbing. So, we found no association between the DNA level and the ALT level.

[Slide.]

In conclusion, TTV is found in a high and equal frequency in donors and in patients. It is transfusion transmitted and the risk increases with increasing numbers of transfusions. There is frequent nosocomial spread and probable non-parenteral spread in the community accounting for the very high levels. It may be fecal orally spread as postulated by the Japanese.

[Slide.]

It can lead to persistent infection. I didn't show you that data, but we have patients who have this virus for up to a year after acquisition, but importantly, I think

this is the key to the study, that it is in the same frequency in patients who develop non-A-to-G hepatitis or non-A-B-C as those who do not develop hepatitis at all.

There is a generally poor correlation between the ALT level and the TTV level, and it doesn't influence the course of coexistent hepatitis C.

[Slide.]

So, to put this all together, what I think is that this is deja-G all over again.

[Laughter.]

DR. ALTER: I want to acknowledge Aki Matsumoto who not only did all the work, but who told me what it meant. So, Aki, thank you very much.

DR. HOLLINGER: Thank you, Harvey.

The next speaker is Isa Mushahwar from Abbott Laboratories.

Isa Mushahwar, Ph.D.

DR. MUSHAHWAR: I would like to thank Dr. Tabor for inviting me to share with you some data we generated recently. I haven't been to a BPAC meeting since 1984 when I defended the need for an anti-delta test. I was correct then and I hope I will be correct today.

The initial TT virus perhaps should be the wandering nomad virus, or WN virus, and I will try to explain what I mean by that. If you want to not listen to

what I have to say for the next 10 minutes, I will summarize my presentation in three statements.

Number one. TT virus or WN virus is not a single-stranded linear virus that belongs to the parvovirus family. In our hands, TT virus is a double-stranded, circular virus that belongs to the papova family of viruses, and those viruses, as you know, they are the papilloma virus, the vacuolating virus, and the papova virus. They are spread all over the place like a nomad, hence, the name the wandering nomad.

The second point I want to say that using current primers and probes that have been described in the literature significantly underestimate the prevalence of this virus especially among blood donors.

The third statement was already touched on by Dr. Alter, that most of those infections I think are not parenterally acquired, but they are non-parenterally acquired because we found a carrier chimpanzee with the virus, and preliminary evidence shows that the stool of this chimpanzee have the TT virus.

I would like to apologize for trying to prove my point. I have to use a little molecular biology and some repeat slides that Dr. Tabor showed.

[Slide.]

This is the summary of the Japanese Nishizawa's

paper that appeared in BBRC. The points I want to mention in here, that in this paper, they mentioned the buoyant density of the virus as 1.26 g/ml, and had one open reading frame encoding 166 amino acids.

The method of detection, of course, was the PCR method that Harvey described, and by RDA the isolated sequence with 500 base pairs.

[Slide.]

In the second paper that was submitted to general viral hepatitis on December 5 and accepted on December 5, they claim that the virus in nonenveloped single-stranded DNA virus have a genome of roughly around 4,000 bases, and now they claim two open reading frames - 770 and 202 amino acids, and has a high divergence for a DNA virus, 30 percent. Then, they have a new primer set that detects a second genotype of the virus.

[Slide.]

This is the data for the prevalence of the virus, and this was done by Dr. Tabor, but what I would like to emphasize in here, this is the selected population, mostly liver disease patients and hemophiliacs, and also they don't have a randomly selected population. In our hands, randomly selected population of Japanese in Okinawa and also in Nagasaki, the random population, the prevalence of TTV is something around 52 percent, and I will show you the data

later on.

Also, the blood donors here are highly underestimated. In a meeting in California at the Hepatitis Panel meeting between Japanese and the Americans, I have been told that this percent in certain areas in Japan go as high as 45 percent, and in certain areas in China, this percent goes as high as 82 percent.

[Slide.]

Now, we at Abbott in the Virus Discovery Group were interested in this virus, and we have sequenced to near completion an isolate from Ghana, Africa, and it has similar base pair sequence, and at least so far two open reading frames, but no significant similarities to known sequences, and we believe that the genome of this virus appears to be circular.

[Slide.]

Why do we believe so, we took the 2,900 base pairs known, 22 as described by the Japanese, and we have been working upstream and downstream of the genome, and we selected nested primers from the ends of this clone, and the question we wanted to answer is this virus linear or is it circular.

In order to do so, when we built those nested PCR, you look for a PCR product. If it is there, then, you know it is circular; if it is not there, then, it means it's

linear, and that is also the experiment plus other experiments I don't want to bother you with, is that we found the product, that it is 1,500 kilo base, therefore, we believe that the virus is circular, and when this fragment was cloned and sequenced, the sequence completely resembled the sequences of the TTV virus.

We have done other experiments like cesium chloride, the classification in the presence of papillomavirus, and so on, and so forth. We have other physical evidence to prove that this virus is double-stranded DNA or circular virus.

[Slide.]

Now, these are the physical characteristics of the virus and some chemical characteristics. As I said before, this is the buoyant density in cesium chloride, and it did pass through a 100 nanometer filter, but not through a 20 nanometer filter, so it is sized somewhere between 20 and 100, and I think most polyoma viruses are around 45 to 50 nanometers in size.

There has been reported by us and others a sequence variability not consistent with human genomic DNA. That means that the decoding region high rate of cell position changes, and our data -- and some of it I didn't show you -- suggests that the TT virus is a member of the Polyomavirinae family, and not the Parvoviridae family.

[Slide.]

What is this family of viruses? As most of you know, they are members of the Papovaviridae, a 45 nanometer non-enveloped viral particles. Look at the buoyant density. It is 1.34, like we found, and not 1.26, and it has 4.7 to 5.3 kilo base pair circular, double-strand DNA genome, capable of latent and persistent infection.

I don't want to bother you anymore, it is mostly tissue tropism, it is not in the liver. When you find more TT virus in the liver than in the serum, that does not mean this virus is hepatotropic. You have to do other studies to prove so.

Anyhow, it is distributed mostly in lung and kidney epithelium, and let me tell you about BK virus infection in kids in the United States between the age of 5 and 10, around 50 percent, and more than half of them acquire a BK virus persistent infection.

Children between the age of 6 and 14 acquire JC virus infection, 50 percent again, acquire JC virus infection and 15 to 20 percent have a persistent infection of the virus after that acquisition.

So, there is no doubt in our mind that this virus belongs to this family of viruses.

[Slide.]

We have infected those tamarins with the G virus,

but at that time we didn't know that the inoculum had also TT virus, but regardless, we have followed this for one year, and so far we have not seen any replication in either the liver or the blood, although I told you about the chimp that is persistently infected with TTV, the stool of that chimp has the TTV virus.

[Slide.]

Now, with this introduction, I come to my second point, is that we are underestimating the percentage of TTV virus in the blood donors, not only in the United States, but everywhere else.

These are the position of the three published primers for the TTV detection. The pink, the genomic, the sense, anti-sense primers as described by Nishizawa, the green ones are as described by Okamoto, and the white ones are described by Simmonds, and Dr. Simmonds will tell you later, but that is a misprint in this anti-genomic primer in the line position as published in Lancet. There is an extra base. It shouldn't be there. But regardless, that doesn't make too much difference.

[Slide.]

We confirmed by nested PCR and also by southern blotting and sometimes by sequencing, everything is positive that we find, so everything is positive that we find, we confirm, at least by southern blot.

[Slide.]

This is the data for three global populations we tested, from Ghana, West Africa, from Japan, and drug abusers in the United States. Let me describe this Ghana population. These are schoolchildren between the age 6 and 16 that have been surveyed for the presence of hepatitis B and C viruses.

Hepatitis B markers were around 60 percent active viremia, for B it was around 5 percent, hepatitis C virus, prevalence was around 5 percent. They have been cleared as drug abusers. They have not shared needles after vaccinations, there are poor sanitary conditions. We find around 38 percent of those children harbor this virus.

The Japan population I mentioned before, and here is the number, 52 percent, and the drug abusers around the Chicago area, around 21 percent.

[Slide.]

Now, what is the sensitivity of those primers that I described? I did not use the second paper Okamoto primer because in our hands it didn't work, so I used Nishizawa primers and I used Dr. Simmonds' primers, and as I see in this, you find that both primers, the Simmonds' primer and the Nishizawa primer, detected 7, the Nishizawa primers detected 1 that the Simmonds' primer didn't detect, and Simmonds' primer detected 24 more than the Nishizawa primer.

All in all, you have 8 detected by the Nishizawa primer and 32 detected by the Simmonds' primer. This means roughly there is 4-fold difference between those primers, meaning that we still need primers to really come to the true prevalence of TTV.

[Slide.]

How about the normal blood population? Here is a population with high ALT, 165 donors, that have been rejected donating blood in Eastern Maryland. Their ALT level is 2.5 times above the limit of normal, and you find, to my amazement, 9 percent, less than volunteer blood donors in Southeast Wisconsin that are repeat donors, 13 percent, and commercial plasma donors, 13 percent.

[Slide.]

This is a slide just to show you that perhaps there are other transmission of GBV-C or G virus, as mentioned by Dr. Alter, is completely different than other transmission of TTV because you look at this, only one out of those volunteer donors have both TTV and hepatitis G, and only five out of the commercial donors have both TTV and hepatitis G. This means to me evidence that the transmission of G is similar to HCV is mostly parenterally, however the transmission of TTV may be by other management, parenteral products.

[Slide.]

The sensitivity of those primers in this population, it looks like this, 11 positives were detected by Simmonds and Nishizawa, 9 by Nishizawa and not by Simmonds, and 28 by Simmonds, not by Nishizawa, and if you add the numbers, 20 by Nishizawa, 39 by Simmonds, and the difference is 2-fold.

[Slide.]

To summarize, all this data with the blood donors and the other populations, I show you here we have 70 detected by Simmonds' primers, 28 by Nishizawa, so you have 2.5-fold difference in prevalence. Again, I emphasize the need for better primers to detect the true value of this.

[Slide.]

In summary, then, the TTV characteristics are little or no homology with other viruses, genome appears to be circular, filtration and centrifugation data suggest a small, non-enveloped DNA virus. Biophysical and genome characteristics indicate TTV is most similar to polyomaviruses, examples like JCV, BKV, and SV40 in macaques.

[Slide.]

Current assays and epidemiology. Current PCR assays grossly underestimate the true prevalence due to extensive sequence heterogeneity. A standard PCR detection method is needed using highly conserved region primers.

Serologic assay is necessary to determine the true infection rate although I am satisfied with the PCR assay. Routes of transmission may include transfusion, possibly fecal-oral.

A paper is coming in October issue of General Medical Virology from Dr. Mayumi's lab that show that 3 out of 5 patients that are fulminant hepatitis patients that are infected with either hepatitis B or C virus, and also coinfected with TTV, 3 out of those patients they found excretion of the virus in high titers in the stool.

So, this data plus the chimp data plus the West African data apparently agree with the polyomavirus spread by non-parenteral route.

Of course, disease association requires further study. I still believe that this virus is a wandering nomad and I am waiting to be proven wrong.

Thank you very much.

DR. HOLLINGER: Thank you, Isa.

Our next speaker is Dr. Yuwen.

Hao Yuwen, M.D.

DR. YUWEN: I am going to talk about TT virus from a narrow perspective from our laboratory, Division of Transfusion and Transmitted Diseases, CBER, FDA.

[Slide.]

The work was supervised by Dr. Tabor and the work

was done by my coworker Dr. Sieya Momosaki. The topic of this presentation is a population survey of a small population in Japan of the prevalence of TT virus.

[Slide.]

First of all, I would like to mention a few background, and Dr. Alter and Mushahwar also mentioned a lot about this virus, and Dr. Tabor gave a good background introduction of the virus.

This virus is relatively new, it is less than one year since its publication end of last year, and we have a lot of questions and answers, and you just heard Dr. Mushahwar's presentation, and he kept raising questions about whether it is a circular one or double-stranded, and the way of transmission.

You may hear a lot of interesting topics today after this presentation.

[Slide.]

We selected sera from a group of population in Japan. The reason to do this is when work was started almost a few months ago, there was no additional information available outside of Japan except those two papers published in Japanese groups. We are concerned with the fact that regional factor may be contributing to the transfusion of this virus, and so we selected people from Japan instead of from other places.

Also, we used random selected 47 samples out of 2,000 from Miyazaki cohort study, which was conducted years ago, and among those 47 samples, 24 of them are anti-HCV positive and 23 are negative, and their age between 27 up to 80 and the female and the male almost equal numbers.

[Slide.]

We still use the primer described by Dr. Okamoto in the second paper since the TT virus was introduced. People who work with the TT virus will understand those three. It is kind of semi-nested. That means there were two upstream primers and one downstream primer. So, we run the PCR by two round of amplification. In the first round we use one upstream and one downstream. In the second round, you use another upstream and the same downstream primer.

[Slide.]

This work is continuous, still working on it. It is just very preliminary. We got two positives out of the 47 samples by PCR, and we come from those two by DNA sequencing, and it showed that those two samples differ each other by as much as 13 percent DNA sequence-wise.

The first sample is 99 percent homology to the published [TRs], subtype 1B as proposed by Dr. Okamoto, et al., and second positive one showed 88 percent homology. Those two individuals are both male, and they are HCV-

negative, and they are all anti-HTLV-I positive.

[Slide.]

This short work also has a lot of input from the following individuals. Thank you.

F. Blaine Hollinger, M.D.

DR. HOLLINGER: I am going to take the chairman's prerogative of also interjecting some donor studies also with the Okamoto primer and some small study that we had done at the Gulf Coast Regional Blood Center in which we were also looking at donors.

[Slide.]

There were also seronegative donors who were also tested for the TT virus, and the only thing in these donors were their ALT levels that we were looking at. This is a subset of about 6,000 donors that we have looked for previously for HG virus, and so on.

In that, the percent reactivity was fairly similar, was essentially similar regardless of the ALT level. Those 112 patients who had an ALT level equal to or less than 49, 4 of them were positive or 3.6 percent, pretty evenly divided between the males and the females, and those over 90, it was about 5.2 percent, again probably not a statistically significant difference.

It did appear perhaps that females perhaps were a little bit more likely to be positive than males, but again

these are very small numbers, so it is really difficult to say.

Again, we didn't see -- we just happened to look at the blood type -- didn't see a difference basically, a difference in the distribution of the virus according to blood type, again small numbers and regardless of what the particular blood type was.

So, in a group of individuals, 308 individuals or donors that were seronegative, the overall TT virus detected in those donors was 3.6 percent in that group.

Thank you.

We talked about clinical aspects or donors particularly, and we are going to look now at the TT virus in plasma derivatives, and the first one to talk on that is going to be Dr. Yu from the Division of Hematology.

Detection in Plasma Derivatives

DR. YU: The next slide, please.

[Slide.]

The goals of our study is to determine the prevalence of TTV DNA in plasma derivatives currently licensed in the U.S. These products include Factor VIII concentrates - intravenous immune globulins, intramuscular immune globulins, and albumin products.

Now, in Factor VIII concentrates, we include human plasma-derived AHF, porcine AHF, and recombinant AHF

products.

Another goal of this study is to evaluate the possible effect of various viral inactivation removal procedures and the methods of purification on the prevalence of TTV.

[Slide.]

For DNA extraction, for AHF, we reconstituted with half of the specified volume. 0.4 to 2 mL were assayed. For IGIV containing 5 to 10 percent IgG, we use 0.2 to 2 mL. For IGIM containing 18 percent of IgG, we use 0.1 to 2 mL. For albumin products containing 5 to 25 percent of proteins, we use 0.1 to 0.4 mL to assay for DNA extraction.

Now, PCR procedures are similar to that described by Okamoto in Hepatology Research, but with some modifications in amplification and detection.

[Slide.]

We found TTV DNA is prevalent in AHF, I mean was prevalent in AHF (human), but not in recombinant AHF or porcine AHF. We assayed 32 lots of recombinant AHF and 4 lots of porcine AHF. No TTV DNA was detected.

We found all 16 lots of AHF (human) made in 1977 to 1983 positive for TTV DNA. These lots were not subjected to any viral inactivation procedures, and they were lower purity.

Among 6 lots of AHF made in 1987 and 1988 period,

we found 4 lots positive. They were subjected to some viral inactivation removal procedure, but they are inefficient, such as dry heating.

Among 160 lots of AHF (human) made in 1993 through 1998, we found 31 lots positive, so with a prevalence rate of 19 percent. However, the prevalence rate are varied among products made by different manufacturers.

[Slide.]

There are the five manufacturers, current manufacturers of AHF (human). All I want to say is that TTV DNA is least likely to be found in immunoaffinity purified AHF products. In addition, TTV DNA is less likely to be found in AHF product that are virally inactivated by wet heating.

[Slide.]

TTV DNA was not found in IGIV or in albumin products, however, we did find 1 of 45 lots of IGIM was positive for TTV DNA.

[Slide.]

In conclusion, it remains unknown whether positive TTV DNA equates with infectivity in recipients of AHF.

TTV DNA was in most AHF (human) lots made in the 1970s and 1980s from plasma not screened for antibodies to HIV or HCV, and either not subjected to, or not treated effectively, with viral reduction procedures.

TTV is least likely to be found in products purified by immunoaffinity chromatography, and is less likely to be found in products virally inactivated by wet heat than by other methods.

[Slide.]

TTV DNA was not detected in any recombinant AHF, porcine AHF, IGIV, or albumin.

Only 1 of 45 IGIM lots was positive for TTV. This lot was made in 1991 and not subjected to viral inactivation procedures.

[Slide.]

I would like to acknowledge others who contributed to this work: Dr. De Tan, Dr Akihiro Matsumoto, Dr. Anthony Yeo, Dr. James Shih, and Dr. Harvey Alter.

Thank you.

DR. HOLLINGER: Thank you, Dr. Yu.

The next speaker, Peter Simmonds from the University of Edinburgh in Scotland.

Peter Simmonds, M.D.

DR. SIMMONDS: Good morning. Thank you very much for the invitation to the BPAC meeting. I have certainly learned a lot about TTV this morning, and I think it is great to compare notes at this very early stage and see which findings are consistent between groups. I think the blood product data is very interesting taken in the light of

the preliminary studies we have done before.

[Slide.]

I also had a background slide which I will probably skip because I think we have been through this already. It is really just to show the genome of the fragment that has been published so far from Okamoto with the two open reading frames, and, in fact, the idea that it was possibly a single-stranded DNA virus on the basis that it could be digested among nuclease, where, in fact, the evidence now might be that, in fact, it is a quite different type of virus, and that is an intriguing observation, and certainly I agree makes more sense in terms of what we now know about the prevalence and epidemiology of virus.

[Slide.]

I am going to concentrate a bit on our own investigations of TTV and, in particular, look at the impact of the virus on blood transfusions in the UK. Foremost among this was really an investigation of the prevalence of viremia in blood donors and the extent to which that would be then transmitted to recipients of blood, but also to investigate whether some of the exposed risk groups, such as hemophiliacs, showed a greater likelihood of infection that may be attributable to blood products derived from these blood donations.

[Slide.]

This is a basic summary of the findings so far. We screened a total of 1,000 blood donations collected from October to December 1996. These are screened in pools of 10 and then split to identify the individual component donations. Our initial prevalence was 19 out of 1,000, which is 1.9 percent.

I just want to highlight two or three qualifications about this raw prevalence data. As Dr. Mushahwar had mentioned, primers are not optimally designed at this stage. This figure here was actually a composite of our own primers and primers published by Okamoto. In fact, we find samples that were positive with one set, and not with the other, so this is really a composite figure, and an implication might be that the true prevalence may be higher.

The second qualification is that levels of viremia are quite low in TTV, and samples going all the way down to the cutoff of sensitivity of the PCR exist in this and other populations, and I think that we might find a higher prevalence if we were able to screen larger volumes of plasma from the donors. We were testing 100 microliters, but we suspect that if we were testing larger volumes, we might find a higher prevalence of infection.

Thirdly, this is only the frequency of active infection, and as already shown, individuals infected at least as adults with TTV can resolve, so, in fact, this does

not reflect the frequency of donors who have been infected and resolved, and, in fact, the true frequency of exposure to TTV may actually be higher than this.

Consistently, what has been said, we don't find an association with hep G, so, for example, out of the thousands that were screened, 23 were actually positive for hepatitis G, but there were no cases of coinfection.

We noticed an interesting age stratification. So, for example, among the TTV-positive donors, the mean age was 53, and this is quite unrepresentative of the blood donor population generally, where I think our mean age is around 35. So, in fact, we do see an overrepresentation of infection amongst older donors, and, in fact, many of the donors in this study were actually 60 to 65.

[Slide.]

Again, underlying the problem of the primers, if you sequence TTV amongst donors, you can actually show at least with the initial passthrough types 1A and 1B. If you use different primers, you can find examples of other genotypes. This is type 2, which is around 30 percent divergent from the original clone.

There is evidence for a third genotype here, which isn't shown on this tree, but, in fact, we now know there is a variance of TTV that may differ by up to 50 to 60 percent in a immunoacid sequence from the sequence, and these

variants are almost impossible to detect with any of the published primers, so we are clearly underestimating the true prevalence of this infection.

[Slide.]

Despite the limitations of these type of PCR based surveys, we can, nevertheless, show a marked geographical difference in prevalence throughout the world, so if we just review the data here, in Scotland, around 2 percent, probably an underestimate, Japan 12 percent, Pakistan, these are blood donors, 16 percent, but if you actually start looking at African countries, for example, Nigeria, Gambia, and Zaire, we can see prevalences ranging from 44 to 83 percent of that whole population. Similarly, in Papua, New Guinea, we find 74 percent, and Ecuador, a very high frequency of 60 percent.

These samples from wild-caught chimpanzees, we find at least one of these to be infected with TTV, although we really want to get the sequence of that to see how divergent it is from those found in humans.

So, clearly, there are marked geographical variations and accepting limitations of the PCR data, perhaps we may see a universal exposure to TTV in certain populations.

[Slide.]

We were quite interested to look at the sort of

risk factors associated with TTV infection, and we have cohorts of different patient groups where, in fact, we can look at the prevalence of different markers that may be associated with sexual contact or contact in particular drug use, and this, in fact, summarizes the results from testing for three markers - hepatitis C, hepatitis G, and TTV. This is blood donor prevalence data, it is quite low for hepatitis C, hep G, 2.3 percent, TTV around 2 percent.

This is a control group of patients from the STD clinic who have no real disclosed risk factors, they show a low prevalence of hepatitis C and hep G, and, in fact, this 8 percent prevalence, 3 out of 36, seems to be roughly the sort of population norm in this and other studies.

Maybe I should look at those who have a history of sexual exposure, female prostitutes, gay men who are HIV-positive or negative. We find higher frequencies of hep G in these, but we do not see an increase in prevalence of TTV in these groups.

If we look at drug users, we can again show a higher frequency of hepatitis C, say, for example, among HIV-positive drug users, hepatitis C is present in 93 percent. Hepatitis G is also increased in prevalence compared with controls at 46 and 33 percent. When we look at TTV, we actually find only 4, or 7 percent.

So, I am not saying it is parenterally

transmitted, but at least in this risk group we do not see an excess of prevalence over controls, and, of course, there are many possible explanations for this, one of which may be that the amount of blood exchanged during drug abuse may be too small to be infectious, and given the very low titers of TTV in blood, it may also be that, in fact, individuals exposed as adults may be able to resolve infection more easily than those who are exposed later on, but anyway, these do not seem to be classically associated with TTV in the same way that they are with the other virus markers.

[Slide.]

This data was published in the Lancet, and the numbers are much smaller, but they are very consistent with what Mei-ying Yu just presented. If you look at blood products that are not virally inactivated, you can show high frequencies of contamination in Factor VIII and Factor IX, but not in immunoglobulin.

If you look at virally inactivated concentrates, you can still detect TTV, but the frequency is lower, and, in particular, it is lower in some of the concentrates prepared where the heat inactivation has been through heat rather than by solvent detergent.

These are Scottish products which have been inactivated by solvent detergent, and this seems to make no difference to the frequency of detection in marked contrast

to the commercial products which largely were heat treated.

[Slide.]

This is the data that Dr. Tabor presented earlier on. It was nice he showed percentages rather than numbers because these numbers are rather small, but basically, what you can show is an increased prevalence of TTV in hemophiliacs who have been treated with non-inactivated concentrates, and there does seem to be a stratification based on Factor VIII usage.

So, for example, if you look at hemophiliac A patients who have been treated before 1986, we find an increasing prevalence with disease severity going up to 41 percent. Hemophilia B is similar, where moderate to severe cases have 57 percent prevalence.

If we look at hemophilia A patients who have been only treated with non-inactivated concentrates, we find only 1 out of 19 to be viremic, and that is significantly different from the prevalence in the other hemophiliacs, but we really need to have greater numbers to establish this more clearly, because we know, for example, this group has been infected with B19 from the same concentrates as have been exposed to with TTV, so there may be a differential in inactivation between the two viruses.

[Slide.]

Finally, I just want to show some very preliminary

data about dissociation or lack of association with fulminant hepatitis. We have a well-characterized cohort of 21 cases of which 6 had hepatitis G, but, in fact, we decided that this was not likely to be the etiological agent of those cases.

We find TTV in a total of 4 of 21, of which 1 had actually been mostly transfused, and we think that, in fact, TTV is not associated with that. You will notice in the other 3 patients that we do not have a pre-disease sample, so all we can record is the fact that they were positive when they presented with fulminant hepatitis, and I think given that there are only 3 out of 24, this clearly makes TTV not a major etiological agent of fulminant hepatitis, and I think 3 out of 24 isn't too different from the general population prevalence of the virus, so I think probably we would not say this is evidence for a role in fulminant hepatitis.

[Slide.]

The same conclusion. The virus is widely distributed. It has got a DNA genome at least. I don't know whether it is single or double stranded. It is interesting, it may correspond to the chloroform resistant agent that was found in early chimpanzee transmission studies although then again it may not, and obviously, Dan Bradley is very keen to get that material tested to see

whether, in fact, it was TTV in those samples.

[Slide.]

Frequency of infection in blood donors indicates that there is going to be, first of all, exposure to users of blood products, but also ongoing transmission through blood transfusion, and that is clearly an issue, and obviously, the more data we can accumulate that it is non-pathogenic, the more justified we are in not attempting any form of screening for the virus. What we urgently need is some lookback and clinical follow-up of the recipients of positive blood.

I should like to acknowledge the people working in the group. This is the blood transfusion people, the group in the university, Linda Prescott in particular, and also collaborators with the clinical departments.

Thank you very much.

DR. HOLLINGER: Thank you, Peter.

The last group is going to be on the blood transmission studies. The first speaker will be Kevin Brown from the Hematology Branch.

Blood Transmission Studies

Kevin Brown, M.D.

DR. BROWN: Thank you very much, but in contrast to what you have just heard, I am not going to talk on the blood transmission studies, but I am going to show you some

of our other data, which is why we haven't done the blood transmission studies.

[Slide.]

I have no background slides because I am sure you know the background, but I am going to show you our data using PCR to detect this virus. Before I start, I want to just say how we are detecting virus because I think it is probably significant.

We are using a modification of the method that was described in the Okamoto paper, and we are using their primers, but when we originally tried the primers and using the serum that he had provided, we were unable to get very convincing results, so we spent quite a lot of time optimizing this, using different methods of DNA extraction, altering the cycle, the enzymes, the conditions, and we also designed an internal oligonucleotide probe to increase sensitivity.

[Slide.]

This is just an example, so that you know what we are looking at. This is what it looks like by ethidium. This is our positive control that was provided for us, and you can see quite clearly these 2 positives in this group of 18 sera, and you can see how nicely they light up with our oligo probe.

[Slide.]

So, we have gone on and used our assay to look at a variety of different serum groups, and the first lot of data is in collaboration with our colleagues in Viet Nam. These are from Ho Chi Minh City. We looked at blood donors from Viet Nam. These are all volunteers, they are not paid to donate blood, and they have all been screened. We also looked at 18-year-old Army recruits and we also looked at children.

This is the summary of the results. You can see that in these blood donors, we were actually finding about 12 percent were positive, which is very similar to what has been found in Japan and in some of the other countries that have been looked at.

However, when we looked at 18-year-olds, we have found a much higher percentage that were positive, and when we looked at children, these were from a pediatric hospital in Ho Chi Minh City, we also could find much higher rates than in blood donors, suggesting that maybe this is not necessarily a virus that is acquired by blood transfusions, but is acquired by non-parenteral methods, which has been talked about before.

This was strengthened when we broke down the different ages of these children, and you can see even though the numbers are small, quite clearly, the prevalence of viremia is increasing with age, so that we had 11 out of

15, 11 to 15-year-olds that were positive.

[Slide.]

We have also looked at similar groups in the U.S. We looked at blood donors. This is in collaboration with the NHLBI REDS study. We used donors from five different centers, 50 from each. We also looked at lab staff. That is the individuals in the Hematology Branch at NIH, and I should point out that we do not represent the American population as a whole. We have a rather increased number of Asian, Chinese, and Japanese workers. We also looked at children who were less than 15 years old.

This was a special study that we are doing, in fact, looking at HTV. These are children attending Children's Hospital in Washington. What is special about them is that they have been preselected or prescreened to make sure that they have not received any blood products or blood transfusions, so that we know that any that we find are positive have not acquired these at least by those routes.

[Slide.]

This is the results of looking at the different sites of blood donors. There is no significant difference between these, but you can see we are picking up about 9 percent of American blood donors have viremia.

[Slide.]

This shows the other groups that we looked at. The lab staff is slightly higher, and most of these 7 positives were either Chinese or Japanese, well, 3 of them were Caucasian.

The children less than 15 -- and we have not been able to break down the ages yet to look at these -- but 20 percent were positive, so that is twice as high as what we are finding in the blood donors.

[Slide.]

My lab has an interest in hepatitis, specifically aplastic anemia preceded by seronegative hepatitis, and so we did some preliminary studies to look in these individuals. You can see in serum, 2 out of 17, 12 percent, again not significantly different than our blood donors were positive.

Looking at liver samples, we have only looked at 8 from each group. These are control livers. These are seronegative fulminant hepatitis livers, and we are finding exactly the same rates in the two different groups, again suggesting that seronegative hepatitis is not associated with this virus.

[Slide.]

I also give you this data. This was an accidental finding. We were using tamarin inoculations as part of our own studies to find the etiological agent of hepatitis-

associated aplastic anemia, and we had three tamarins, each inoculated with pools of 10 acute hepatitis sera from Viet Nam. They were followed for 12 weeks.

All the animals including the controls remained well, but this monkey 3757, although had no hepatitis, no increase in transaminases at all, no drop in hemoglobin, when we did a retrospective analysis, although initially negative up until week 5, week 7 and 9 clearly were positive for this virus. By week 12, he was negative again. So this, in contrast to what you have just seen, does suggest that in our tamarins at least, we could inoculate them and get evidence of TT viremia.

[Slide.]

This is a representative blot, ethidium and blot of the donors that I have been describing, and I want you to sort of note particularly this one here. You can see clearly that there is a group here of positives. This one clearly has a band of the right size, but when we use our probe, it hardly probes at all, and you can see this one is also similar. In contrast, this is much weak, but we get quite a strong band.

[Slide.]

We have been sequencing quite a lot of the products, and I don't expect you to be able to read any of this, but certainly when we look at the sequence, we can

find again as has already been described, up to 50 percent difference in the sequence of our PCR products compared to the published sequence, and I have, in red, that you may just be able to see, this is the G1A and G1B, and you can see we have clusters.

These are our own sequences. The bulk of these here are the blood donors, but we also have blood donors in this group that matches with 2B, this one which matches with 2A, and we have again, as has been alluded to, what looks like a third genotype which is complete different from these others, and yet with our modified PCR, we can pick them up.

I also want to add, and you might just be able to see that there is three of these that are in blue. This is from the fulminant hepatitis, and these are from two of the hepatitis aplastic anemia. All three of them cluster in these ones that really, the original PCR primers are not designed to pick up, and these give the relatively weak band by PCR. So, I am not sure that we can be quite as confident, as has already been said, that this virus is not associated with hepatitis.

It may be that most of the positives that we are finding so far are the ones that may be nonpathogenic and more associated with TTV G1A and G1B, and I don't think that we can say anything at all until we know more about the conserved areas of this virus and have better PCR before we

can really answer whether this is associated with hepatitis or not. Having said that, I don't think it is.

[Slide.]

So, these are my conclusions. The virus is very common in both Vietnamese and U.S. blood donors, but it is even higher rates in children and young people. So far we have not found any increased prevalence in hepatitis samples, but maybe our PCR primers are not correct, but there is extremely high sequence variability which almost certainly are underestimating the true prevalence of this virus.

Thank you.

DR. HOLLINGER: Thank you very much.

The final speaker then on this topic is Ian Williams from the Hepatitis Branch at the CDC.

Ian Williams, Ph.D.

DR. WILLIAMS: Thank you very much. It is my great pleasure to be here today, but unfortunately, I am the only thing separating you from lunch, so I will try to be direct and to the point.

Again, I am listed as talking about blood transfusion studies, but I am not going to be talking about that. I will be talking about TTV and its role in community-acquired viral hepatitis in the United States.

[Slide.]

Specifically, I am going to be talking about TTV in the sentinel county study of viral hepatitis, and I would like to begin by thanking a number of people, especially Dr. Howie Fields, the chief of the Electroimmune Diagnostics Lab at the Hepatitis Branch who did a tremendous lot of work, as well as Yuri Khudakov, to make sure I had some data to date to show to you guys, specifically, some of the PCR groups being completed on Monday, so my talk should be appropriately titled a research in progress, because the analysis is very quick and dirty, and when we get to the end, the data is so preliminary I hate to draw any conclusions, but I will anyway.

[Slide.]

So, as we heard earlier, the method of PCR may be quite vital to detecting this virus. Here is the method that we used, basically, a 271 base pair were selected from the open reading frame one of the TTV genome. Semi-nested PCR was done to amplify the fragment. The sample was prepared with a phenol chloroform extraction in 50 microliters of serum sample. There was an ethanol precipitation. It was dissolved in 20 microliters of water and then 2 microliters were used for PCR.

PCR conditions in the first and second round are stated over here. So, these are the methods that we use to detect TTV.

[Slide.]

For those of you who may not be familiar with the sentinel county study, this is used for quite a number of purposes within the Hepatitis Branch, and it basically provides a backdrop for almost all we know about community-acquired hepatitis in the United States today. It has been quite vital for a number of functions specifically for the detection of emerging infections. Most notably, it was key in hepatitis C, it has been used to look at hepatitis G, and today I am going to try to use it to talk about TTV.

[Slide.]

So, who are the patients in sentinel counties? Well, specifically, these are patients with acute viral hepatitis, and they reported to six counties throughout the United States, although the analysis today is only going to focus on four.

These are identified through stimulated passive surveillance, and these are really acute clinical cases, that is, they have to have signs and symptoms of viral hepatitis, they have to have an ALT or AST more than two and a half times the upper limit of normal, and they have to exclude other causes of liver injury.

They undergo extensive serologic testing, both locally by the health department and again by our lab. For all the samples that are non-A, non-B hepatitis, PCR for

hepatitis C is also done. In conjunction with all the serologic testing, there is an extensive epidemiologic interview that takes about an hour to complete, to search for risk factors for infection.

[Slide.]

The counties I am going to focus on today are the following four counties: Pinellas County, which is St. Petersburg, Jefferson County, which is Birmingham, Denver, and Pierce County, which is Tacoma, about 30 miles south of Seattle.

[Slide.]

In the sentinel counties between 1982 and 1995, they have identified about 11,000 patients with acute viral hepatitis. About half of these were hepatitis A, about a third were hepatitis B, about 15 percent were hepatitis C, and about 3 percent were non-A-through-E. The first part of the presentation is going to focus on this part of the pie.

[Slide.]

The samples we looked at for the non-A/non-B patients were actually patients selected in two study periods from 1985 to 1986, and 1991 to 1995. These samples, the reasons we used these, they have been previously tested for hepatitis G. The paper was published in the New England Journal by Miriam Alter, et al., and the citation didn't make it, but it was New England Journal in March of 1997.

So, basically, these are samples that have been looked at before for hepatitis G.

[Slide.]

So, let's get to the bottom line. What did we find? Well, basically, we looked at 87 patients with acute non-A/non-B hepatitis, and here is what we found. We found 1 patient who had TTV alone, which represented about 1 percent of the samples tested, 1 person had both TTV and HGV infection, 7 people had TTV and hepatitis C infection as compared to about half the sample which is HCV alone, and about 16 percent of the sample which had HCV and HGV infection as compared to about 17 people that were non-A through G and TTV.

Based on these findings, if you sum up all those that were non-A through G prior to starting the study, we identified possibly 1 additional patient with non-A through G hepatitis. If this acute viral hepatitis was caused by TTV, that would mean roughly 5 percent acute viral hepatitis in community-acquired settings caused by TTV if you want to believe that TTV actually caused acute viral hepatitis.

[Slide.]

So, let's look at some of the demographic characteristics of these patients with non-A/non-B hepatitis that we looked at.

Basically, the 1 patient with TTV alone was 40

years of age, the 1 with TTV and hepatitis G was under 40 years of age, and if you look at the bulk of the group, which is the TTV/HCV infected group, and compared their distribution with that of hepatitis C alone and with hepatitis C and HGV, and compare it against those who don't have hepatitis A through G or TTV, you will find that the age characteristics are very similar between the TTV and HCV groups, as well as those with HCV alone and HCV and hepatitis G.

[Slide.]

The same thing is seen for the distribution of gender. Basically, the two TTV, the TTV alone, TTV/hepatitis G person, the people were both males. If you look at the distribution of TTV and hepatitis C, about 60 percent of them were males. Interestingly, and we don't have a good explanation for this, there is this interesting gender reversal where about 60 percent of our hepatitis C patients were female.

We are not quite sure why this true, but if you look at the hepatitis C and hepatitis G group, they are very similar to the TTV and hepatitis C group, about 60 percent are male, and if you look at the non-A through G and TTV group, they are about 50-50.

[Slide.]

If we look at the racial breakdown, basically, you

see a similar thing. The distribution of TTV versus white and non-white is very similar for those that have TTV and HCV compared with those that have HCV alone, and actually, if you do tests for statistical significance you don't see any differences between these groups.

These groups look somewhat different than you see with the people who have non-A through G and not TTV. There is about a 50-50 breakdown here where it is closer to 80 or 90 percent of the people with hepatitis C or hepatitis C and TTV have.

[Slide.]

Let's look at some of the clinical characteristics of these patients. The TTV alone patient had moderately elevated ALTs. The groups here, for those who can't read it in the back, we looked at ALT times upper limit of normal, and we grouped them into three groups - those who were between 2.5 and 5.9 times upper limit of normal, those who were between 6 and 15.9 times upper limit of normal, and those who were 16 times the upper limit of normal.

The TTV alone had moderately elevated ALTs. Our TTV and HGV person had significantly elevated ALTs. If you look at the distribution of those who were coinfected with HCV and TTV, all of them had more than 16 times upper limit of normal, and this is pretty similar to what we see among the HCV-alone group, as well as those that are HCV and HGV

coinfected.

This is somewhat different than what you see with the people who aren't infected with any of those. We see more equal distribution among ALT.

[Slide.]

One last look. Let's look at bilirubin. We broke it again into three groups - bilis less than 1.5, 1.5 to 2.9, and greater than 3. Our TTV-alone was actually not jaundiced, however, our TTV and HGV case had bilirubins of greater than 3.

If you look at those that are coinfected with TTV and HCV, the bulk of them had bilirubins greater than 3, and this is again what we see with HCV alone, as well as with HCV and hepatitis G. But this is somewhat different than we see with those who don't have any of the above.

[Slide.]

Let's get to the bottom line and look at risk factors. Let's look at risk factors within the previous six months prior to onset of illness in these cases.

Basically, we looked at these in mutually exclusive groups going from left to right, and what does that mean. Basically, we took everybody with blood transfusions, if they had that risk factor, they didn't appear in a subsequent group, and we worked our way down here.

So, these are drug users in exclusion of people who had blood transfusion, and these are health care workers who aren't injecting drug users and haven't had a blood transfusion.

With that said, our TTV-alone had none of these risk factors, and actually when you look at this case, he had no risk factors at all as far as we could discern. He is a 55-year-old male who was very mildly ill. His blood was drawn four days post-onset of symptoms, which were very mild. He was found to be TTV-positive at that point and HCV-negative based on HCV RNA. When he was tested six months later, he was still HCV RNA negative, and TTV negative at that point. So, it looked like a very mild infection, if any.

When you look at his distribution of all the other risk factors, he is not a gay man, he had one sex partner who was his wife. The only thing interesting we could find in terms of looking at his detailed interview, his wife was a home health aide who said she had contact with human blood. Her specimen hasn't been tested yet. So, this raises the possibility of maybe sexual or household transmission to him, if you believe that TTV was the source of his infection.

When you look at the other risk factors for TTV, of hepatitis G and TTV with hepatitis C, you basically see

what you would expect for hepatitis C, that is, the TTV and hepatitis G person was a dialysis patient, had dialysis in the previous six months.

Those with TTV and hepatitis C, one had a blood transfusion. More than half of them were injecting blood users, and two of them were previous injecting drug users, which we believe is a proxy for people who don't admit to current injection drug use.

Again, if you look at the distribution of these risk factors and compare them against those who have hepatitis C alone and those who have hepatitis C and HGV, you basically see very similar distribution of risk factors. Again, when you look at the distribution of these risk factors compared to those who don't have any of the above, they look quite different.

[Slide.]

Very quickly, let's turn and look at those patients who have acute hepatitis A and acute hepatitis B in this study. Basically, the idea here is to look at two different comparison groups. Specifically, we picked consecutive samples from the four sentinel counties from the period of 1991 to 1995.

We purposely oversampled hepatitis A and hepatitis B patients to basically match injection drug use, since injection drug use is not a typical risk factor for

hepatitis A, and we had some suspicions that TTV might be parenterally transmitted, we wanted to make sure we had enough injecting blood users to look at that potential risk factor.

[Slide.]

So, what did we find? Basically, of the 149 patients with acute hepatitis A, we found 8 of them, or about 5 percent, were viremic for TTV. Among the 137 people with acute hepatitis B, we found a seroprevalence of about 7 percent. Keep in mind that we oversampled purposely for injecting drug users, so these seroprevalences may actually overestimate what we would find in the general community among community-acquired acute hepatitis A and acute hepatitis B.

[Slide.]

I am not going to present to you the clinical and demographic characteristics, but they looked very similar. The bottom line is let's look at people with hepatitis A, the 149 people with hepatitis A, and look at those who were viremic for TTV compared to those weren't viremic for TTV.

I am going to present you the same risk factors we saw for non-A/non-B hepatitis. What did we find? Interestingly, half of these people that were viremic for TTV were actually current injecting drug users, and that is pretty much what you find among those who were TTV-negative.

When you look down here, about half these people didn't have any of these risk factors. Keep in mind that these aren't traditional risk factors for acute hepatitis A, so I took the liberty of just sort of looking at some of the other data to try to see where risk factors may have clustered.

Basically, what we found is the bulk of the rest of these people, all four of the other TTV cases reported they had contact with a known case of hepatitis A in the two to six weeks prior to their onset of illness. This is much higher than you would find with those who are TTV-negative.

One of these patients had a household member who had hepatitis A. One of them had a household member who was an injecting drug user. One of them had a history of an STD. What this exactly means, I am not sure. It suggests that there may be a non-parenteral means of transmission, however keep in mind that these are people with acute hepatitis A, and we have no idea whether TTV occurred as a coinfection or they had existing TTV viremia and hepatitis A occurred as a superinfection.

[Slide.]

Let's quickly turn to hepatitis B people and do the same thing. Basically, here are the 10 people among the 137 that we found with acute hepatitis B that had TTV. Basically, we found that a similar thing that we saw in

hepatitis A. About 30 percent of them were current injecting drug users. However, about 70 percent of them had no other risk factors.

The hepatitis B group is a little more ill defined. It was sort of hard to find any sort of any common risk factor that caused them all to cluster together. One of them had contact with a hepatitis B case, which is about what we found for the TTV-negative group. One of them had a household member who had hepatitis B, and one of them had a history of STD.

My conclusions are -- I purposely left it blank because I think we have a lot more work until we can draw really firm conclusions based on this data, but I think we can probably draw three probable conclusions.

One is it doesn't look like TTV is associated with disease in community-acquired viral hepatitis in the United States.

Two, it seems likely that TTV is transmitted in about the same ways that hepatitis C is, and finally, it doesn't look like TTV is an important component of community-acquired non-A through G hepatitis in the United States based on our study.

Thank you very much.

DR. HOLLINGER: Thank you.

We now know all there is to know and maybe more

than we wanted to know about TT virus.

We have some time and so I want to open it up to the committee. I will ask some of the presenters if they will be around to respond to any question that might be raised.

Is there anyone from the committee with questions? Yes, John.

Open Committee Discussion

DR. BOYLE: I would like to ask this question of Dr. Tabor. When we have a newly recognized disease like TT virus, which can either be just newly recognized or newly emergent, and the only way you are going to know is to go back and look at blood 20 years earlier and see whether you have got the same type of prevalence, does any public health agency have a responsibility to do that, to determine whether it is newly recognized or newly emergent, or is it left to individual investigators to look at that?

DR. TABOR: There are times when this advisory committee sounds more like a congressional hearing than a scientific forum. I certainly prefer the latter.

There are a number of mechanisms set up for looking at the situation you describe. Of course, for many decades, probably throughout the existence of the Public Health Service, people have followed up newly-recognized diseases, whether they are infectious or not. This is in

the nature of the organization and in the nature of the directive that they were given when they were founded. It is also in the nature of the people who are working here.

Given those facts, there is a much greater attention being paid to emerging infectious diseases today with regard to the blood supply. The reason for this is everyone wants to avoid "another AIDS" or at least if we can't avoid it, be there with whatever tools we have before things get out of hand.

To do this with regard to blood, we have a number of forums. One of these is something called the PHS blood conference call at which these topics are discussed in I think it is a biweekly conference call involving the CDC, NIH, FDA, and sometimes members of the other government agencies, such as the Army, other interested parties.

In addition, at a recent BPAC meeting -- I don't remember if it was the last one or the one before -- I gave a presentation about something called the Emerging Infectious Disease Committee. If you go back to your notes, you will see that. This is a much smaller group that is co-chaired by myself and Dr. Mary Chamberlin, CDC, who happens to be here today. It also has representation from NIH.

The purpose of this group is several-fold. One is we have a sort of algorithm, very formal written algorithm for following up newly-recognized infections, but I will

have to say, frankly, I think even though we did that, and wrote a very good one, it is gilding the lily because what we would all do anyway, if we were properly trained scientists and physicians, but nonetheless, we have a good written plan for how to approach these things.

In addition, this committee maintains a database of all infectious agents that we think could be a threat to the blood supply with a kind of tabulated summary of what is known about them with regard to blood infections, and that was also handed out at BPAC.

Finally, the committee meets periodically, generally by conference call, to discuss newly-recognized agents, such at TT virus.

DR. HOLLINGER: Dr. Hamilton.

DR. HAMILTON: It is pretty obvious that the measurement of nucleic acids has revolutionized the diagnostic technologies applied to patient care issues, but I would like to ask any of a number of the presenters, probably all of whom know the answer, what the progress is with respect to defining some antibody response to this new agent. I think it has implications perhaps less for diagnosis than for pathogenesis, but I would be very interested to know what the progress is there.

In a related question of a series, if I may, I wonder if someone could tell me, if they know, what the

typical duration of persistence of these coincidentally found nucleic acid positive patients is.

Is there something that we can hang our hat on, both good news and bad news, if there is a disease that is a consequence here? Are there epidemiologic-linked clusters that have been identified?

Those three issues, I would like to hear about.

DR. HOLLINGER: I think one of the issues regarding the time, I think someone has mentioned, I think Dr. Alter maybe mentioned they had seen it for up to a year in a patient.

DR. WILLIAMS: I think I can answer questions 2 and 3. Those are questions we have the data to look at. I didn't present it because we haven't really done a detailed analysis, but based on the samples we have done so far, it looks like viremia can be intermittent. Based on the one sample that was TTV-positive alone, viremia persisted for less than six months. We haven't gone on and looked. We have serial follow-up on these people that go up to 16 years, haven't done a detailed analysis, but that will be coming soon.

In terms of looking at epidemiologic clusters, we also have that data within sentinel counties to look at. We haven't really started doing that yet, but it is something we can do.

I think Howie Fields can probably comment on the antibody testing thing. I know that they are in the process of developing one. Is Howie out there someplace? Do you want to take a crack at it, Howie?

DR. FIELDS: At this time, there is not a lot to report. We have cloned and expressed recombinant antigens from the two open reading frames. We are evaluating the expressed antigens as an antigenic target for antibody development.

It is just much too early for me to report on whether we have an antibody assay at this time. We are hopeful that we will, and we will also apply the antibody test to the same kinds of epidemiologically-defined specimens as you heard this morning.

DR. HOLLINGER: Both of those responses came from the CDC.

Harvey, did you use the Okamoto primers in your group, do you know?

DR. ALTER: Basically, yes, but, Aki, are you here? The primers we used, how close were they to the Okamoto primers? The same as in the second paper from that group.

DR. HOLLINGER: Also, just my comment about Dr. Yu's talk, too. Although there were seemingly some information that would suggest that certain inactivation

procedures or properties might lead to less virus, I guess the real caveat with that would be you don't know the donor population from which these bloods were drawn. They could be lower levels, they could be different, probably the same, but I think that is a caveat -- unless you know that from that, so you are really looking at the end product, not perhaps the donors that made up the first product.

DR. SIMMONDS: Could I answer question 2?

DR. HOLLINGER: Yes, please. Dr. Simmonds.

DR. SIMMONDS: I agree there isn't much data about persistence, but I mean I think there are two things that suggest that it can be. Firstly, you can't have a population prevalence of 80 percent unless it is persistent, unless something remarkable happens just before you sample them. So, clearly, in that percent of the population it will be persistent.

I think the other evidence would be if you accept that there is an increased prevalence in hemophiliacs, and also that virus inactivation procedures may be effective for TTV, then, the fact that you can actually observe an increased prevalence about 10 years after inactivation started, suggests that at least in these hemophiliacs, it has been persistent for at least 10 years.

DR. HOLLINGER: Thank you. Did you have something else?

DR. ALTER: I wanted to make a separate comment. One thing that I had meant to mention in my talk, and actually has come up, but just the fact that this is a non-envelope virus is relevant today. Even though I don't think this is a hepatitis agent, but now you have G and TTV two, quote, "new," although they are old agents probably, non-enveloped, blood transmissible agents, it does speak that solvent detergent could potentially not inactivate these agents, and it gets into that dispute about inactivation.

We don't know. One advantage of being able to measure antibody would be that there may be enough antibody in the population since it is so prevalent to be protective, or it may be gamma globulins that would have protective levels since it is so common.

DR. HOLLINGER: Thank you.

Yes, please.

DR. OHENE-FREMPONG: You mentioned some patients who seem to have acquired the infection either during their hospitalization in which they were not infused, and then those who were transfused. Were these previously tested negative patients?

DR. ALTER: Those data were all acute infections. We had pre-samples on all these people, so these were infections that happened in the hospital at remarkable rates.

DR. HOLLINGER: Yes, Dr. Koerper.

DR. KOERPER: I have a question for Dr. Brown.

The data that you showed for children -- it is sort of a question/comment -- I believe I heard you say that that was taken from children who were being seen at a children's hospital, and so while it is of concern that they had a 20 percent incidence as opposed to 10 percent incidence in blood donors, I am not sure that that group of children represents the majority of children in the United States, because by virtue of being at a hospital, they either had a disease that might be caused by this virus that caused them to come in, or they were getting injections, procedures, crawling around on the floor where blood had spilled, et cetera, so I hope that doesn't represent an increasing incidence in children in general.

DR. BROWN: You are right. Some of those facts can occur for some of them, but the majority of the individuals that we tested were trauma cases, and this was blood that was taken, I would say actually came through casualty, so that would suggest that this is more representative of what is happening certainly in the Washington population as opposed to in patients in the hospital.

DR. KOERPER: Right. I wanted to clarify. These were just children at D.C. Children's.

DR. BROWN: They weren't entirely, but the majority were casualty patients.

DR. KOERPER: Thank you.

DR. HOLLINGER: Go ahead, Howie.

DR. FIELDS: Howard Fields from the CDC. I would like to ask Dr. Mushahwar, maybe I missed it, Isa, you presented evidence using inverse PCR that TTV is probably single-stranded, but what is the evidence that you have that it is double? I mean you presented using inverse PCR, you presented evidence that it is circular. What is the evidence that you have that it is double-stranded and circular?

DR. MUSHAHWAR: The enzyme that Dr. Simmonds mentioned is highly sensitive to zinc. The experiment that was done in Japan is mostly questionable as to the concentration of the zinc to activate that virus.

Our other experiments that we have done, we run cesium chloride with papillomavirus N, which is a double-stranded virus, and the TTV virus and the chemo together.

DR. HOLLINGER: Isa, speak into the microphone, would you, please.

DR. MUSHAHWAR: Cesium chloride experiments, we run one with papillomavirus and one with parvo B19. You can't separate the parvo B19 from TTV, but you can separate the TTV from the papillomavirus. So, that is another set of

experiments, plus the cyclization experiments I described. I don't know if you were here, but we described that in detail.

DR. HOLLINGER: Yes. Please state your name.

DR. GOLDING: I am Basil Golding from the Division of Hematology in the Office of Blood.

Regarding what you said about the caveat related to donor used data, I would like to get back to that because I think there is an important point in the data, and the important point is the potential for using certain steps in manufacture which will reduce non-enveloped viruses. I know this is early in the game, but that is what her data point to and you can use TTV as a marker virus whether it causes disease or not.

But your point as to looking at the donors and perhaps there is a difference between the donors in the populations, I know from other data that, Doctor, you have generated looking at other viruses in product from those companies which we are talking about, that there were high incidences of HCV in the products from those donors in her previous studies prior to viral inactivation, so I don't think that the donors are really different, and that is not the explanation for her finding. I think it is very likely that her finding relates to the immunoaffinity columns, and that is a potential thing that we should look into because

it might be a way of removing non-enveloped viruses from these viruses.

DR. HOLLINGER: I agree. I did not mean to imply that we shouldn't look into it. I am just stating -- and I still stand by the point -- that unless you know what the starting factor is, you can't really relate to the endpoint.

Yes, Dr. Brown.

DR. BROWN: I come back to this issue of the nature of the virus. You show that it is a circular virus, and you say you compared it to polyomavirus, but it is possible this could be a circular, single-stranded virus, such as chicken anemia virus, did you compare it with this, or the data with the restriction enzyme I guess also suggested it was single-stranded. Could you confirm that data?

DR. MUSHAHWAR: No, we did not run that experiment, but the most important thing, that it is not a linear virus, so circular is not linear.

DR. DE TAN: Dr. De Tan from Mei-ying Yu's group. I am answering Dr. Hollinger's last question. In fact, we did make some preliminary analysis between the paid blood donors and volunteer blood donors in one specific company, and we could not find any difference from the Factor VIII from these different populations.

Also, we did perform the logistic analysis among

all of the different kinds of manufacturing procedures and inactivation procedure, and we found that the solvent detergent is the least effective way to remove the TTV.

Also, the dry heat is not effective at all, and we found, like she already mentioned, that the immunoaffinity column is the most effective way to remove the TTV and also followed by the pasteurization, and also chromatographic methods are also effective, partly effective, to remove the TTV. In fact, we have a lot of information regarding the inactivation procedure and the manufacturing procedures.

DR. HOLLINGER: While you are still there, again, give me some numbers. What was the prevalence of TT virus in the immunoaffinity donors before the material was manufactured? What was the prevalence of TT virus in the plasma donors from the manufacturer that makes the immunoaffinity product?

DR. DE TAN: We did not look into the original blood donors, but we did find that the plasma product from either paid blood donors or volunteer blood donors, the TTV positive rate has no difference. We did not look into the original blood donor samples.

DR. HOLLINGER: Thank you.

Yes, Dr. McCurdy.

DR. McCURDY: Isn't it likely true that the derivatives made from volunteer donors, however, contain

close to two or three times as many donors because they were made as a byproduct or the plasma came as a byproduct of whole blood? I mean I don't want to condemn one or the other, I just think it is no ballgame yet.

DR. HOLLINGER: Dr. Yu.

DR. YU: I really didn't want to say very much at all for the data that I presented a while ago, but I just want to say that, you know, the recovered plasma versus source plasma, we are looking in one particular manufacturer's product, and as Dr. De Tan just mentioned, he said there is no difference. What happened is that that particular manufacturer's product, we didn't find any. I just want to straighten that point. So, there is no way we can find the difference between recovered plasma donors versus source plasma donors, so we cannot find the difference in that aspect.

DR. HOLLINGER: Thank you, Dr. Yu.

Yes, Dr. Hamilton.

DR. HAMILTON: I am wondering if, in sort of a reverse version of a scientific event that occurred over 32 years ago, at which Dr. Bloomberg discovered the cause of hepatitis B in the course of investigating I think children with leukemia, perhaps we are not looking at the right thing as to what this virus is doing.

Dr. Mushahwar said that he doesn't even think it

is in the liver preferentially, but found it evidently elsewhere, and I would be interested to know where.

Let me just pose the question what other diseases are under consideration as a consequence of this virus, if any, and what are the relative likelihoods there? I don't know who to ask that question to.

DR. MUSHAHWAR: In order to prove that a virus is a hepatotropic, you really need to do in situ hybridization and immunohistochemical staining because of the contamination of blood in the liver, you are not sure that you are getting rid of all the blood when you do just comparative study plasma to blood even though it is twice as much as in the blood.

So, based on that, we don't think it is a hepatotropic virus unless you do those experiments.

Secondly, we have done a lot of studies with the G virus, and we could prove that is a hepatotropic, and even so, it may be not an ideal virus.

As far as disease association, I have mentioned that the polyomaviruses cause those type in the same type of diseases among children between age of three and five, and 10 and 15. Fifty percent of those children are infected with those harmless viruses and persistence of that around 15 percent, but a particular disease with this virus remain to be seen.

I don't know if I answered your question.

DR. HOLLINGER: Isa, you were among the first ones to develop also a good antibody test for the GBB-C agent. Are you able to tell us whether you have been developing or have developed anything?

DR. MUSHAHWAR: With this high a viremia, it is really not the proper way to look for an antibody. It is high viremia, that means there is no antibody. Dr. [Myome] mentions around 10^4, 10^5. We have seen as high as 10^6, as low as 10^1. When you have persistent viremia, this is not an XCV type of virus, so there is no antibody. I would doubt very much there will be an antibody capable of testing the epidemiology of this virus as the PCR for the virus itself. I may be wrong.

DR. HOLLINGER: Thank you.

Dr. Alter.

DR. ALTER: John, it is obviously almost impossible to rule out that a virus doesn't cause anything. That was the question that kept coming up for G. But what we know is we have 25 percent of people who are getting transfused who are getting this infection, and clearly, not only is it not causing hepatitis, but there is nothing apparent in this huge number of people. You can't say that in 1 percent of those people it isn't doing something. I doubt that it is, but when viruses are this common,

infection is this common, if it was doing something relevant, you should be seeing it.

DR. WILLIAMS: I want to add that epidemiologic studies are extremely difficult to do unless you know there is a disease you are looking for. Otherwise, you are just doing prevalent studies and trying to work backwards and making some guesses, so this seems to be sensible place to start.

I also want to echo that like hepatitis G, it is important to look at cofactors and whether this is actually a cofactor in causation of disease and something that needs to be looked into. Hopefully, all of us will be trying to evaluate that a little more closely in the future.

DR. HOLLINGER: Any other comments from the committee? Yes, Dr. Mitchell.

DR. MITCHELL: I think that it was interesting to bring this virus to us. It sounds like it is compelling that there is not clear association between hepatitis and this virus, and it looks from what we have heard that although it can be transmitted through the blood, that it can also be transmitted other ways.

So, I think that the conclusions are that it is something that I would not recommend that the FDA spend a lot of future time focusing on this as a source of disease, blood-transfused disease. I mean it will be interesting to

see what happens, what information we have in a year or two years, but it sounds like this is not going to be a major problem for the blood supply.

DR. HOLLINGER: Dr. Smallwood has a comment to make before we break for lunch.

DR. SMALLWOOD: Yes. I just wanted to announce for the session after lunch, the open public hearing that I have identified speakers, and I would just like to let you know which order, so that you will be prepared. Just quickly, the Sacramento Blood Center, DiaSorin, America's Blood Centers, Blood Systems Laboratory, the Blood Bank of Rotterdam, the Netherlands, the WP Blood Transfusion Service, South Africa, and Organon-Teknika.

Now, these are the speakers that have been identified to me, and if there is anyone else that would like to speak, would you please come forward after we break for lunch to speak with myself and the committee chair.

Thank you.

DR. HOLLINGER: We will reconvene at 2 o'clock.

[Whereupon, at 12:40 p.m., the proceedings were recessed, to be resumed at 2:00 p.m.]

AFTERNOON SESSION

[2:00 p.m.]

DR. SMALLWOOD: Before we start I would just like to again announce the order of speakers for the open public hearing, and just to tell you that you are limited to five to seven minutes, and if you don't hear what I am saying because you are talking I will not repeat it again.

The order of speakers for the open public hearing: the Sacramento Blood Center, DiaSorin, America's Blood Centers, Blood Systems Laboratory, Blood Bank, Rotterdam, WP Blood Transfusion Service, Organon-Teknika, and the AABB.

I would also like to announce for the record that the committee members were given information from the Immunedeficiency Foundation concerning additional information regarding shortages in IGIV. It will not be publicly presented during the open public hearing because it was not a topic that was for discussion at this meeting, but I am announcing that the advisory committee has been given that information as a follow-up to a previous meeting.

Thank you.

Dr. Hollinger.

DR. HOLLINGER: Thank you, Dr. Smallwood.

This afternoon is devoted to the topic on the Abbott Laboratories PRISM detection assay of HBsAg, Anti-HCV, and Anti-HTLV-I/II.

We are going to start off with an introduction and background by Janet Claggett from the Division of Transfusion Transmitted Diseases.

Abbott Laboratories PRISM Detection Assay of HBsAg, Anti-HCV, and Anti-HTLV-I/II

Introduction and Background

MS. CLAGGETT: Thank you, Dr. Hollinger. Thank you and good afternoon.

The subject for discussion this afternoon is Abbott's PRISM systems assay. I am going to begin with an introduction, which will be followed by a presentation by Abbott. Following Abbott's presentation, I will give a brief summary of the Abbott PRISM HBsAg, and a summary of PRISM's HCV assay will then be presented by Dr. Fashid, and Dr. Cowan's summary of the PRISM HTLV/I, HTLV/II assay will follow.

[Slide.]

Abbott Laboratories has submitted Product License Applications to the Food and Drug Administration for the Abbott PRISM systems assays for the detection of hepatitis B surface antigen, antibodies to hepatitis C virus, antibodies to the human T lymphotropic virus types I and II, and antibodies to Hepatitis B core antigen.

Although all of these submissions are currently under review, we will limit our presentations and discussion

today to the first three of these four, because the initial reviews are nearing completion and the due dates for these three fall before the next advisory committee.

[Slide.]

These products are in vitro chemiluminescent immunoassays for the qualitative detection of viral marker antigens or antibodies. All of these assays are designed to be performed on the Abbott PRISM instrument, which is an automated immunoassay analyzer.

[Slide.]

The PRISM instrument is designed to perform sample identification and pipetting, reagent dispensing, tray transport and incubation, sample reading, and result reporting.

[Slide.]

The system design includes numerous sensors and built-in redundant checks to ensure the integrity of the testing process. In addition, the system can perform continuous access, as well as immediate or STAT processing, and monitors and records critical batch information necessary for compliance with current good manufacturing practices.

Because the instrument is reviewed as a device, Abbott Laboratories has submitted a premarket notification for the PRISM instrument in accordance with Section 510(k)

of the Federal Food, Drug and Cosmetic Act. This submission is also currently under review.

A PRISM instrument has been installed in FDA's Product Testing Laboratory for the purpose of testing kits submitted in support of licensure and for lot release testing.

[Slide.]

The reason we are bringing this to the advisory committee for discussion is that the PRISM system is a novel system for the screening of blood for viral markers and comprises technology that has never been used in this way in the U.S.

The manufacturer states that the PRISM system is capable of high throughput and that it provides improved detection in some cases, increased GMP compliance by having process controls to reduce opportunity for errors, breakthrough device safety features, and tamper-resistant design to prevent overt tampering and human error.

Because of the technology involved and because of the manufacturer's claims for advances in testing and control, we seek the advice of the committee on whether the safety and efficacy data under review support a decision to approve the test for donor screening.

Thank you.

DR. HOLLINGER: The next presentation then is by

Dr. James Stewart from Abbott Laboratories.

Presentation

James Stewart, Ph.D.

DR. STEWART: Good afternoon. My name is Jim Stewart, and I am the PRISM program director for the Abbott Diagnostics Division. Today, I would like to provide the committee with an overview of the Abbott PRISM system, its performance characteristics, features, and benefits.

As an introduction to this new technology, I would like to present a short video that highlights the basic features of the instrument.

[Video played for committee members.]

DR. STEWART: Following that visual introduction, I would like to share with you some more detailed information and data on PRISM.

[Slide.]

The presentation today is divided into three parts. First, I would like to supplement the video you just watched with additional details on the PRISM system. Then, I will focus on examples of the process control and GMP features which make this device unique and which represent a significant step forward for blood screening and laboratory compliance.

Finally, I will briefly describe some of the performance characteristics for the assays submitted to FDA.

Additional details on the performance of the PRISM HTLV-I, HTLV-II, HCV, and HBsAg assays will be provided by the CBER review scientists in the next session.

[Slide.]

Before I embark on the description of PRISM and the associated data, I wanted to emphasize that our experience with PRISM comes from two sources. Most of the data today is derived from clinical evaluations conducted in support of U.S. FDA submissions and approval. In addition, however, we have the combined experience of a large number of PRISM users outside the U.S.

PRISM was launched with a five-assay menu in early 1996. There are now 88 PRISM instruments installed in customer sites and regulatory agencies in over 25 countries. Sixty-two sites are now using PRISM as test of record with cumulatively over 7 million donations per year being screened using this technology.

Based on our rest-of-world clinicals and submissions, we have received approval for PRISM in all of the major regulated countries outside the U.S. including the Paul Ehrlich Institute, the Agence du Medicament, the National Blood Authority, and several others.

Our focus over the last two years has been gathering and submitting the necessary data for U.S. approval of the system.

[Slide.]

A picture of the PRISM is shown on the left side of this slide. All of the functionality required to test a blood sample for multiple infectious disease markers from sample pipetting to assay processing to final result determination is contained and controlled within this single instrument. This is in contrast to the multiple pieces of equipment which are required to process EIA technologies, bead, and/or microtiter which are in use today in the U.S.

[Slide.]

This cross-sectional view shows the layout of subsystems within the PRISM that manage various steps within the testing process including automatic tray loading, on-board refrigerated storage of reagents up to 5,000 tests per assay, automatic sample bar code reading and pipetting, fluidic pumps for reagent delivery, optics for chemiluminescent detection, and computer controls and interface.

PRISM processes multiple assays simultaneously using six independent and dedicated immunoassay channels.

[Slide.]

This is a cross-sectional view of a three-step assay channel on PRISM, such as would be used, for example, to test for antibodies to HTLV-I and HTLV-II. The channel is surrounded by a heater blanket for continuous temperature

control.

Once trays are loaded into the PRISM, they are moved along the assay channel in carefully timed increments. This allows for precise control of both incubation time and temperature without relying on manual movement and documentation of trays by the operator. In fact, once a tray is loaded into the instrument, an operator does not physically touch the tray until the test is completed and the tray is dropped into the waste container.

As the tray moves along the channel, sample reagent and wash additions are completed at a series of fixed dispensed positions or stations. Meanwhile, the tray is continuously incubating and processing. Viral markers present in the sample are captured by microparticles which are coated with specific antigens or antibodies for that marker.

These particles are contained by a glass fiber membrane matrix, and as shown in the video, a photomultiplier tube reader is used to detect photons of light emanating from the bound chemiluminescent conjugate.

Current technologies require that many of these types of steps be performed or documented manually. Indeed, processing current bead and microtiter assays is complex. Each assay has its unique set of requirements. Some require pre-dilution of samples, some require pre-mixing of

reagents. Sample volumes and incubation temperature requirements can vary greatly.

Some incubations require shaking, others do not. Calibrator and control strategies vary greatly, and the data reduction and validity criteria present a maze of options. PRISM, by its design, is intended to help simplify these processes and reduce the reliance on manual processing and documentation.

PRISM helps to simplify testing in two fundamental ways.

[Slide.]

First, all of the PRISM assays are standardized around a common technology. This technology, referred to as chemiluminescent immunoassay, or ChLIA, is employed by all of the PRISM assays. Each employs microparticle capture and chemiluminescent detection through acridinium labeled conjugates. Assays are formatted in either two-step or three-step formats, but with similar throughput as you saw in the video giving 160 samples per hour or up to 800 tests per hour on five channels.

Once calibrators are processed, results can be made available for a sample within 54 minutes of pipetting.

[Slide.]

Secondly, standardization in assays also allows for simplification in the overall system architecture. Each

of the six channels functions similarly, creating an immunoassay processing factory where repetitive steps like reagent identification and tracking, reagent dispense and verification, reaction tray transport and incubation, and result reading determination are automated and controlled.

The sixth channel is capable of running any assay, thereby providing backup capability. Common functions are centralized, like sample pipetting, software control, and host interface.

[Slide.]

PRISM includes detection for both hepatitis and retrovirus markers that are currently tested in U.S. blood banks. Assays for detection of hepatitis B surface antigen including a specific neutralization assay, anti-HCV, anti-HBC, and anti-HTLV-I/HTLV-II are currently submitted to FDA.

An assay for detection of anti-HIV-I/HIV-II, including specific detection of group O, is currently in clinical trials, and a test for HIV-I p24 antigen is well into development.

The PRISM system outside the United States has an HIV-I/HIV-II assay, which was included originally in the U.S. clinical trials, but we have since modified for the detection of group O, which explains why this particular assay is just currently now in clinical evaluations.

[Slide.]

In the next few minutes, we will discuss ways in which PRISM can positively impact each of the key contributors to test integrity and blood safety. Specifically, we will review elements of PRISM's process control which can reduce the opportunity for errors, both errors of omission and errors of commission.

We will see evidence of how PRISM reduces the amount of manual manipulations and the level of operator intervention in the testing process. We will also touch on the performance of the system relative to sensitivity, specificity, and reproducibility.

[Slide.]

The fundamental safety objective of PRISM's automation and process controls is to reduce the opportunity for errors. This is accomplished through automation which eliminates numerous manual steps, provides standardization to the test methodology, makes use of electronic monitoring of test process steps, and maintains a complete electronic batch record.

PRISM provides a range of reports to the user to document the run including tracking of operator, instrument, reagent lot numbers, expiration dates, detailed sample results and retest reports, or if a sample result was not provided, documentation of the specific error code associated with the processing of that sample.

[Slide.]

An analysis of the extent to which PRISM can reduce operator and instrument related steps has been described by the blood bank Leuven in Belgium. This laboratory runs three viral markers for a total of approximately 250 samples per day.

During their evaluation of PRISM, they compared the number of process steps required with their microtiter technology, here utilizing a Summit processor and two T-cam pipetters versus the steps required when using PRISM. Although each of the detailed steps is obviously not readable, the fundamental conclusion of this site was that PRISM reduced the number of technician and instrument related steps by about two thirds. Similar dramatic reductions have been documented in numerous PRISM sites with various test volumes around the world.

[Slide.]

To better understand the types of process controls that are designed in the PRISM, we first must take a look at the process that we are trying to control. This diagram schematically illustrates the key steps in immunoassay test processing. Each blood donation must go through this test cycle at least once; multiple times if retesting is required.

Sample identification relates to uniquely

identifying each blood donation and maintaining that positive identification throughout the testing process. Sample pipetting refers to the accurate delivery of serum or plasma into the assay reaction.

Reagent management refers to the correct identification of reagents used to do the various immunoassays particularly with respect to using matched components in a master lot and verifying expiration dating. Reagent dispense involves the steps of delivering assay kit reagents like microparticles, probes, and conjugates at the appropriate times and in the appropriate volumes.

Reaction tray transport and incubation refers to the physical movement of reaction trays to ensure that incubation times and temperatures are met and to prevent any physical interference with the trays.

Sample reading to result determination covers the multiple steps required to measure the signal from a sample compared to an established cutoff, determine a result, and verify the validity of the run.

All of this leads to determination of whether retesting is require which could lead to another cycle through this process.

[Slide.]

Time today does not permit to cover all of these steps in the various modes of control employed by PRISM. I

would, however, like to give you some brief examples of the most unique and important process controls employed by the instrument.

As you saw in the video, when an operator loads samples onto the PRISM, bar codes on the tubes are automatically scanned into the PRISM computer. The PRISM verifies that there are tubes in the rack positions, that these tubes have bar codes, and that the bar codes are readable.

Once loaded into the system, sample tubes cannot be switched or I.D.'s altered. Sample are simultaneously pipetted into trays on each of the assay channels using disposable tips to prevent cross-contamination. The PRISM sample manager position is monitored and pressure sensors are used to verify volume of delivery on both the aspiration and dispensing of the sample. These pressure algorithms can effectively detect clots, bubbles, or other sample conditions that affect pipetting.

The operator loads reagents onto the PRISM by scanning bar codes on both the reagent bottle and the fluidics lines. The PRISM automatically verifies that the lot numbers match those of the kit master lot being loaded, so mismatched components cannot be used. It also verifies expiration dating of the kit reagents.

There are multiple redundant checks on dispensing

of reagents. Pumps are continuously monitored to ensure accurate delivery. Optical sensors are employed to check reagent delivery at the point of dispense. The time it takes fluid to drain through the membrane is also electronically measured to verify proper dispensing and transfer of sample and microparticles.

Reaction tray position and well positions are monitored, as are incubation temperatures on the channel. Any disruption to the timing or temperature of the channel will take that channel off-line, effectively aborting the run without allowing release of sample results for that assay.

PRISM monitors the background counts and performs a series of data reduction checks to ensure reagents perform within expected limits. These checks are done automatically. Invalid results do not require an operator's decisionmaking, they are automatically invalidated and thereby not released.

PRISM is also unique in that in addition to assay calibrators, the system employs an end-of-batch release control. This multi-constituent control is a low-level, positive sample which is run as a system check at the end of the run and must be detected or the run is validated.

Finally, while not mentioned on this slide, is the manner in which PRISM controls retest management. Sample

results on PRISM are maintained in a database that is then referenced the next time the system sees a sample.

If a sample which tested negative on PRISM is inadvertently introduced again to the system, it will automatically recommend that no further testing be done. If an initially reactive sample is loaded, it will automatically recommend that this sample be tested in duplicate per the package insert. In this way, PRISM helps to avoid errors in management of retest.

[Slide.]

In order to describe in more detail some of these mechanisms, I will focus specifically on a couple of the test process steps including reagent management, reagent dispense, and tray transport.

Contrast the controls employed by PRISM, required bar code entry, automatic verification, and documentation of components and expiration dates, automatic monitoring of reagent usage, on-board refrigeration of reagents. This compares with current technologies where you see a heavy reliance on visual checks, manual recording and monitoring, and the daily movement of reagents back and forth to refrigeration, leading to temperature stresses and the potential for mixup.

[Slide.]

When PRISM detects an error condition, the system

reports an error, defines a response, and requires some action by the operator. For example, in the circumstance where a wrong kit component or fluidic line would be scanned, the instrument would report a wrong product I.D. error. The operator would not be allowed to proceed with sample processing until they had installed the correct reagent and rescanned.

[Slide.]

Similarly, modes of control have been established for other test processing steps, like reagent dispensing. PRISM forces priming of fluidics lines prior to processing samples. The system automatically monitors pump operation and provides direct verification of kit reagent dispense. Current technologies do not uniformly ensure fluidics priming and typically rely on qualitative or visual verification of reagent dispensers.

Let me show you examples of some patented mechanisms which have been incorporated into PRISM to provide additional process control of reagent dispensing steps.

[Slide.]

This schematic shows how optical dispense monitoring works. A light path is created between an LED source and a photo transistor receiver. As the fluid stream is dispensed, this light path is broken and a characteristic

optical pattern is created.

[Slide.]

This graph shows examples of a nominal dispense profile on top, in which the amount of light measured in relative counts is disrupted for a fixed period of time. The example at the bottom shows a short dispense profile that might result, for example, from a reagent bottle running dry. This disruption in the profile would cause a dispense verification error, thereby invalidating the results from that sample.

[Slide.]

Similarly, this slide shows how the monitoring of drain times is accomplished. A beam of light is reflected from a light source off the reaction well into a photo transistor receiver. As fluid is transferred or added to the reaction well, the light beam is scattered. This continues for the time period it takes for the fluid to drain through the membrane. As a result, again, characteristic profiles can be measured.

[Slide.]

At top is a nominal profile in which the light beam is disrupted for a defined period of time. The middle panel shows an example of a slow drain time error condition as might be caused by having no blotter in the tray or perhaps a highly viscous sample.

The bottom panel is the opposite condition, a fast drain time error. This might occur if no matrix were present in the tray or if no sample microparticle mixture were added.

[Slide.]

The error conditions created by these examples lead to reported error codes and either a stop to processing or invalidation and no reporting of the sample result.

[Slide.]

As a last set of examples, tray transport and incubation is completely under the control of the system. The operator does not have access to tamper in any way with the tray once the assay has started. Times and temperatures are automatically controlled and monitored.

In contrast, current technologies require that the operator handle and move the tray many times during testing. Incubation times and temperatures are frequently documented manually, leading to thousands of hand entries per day, at the same time that the technician is trying to maintain continuous and accurate processing of the assays.

[Slide.]

Like the previous examples, PRISM is engineered to not allow samples or assays to deliver results when error conditions are detected. Some errors, like the heater element malfunction or stalled transport, will invalidate an

entire channel or assay. Others, like improper loading of a tray, will only affect the samples scheduled for that tray.

[Slide.]

As you look at the steps required for testing, and reflect on the fact that this flow diagram is followed over 50,000 times per day in labs across the country, it is not surprising that there is significant opportunity for errors both in accurately completing and documenting the tests.

Evidence that this is the case comes from various indicators of process control and compliance deficiencies including reportable incidents and citations, as well as recordable incidents, like quality incident reports within a laboratory.

Combined, these represent thousands of events each year investigated by the blood industry and FDA. Underlying all of this is the very real potential that many more such events each year go undetected and unrecorded.

[Slide.]

Let me share a few examples of citations available through the Freedom of Information Act, which relate to the process control steps just discussed.

This is an example of an error related to reagent management. In this particular circumstance, kit components from different master lots have been combined to complete testing for hepatitis B surface antigen. While the testing

was subsequently considered invalid, some of the blood products had been distributed prior to retest.

PRISM would control for this situation and that kit components within a master lot are automatically verified, tracked, and documented as master lot kit information.

[Slide.]

This is an example of a reagent dispense citation in which an inspector observed a laboratory technician pipetting 200 microliters of conjugate for anti-HBC and anti-HCV. While the volumes were obviously varying, the supervisor retrieved the microplate and added 50 microliters via single-channel pipette to the wells, using the same tip for all of the wells, which was a violation of the package insert.

When asked why 50 microliters was used, the supervisor responded that by his experience he could tell how much volume needed to be added. PRISM controls for this situation in the manner in which it automatically pipettes appropriate reagent volumes and incorporates multiple redundant checks to ensure reagent dispensing occurs appropriately.

[Slide.]

Finally, as an example of a reaction tray transport citation, in this particular case, an HTLV-I test

batch, the technician accidently jarred the tray, causing liquid to splash out of some of the wells. The operator reported the tests and the plate had been completely processed, the run had met all of the manufacturer's requirements and that repeat testing was not considered necessary.

PRISM eliminates this situation in that the reagent dispense and incubation steps occur entirely within the enclosed channel. The reaction trays are not accessible to the operator, thereby preventing interference with assay processing.

[Slide.]

We further tried to measure the potential impact of PRISM on these types of errors at sites involved in our U.S. clinical trial. Three sites provided details relating to the number and types of errors on test of record including microtiter and bead assays.

During the two months of integrated PRISM testing, there were 4,147 total test of record runs at the three sites. Of these, 187 runs failed, or 4.5 percent. Of these failures, 20 percent, or 38 runs, failed due to causes that were categorized as preventable or technician errors.

In evaluating the causes for these common failure modes, it became clear that PRISM process controls could have prevented all 38 of these errors.

[Slide.]

This slide shows the details of these 38 failed runs and the applicable process controls employed by PRISM. In 8 runs, the technician erred in some aspect of planning and calculating the tray or reagent requirements. PRISM automatically controls tray usage and reagent management.

In 6 runs, the operator made an error related to physically handling the tray during reading or processing. On PRISM, the technicians never handles the tray once processing begins. In 5 cases, clerical errors were made as part of manual batch documentation. PRISM maintains critical information internally and provides detailed batch reports.

The largest category of error, 17 runs, involved the addition of incorrect reagents or reagent volume. PRISM manages both the order of dispense and correct volume of reagents.

[Slide.]

Finally, there were two cases where the wrong lot of reagent was used. PRISM automatically verifies that the correct master lot reagents are utilized. Human error is a common denominator in these examples, and the potential impact of PRISM is clear from this comparison. These same control features also make it significantly more difficult to intentionally tamper with assay processing or results,

are less common but no less important issue for today's blood bank laboratory.

[Slide.]

Let's briefly summarize the potential impact of PRISM on the key contributors to blood safety. PRISM provides an unprecedented level of process control and a design that is resistant to tampering. Testing with PRISM requires significantly fewer steps than current technology, thereby minimizing the amount of manual intervention in the test process.

[Slide.]

Now, we will turn our attention briefly to assay performance. PRISM U.S. clinicals were conducted at 6 sites including 4 U.S. blood centers, 1 Canadian blood center, and 1 U.S. plasmapheresis center. Each assay evaluation included 18,000 to 28,000 samples against various licensed comparator methods. These studies evaluated reproducibility, specificity, and sensitivity with individual and multiple assay channels running. I will show a brief overview of performance testing.

[Slide.]

One hallmark of PRISM is the high level of reproducibility which accompanies the process controls inherent in the system. This slide shows the reproducibility observed on low to moderate reactive samples

for each of the four submitted PRISM assays. CVs were typically less than 6 percent, and in all cases, less than 10 percent.

[Slide.]

Specificity across the four assays ranged from 99.68 percent for anti-HBC to 99.99 percent for hepatitis B surface antigen. In all cases, specificity was comparable to or better than the comparator test of record assay. Overall, sensitivity was improved relative to test of record. There were two incremental pickups for PRISM surface antigen, one for HCV and one for anti-HBC.

In the case of anti-HBC, there were three discordants confirmed positive in favor of PRISM, two in favor of test of record. The improved reproducibility of PRISM is also evident in the cumulative specificity of the four assays.

Unlike current technology, which frequently shows a significant disparity between initial reactive rates and repeat reactive rates, PRISM shows much better agreement. The overall initial reactive rate for PRISM was 1.04 percent, significantly less than the 1.58 percent for test of record. The repeat reactive rate was also slightly lower, at 0.91 percent versus 1.05 percent.

[Slide.]

These differences, particularly for initial

reactives, become significant when you consider the impact such a reduction can have across a large screening population.

Projected across 14 million whole blood donations collected in the U.S., PRISM would yield over 75,000 fewer donations requiring retest. Likewise, a reduction of 0.14 percent in repeat reactive rate translates into over 19,000 fewer donations requiring supplemental testing.

Obviously, the magnitude of these improvements depend on the specific assays employed by a laboratory today, and the rates observed on those assays in a particular lab.

[Slide.]

Sensitivity on pedigreed positive samples was 100 percent for surface antigen, HCV, and anti-HTLV-I, HTLV-II. There was one incremental pickup for PRISM surface antigen versus the test of record. Conversely, there was one additional anti-HBC sample detected by test of record with neither product showing 100 percent sensitivity.

[Slide.]

Seroconversion sensitivity was compared on multiple panels for surface antigen and anti-HCV. PRISM showed additional bleeds in earlier detection than test of record in 10 of the 12 HBV panels and 5 of the 10 anti-HCV panels. The most dramatic sensitivity improvement for PRISM

was seen with surface antigen, where every panel showed additional bleeds detected. We will examine the surface antigen sensitivity performance in greater detail in just a moment.

[Slide.]

Since seroconversion panels for HTLV are not widely available, we looked at the limits of detectability for this assay via dilutional panels. PRISM HTLV detected 1 of 3 HTLV-I-positive samples at 2-fold greater dilution than the HTLV-I/HTLV-II specific EIA.

On HTLV-II-positives, PRISM detected all 3 samples at greater dilution by anywhere from 4- to 16-fold. The PRISM ChLIA assays frequently show an improved dynamic range in dilutional sensitivity than comparable enzyme immunoassays.

[Slide.]

Coming back to surface antigen detection, the PRISM assay show clear superiority and sensitivity to the test of record. In our clinical trials, excluding seroconversion panels, PRISM detected 28 incremental positives. These included two blood donor samples, as well as specimens from patient groups including acute, chronic, and recovered HBV infection, individuals at increased risk for infection with HBV and populations with other medical conditions or interferences.

This enhanced clinical sensitivity was paralleled by significantly improved analytical sensitivity as measured by panels prepared from purified ad and ay antigens. PRISM routinely achieved sensitivities of less than or equal to 0.1 ng/mL. This was compared with the test of record assays which typically ran between 0.4 and 0.6 ng/mL.

[Slide.]

As I showed earlier, this enhanced sensitivity was observed in increased detection of serial bleeds from seroconverting donors infected with HBV. Separate from the seroconversion data provided in our PLA submission, we have now added to that data and evaluated a total of 25 commercially available seroconversion panels.

In conjunction with Dr. Glen Satten at Emory University, these data were modeled using a five-stage Markov model, using a 95 percent confidence interval. The modeling calculations were based on 12 million U.S. donations per year assuming an HBsAg incidence of 4.120 conversions per 100,000 persons per year.

The results of this modeling show that PRISM could detect HBV infection a week earlier than the test of record and continued to detect surface antigen for almost two weeks on the back end of the window.

This projects to approximately 26 additional surface antigen positive and potentially infectious units

which could be detected using the PRISM surface antigen assay. This represents a significant reduction in the window period for HBV, which is the highest residual risk marker among the viruses currently being screened for.

[Slide.]

In addition to sensitive early detection of the common wild-type hepatitis B virus, PRISM surface antigen also shows strong performance in detecting the most common of the emerging mutant HBV strains.

HBV escape mutants having altered sequences in the "a" determinant of HBsAg have been identified in some children born to HBV-infected mothers. These children become infected with HBV despite active and passive immunization at birth. The most common escape mutant is a Gly to Arg substitution in amino acid position 145 in the "a" determinant.

In a study by Oon, et al., looking at a large number of infants born to infected mothers, this Gly/Arg mutation represented 75 percent of the vaccine escape mutants identified. From this information, we have produced a surface antigen molecule matching the sequence and utilized it as a panel to evaluate the mutant detectability of the PRISM surface antigen assay. This testing was performed at one of our U.S. clinical sites.

[Slide.]

The PRISM assay was capable of detecting the Gly/Arg mutant at the same level of sensitivity as the wild-type, that is, approximately 0.1 ng/mL. In contrast, a competitor's licensed test was unable to detect this mutant at any of the concentrations studied. In fact, the licensed test remained negative even at a concentration of 100 ng/mL of the Gly/Arg substituted antigen.

This reinforces the importance for us as manufacturers to continuously evaluate the performance of assays against these types of samples. At present, however, from these data, the PRISM surface antigen assay shows strong detectability for this particular HBV mutant.

[Slide.]

Let me briefly summarize. Our clinical and field experience with PRISM supports that this technology offers significant public health benefits. PRISM restricts access to key processing steps and does not allow operator intervention in failed runs. This level of tamper resistance is unprecedented in blood screening and provides for a significant advance in safety and effectiveness over existing alternatives.

PRISM automation and process controls minimize the number of manual steps, reduce the opportunity for errors and enhanced laboratory compliance with GMP requirements. These features, coupled with the potential to reduce the

number of false reactive samples, support both increased effectiveness and reduced risk.

Finally, the PRISM also provides for earlier diagnosis through improved detection of surface antigen and HBV mutants.

While our clinical data provides primary support for the points discussed today, these conclusion have also been substantiated by user experiences and the demonstrated performance of this technology around the world.

One such user, the Bavarian Red Cross in Munich, informed us that they would be submitting a report to FDA and the BPAC Committee describing their experiences with the PRISM over the last two years. The BRK is one of the largest sites utilizing PRISM and represents a site that has historically performed both European and U.S. licensed tests.

Professor Weise from the BRK could not be present today, as he is chairing another meeting, so I would like to conclude my presentation by sharing a brief summary from his report.

The report is entitled, "The Safety of Blood and Blood Products with Special Regard to the PRISM System," by Professor Wilhelm Weise and his colleague, Dr. Uve Backer.

One paragraph summary reads as follows: "In Germany, the PRISM assays hepatitis B surface antigen, anti-

HIV-I, HIV-II, anti-HCV, and anti-HBC are approved by the Paul Ehrlich Institute and can be used for routine screening of blood donations. These assays have proven to give reliable results with both high sensitivity and specificity. The PRISM system does give a high level of safety for screening blood donations especially in regard to GMP and GLP. We are now using PRISM routinely for more than two years and therefore we are able to recommend the use of PRISM in high-volume blood bank screening."

Thank you very much.

DR. HOLLINGER: Thank you, Dr. Stewart.

We now have the FDA critiques, first, by Janet Claggett, Division of Transfusion Transmitted Diseases.

FDA Critiques

HBsAg

MS. CLAGGETT: Thank you.

Most of what I am going to say you have just heard, but you will hear it again.

[Slide.]

As previously mentioned, Abbott Laboratories has submitted a Product License Application for the Abbott PRISM HBsAG, an in vitro chemiluminescent immunoassay for the qualitative detection of hepatitis B surface antigen in human serum or plasma, and the accompanying Abbott PRISM HBsAG confirmatory assay. As mentioned in my introduction,

this assay is designed to be performed on the PRISM instrument.

[Slide.]

The PRISM HBsAg assay is a two-step sandwich chemiluminescent assay that detects HBsAg in human plasma or serum. First, the microparticles coated with mouse monoclonal anti-HBs are incubated with sample in the incubation well of the reaction tray. The sample can be serum, plasma, calibrater or control.

During incubation, HBsAg present in the sample binds to the antibody on the microparticles. When this incubation is complete, the reaction mixture is transferred to the glass fiber matrix of the reaction tray using the transfer wash.

The microparticles are captured by the matrix while the remaining mixture flows through to the absorbent blotter. Acridinium-labeled goat polyclonal anti-HBs conjugate is added to the microparticles on the matrix and incubated.

At the end of this incubation, the unbound conjugate is washed into the blotter with a conjugate wash. The chemiluminescent signal is generated by adding hydrogen peroxide solution and the resultant photons are counted. The number of photons in the sample is compared to a cutoff value, which is determined from the calibrator, which must

be run with patient samples and controls. The number of protons is proportional to the amount of HBsAg in the sample.

[Slide.]

All specimens that are repeatedly reactive for HBsAg must be further tested with a licensed neutralizing confirmatory test. The PRISM HBsAg confirmatory assay involves two steps. The neutralization consists of combining the sample with confirmatory diluent and pipetting this mixture into two sample cups.

The specimen transplant buffer is added to both cups. Neutralizing reagent is added to the first sample cup, and a blank is added to the second sample cup. After the appropriate incubation period, the samples are processed using the Abbott PRISM.

[Slide.]

The mouse monoclonal IgM anti-HBs in this product is the same as used in the currently licensed Abbott AUSZYME monoclonal assay and the Abbott IMx HBsAg assay. Therefore, this is not a new component, but one with an established reliable track record as used in the currently licensed test kit.

[Slide.]

Clinical studies were conducted at four U.S. blood centers, one Canadian blood center, and one U.S.

plasmapheresis center.

The test of record or comparator assay was Abbott's currently licensed AUSZYME Monoclonal assay, procedure C, which has the shortest incubation of 75 to 80 minutes, and is the least sensitive of the four procedures.

[Slide.]

A total of 25,238 serum and plasma specimens from volunteer whole blood and plasmapheresis donors were collected and tested at six donor centers. Based on an assumed zero prevalence of HBV infection in voluntary blood donors, Abbott claims the specificity of the PRISM HBsAg is estimated to be 99.99 percent. The specificity of the AUSZYME monoclonal assay is estimated to be 99.98 percent according to Abbott. Further review will assess these claims.

[Slide.]

Clinical sensitivity was assessed by testing samples from the following selected populations with HBV infection: individuals previously characterized as positive for HBsAg, individuals with acute HBV infection, individuals with chronic HB infections.

Also included were samples from individuals recovered from HBV infection, as well as samples from individuals at increased risk for HBV, such as intravenous drug users, dialysis patients, hemophiliacs, and patients

with sexually transmitted diseases. The claimed sensitivity is 100 percent and, as mentioned before, further review will assess these claims, as well.

[Slide.]

In patients with acute HBV infection, HBsAg appears temporarily. In order to study analytical sensitivity, Abbott analyzed seroconversion panels composed of 244 serial bleeds from 12 whole blood and plasmapheresis donors with both the PRISM HBsAg assay and the comparator assay, AUSZYME monoclonal assay procedure C.

The PRISM HBsAg detected antigen 3 to 13 days earlier than the AUSZYME monoclonal in 10 of the 12 seroconversion panels. Both assays detected HBsAg in the first available bleed in 2 of the 12 panels, and the PRISM HBsAg detected 5 to 48 days longer than the AUSZYME monoclonal assay in 4 of the 12 panels.

[Slide.]

Abbott Laboratories investigated the PRISM HBsAg assay with regard to HBsAg mutant detectability by testing dilutions of a sample containing the Glycine to Arginine 145 mutation diluted in HBsAg nonreactive human plasma to prepare 1:5, 1:10, 1:100, and 1:1,000 dilutions.

Testing these dilutions with one lot of PRISM HBsAg and one lot of AUSZYME monoclonal using procedure C revealed that both assays detected the mutant at the 1:5,

1:10, and 1:100 dilutions. Neither assay detected the mutant at 1:1,000.

[Slide.]

Detection of HBsAg ad and ay subtypes was evaluated with an 11-member subtype panel. The PRISM HBsAg could detect HBsAg in all panel members at least to about 0.25 ng/mL. The AUSZYME monoclonal assay, using procedure B this time, the long incubation, the most sensitive procedure, could detect all panel members at least to about 0.50 ng/mL.

In conclusion, the review of the Abbott PRISM HBsAg is proceeding on schedule and is expected to be completed by the managed review date.

Thank you.

DR. HOLLINGER: Thank you.

The next critique is going to be done by Dr. Fashid from the Division of Transfusion Transmitted Diseases.

Anti-HCV

DR. FASHID: Could I have the first slide, please.

[Slide.]

Basically, I would repeat what you already heard, but here, I present application regarding PRISM HCV, which is in vitro chemiluminescent immunoassay for qualitative detection of antibody to hepatitis C virus in human serum or

plasma. As mentioned earlier by the manufacturer and Janet, the assay is designed to be used by Abbott PRISM instrument.

[Slide.]

PRISM HCD is designed to detect antibodies to recombinant antigen covering the portion of core, NS3, NS4, and NS5 region of HCV genome. This includes C100, which is constructed from portion of NS3, NS4, NS5, and HCr 43, which is a fusion of NS3 and core. C100 and NS5, you are familiar with. They are currently using the license assay.

HCr 43 is also actually part of the recombinant that you see in current assay except the clonic structure here is a little different, and is a fusion of the core and S3, so you have here core and C33 combined as one single recombinant antigen.

[Slide.]

Again, as mentioned earlier, the assay is a two-step sandwich chemiluminescent assay. In the first step, the sample will be incubated with solid phase microparticles which are coated with the recombinant antigen that I just mentioned.

After a period of incubation and wash, the second step would be that the antigen antibody complex will be incubated with a secondary antibody biotinylated anti-human IgG with acridinium label. Then, after exposure of acridinium to basic pH and hydrogen peroxide, photon will be

produced and the light will be proportioned to the amount of the antibody in the serum.

[Slide.]

The Abbott PRISM system, the clinical evaluation of the system, the assay was compared with also 3.0 and Abbott HCV EIA 2.0. For the specificity studies, they used 25,595 specimens, out of which 8,252 were serum and 14,262 were plasma, and there were also 3,081 commercial plasmapheresis specimens.

[Slide.]

This table summarizes the specificity studies. In 25,595, HCV PRISM initially detected 78 positives, and out of this 78, 76 were repeat reactives, and 47 of those, or 59.49 percent, were supplementary tests positive, and the remaining were indeterminant.

The supplementary test they used RIBA 2.0 or Abbott matrix 2.0. In comparison, HCV 2.0 and 3.0, detected 86 initial reactive, and 70 of those, they were repeat reactive, and 45, or 65.71 percent, of the repeat reactives were supplementary test positive.

The specificity calculated by Abbott is 99.89 percent for PRISM HCV and 99.91 percent for the test of records.

[Slide.]

The sensitivity studied 834 specimens, they were

tested. These include HCV antibody-positive specimens. These are repository samples, and acute HCV infection and chronic HCV infection and at risk for HCV infection. They were including the I.V. drug users and also hemophiliacs. The number is in the next slide.

[Slide.]

Anti-HCV-positive, 400 samples were used. The result that we see here, there was 100 percent concordance between the result of PRISM HCV and test of record, which was HCV 2.0 or 3.0, and I put the result in the same table.

Anti-HCV-positive sample, 100 percent of those they are repeat reactive, and all were confirmed by supplementary test. The same goes for the acute infection, which there were 20 samples, and all were confirmed, and chronic were 154, and all 154 were repeat reactive and confirmed by supplementary test.

At risk, there were 260, 210 of those, they were I.V. drug users, and the remaining were hemophiliacs. 151 were repeat reactive, and 149 were supplementary test positive. As I mentioned earlier, this result was for both PRISM HCV and for the test of record.

[Slide.]

In conclusion, in comparing PRISM HCV with test of records, which is licensed EIA 2.0 or also HCV 3.0, the clinical evaluation indicate comparable specificity in donor

populations compared to licensed EIAs, and also comparable sensitivity in positive specimen population compared to EIAs, and improved detectability in HCV seroconversion panels compared to licensed EIAs.

I did not have a table for the seroconversion, but as mentioned by the manufacturer, that there were 10 seroconversion panels and PRISM HCV detected five of those panels earlier than test of records. The other five, they were detected at the same time.

As Janet mentioned earlier, the review is underway and is expected to be completed by managed review deadline.

DR. HOLLINGER: Thank you.

The final critique on the anti-HTLV-I/II is by Dr. Elliot Cowan, also from the Division of Transfusion Transmitted Diseases.

Anti-HTLV-I/II

DR. COWAN: Thank you very much. Pardon me if I am a little redundant.

[Slide.]

My purpose this afternoon is to provide the committee with a status report on the review of the Product License Application for the Abbott PRISM HTLV-I/HTLV-II.

This test, as you heard from the manufacturer, is an in vitro chemiluminescent immunoassay for the qualitative detection of antibodies to HTLV-I and/or HTLV-II in human

serum or plasma, and, of course, it is designed to be used with the Abbott PRISM instrument.

[Slide.]

This is a three-step automated sandwich chemiluminescent immunoassay. In step one, the sample is mixed with microparticles coated with sonicated and detergent inactivated HTLV-I and HTLV-II whole viral lysates for each.

After incubation and washing, in step 2, a probe is added, which consists of biotinylated HTLV-I and HTLV-II proteins. This probe will bind to free-binding sites of antibody bound to the antigen on the microparticles.

After another incubation and wash, an acridinium-labeled anti-biotin conjugate is added in the third step, following which the chemiluminescent signal is generated by chemical treatment. The chemiluminescent signal is proportional to the amount of antibody in the sample, in the calibrator, or in the control.

[Slide.]

As you can see, this test uses yet another type of probe compared to the other tests which have been discussed. Whereas, the anti-HCV test makes use of biotinylated anti-human IgG and the HBsAg test used an acridinium-labeled anti-surface antigen conjugate, the anti-HTLV-I/HTLV-II test uses biotinylated HTLV-I and HTLV-II proteins.

These proteins are made up of inactivated HTLV-I and HTLV-II whole viral lysates, as well as an HTLV-I envelope enriched viral lysate. According to the manufacturer, this type of probe is designed to improve the specificity of this screening test.

There are separate calibrators and controls in this test for HTLV-I and HTLV-II, which have limited, defined cross-reactivity. Consistent with CLIA recommendations, calibrators are used to generate the cutoff value for a given batch, whereas, controls are used to determine run validity.

[Slide.]

To examine the specificity of the Abbott PRISM HTLV-I/HTLV-II assay, 21,943 serum or plasma specimens from volunteer whole blood donors were tested at a total of five clinical test sites. The PRISM assay was compared to two currently licensed HTLV screening tests, the Abbott HTLV-I/HTLV-II EIA, and the Abbott HTLV-I 2.0 EIA.

The results of this clinical study showed an increase in specificity with the PRISM assay relative to the two comparator tests, a claimed specificity of 99.93 percent for PRISM versus 99.74 percent for the HTLV-I/HTLV-II EIA, and 99.9 percent for the HTLV-I 2.0 EIA according to Abbott, based on an assumed zero prevalence for HTLV-I and HTLV-II in this donor population.

Thus, it would be anticipated that use of the PRISM HTLV-I/HTLV-II assay will result in a slightly reduced rate of false positive reactions.

To evaluate the sensitivity of the PRISM assay, a total of 1,856 specimens were analyzed from a number of different populations using the PRISM HTLV-I/II assay, the Abbott HTLV-I/II EIA, and the Abbott HTLV-I 2.0 EIA.

[Slide.]

I am showing you here the number of confirmed positive specimens out of the total number that were screened. Confirmation of infection was determined by positive results on investigational blood and on RIBA.

The population studied included repository specimens previously determined to be positive for antibodies to HTLV-I or HTLV-II, and specimens from individuals with HTLV-I and/or HTLV-II, associated diseases, that is, ATL, adult T-cell leukemia and MTSP.

All of the preselected HTLV-I and HTLV-II specimens were initially detected on the basis of repeat reactivity using an HTLV-I-only test. In addition, a prospective study was performed consisting of 341 specimens from individuals at increased risk for HTLV-I and/or HTLV-II infection, that is, intravenous drug users and patients with sexually transmitted diseases, and 800 specimens from individuals residing in areas endemic for HTLV-I or HTLV-II,

the Caribbean, West Africa, Japan, and Brazil.

[Slide.]

A total of 751 out of the 1,856 specimens were determined to be positive for HTLV-I or HTLV-II by investigational western blot and RIBA. All of these supplemental test-positive samples were detected by all of the test kits in this clinical study, that is, by the PRISM HTLV-I/II, by the HTLV-I/II EIA, and the HTLV-I 2.0 EIA.

The sensitivity of the PRISM assay was based on the 714 supplemental test-positive specimens from the preselected anti-HTLV-I/HTLV-II-positive, and HTLV-I and/or suspected HTLV-II associated disease categories. Sensitivity was estimated by Abbott to be 100 percent with a 95 percent confidence interval of 99.51 percent to 100 percent. Claimed increases in analytical sensitivity using the PRISM assay through the testing of dilution panels was already discussed earlier by Dr. Stewart from Abbott.

[Slide.]

In conclusion, the status of the review of the PLA for the PRISM HTLV-I/HTLV-II PLA is such that it is expected to be completed by the managed due date of October 20th, 1998.

Thank you very much.

DR. HOLLINGER: Thank you.

That concludes the formal presentations, so at

this time we will go to the open public hearing. The first speaker is from the Sacramento Blood, Mr. Ken Kuramoto.

We need to really stick to the time here on this, so we will be a little harsh if you go over your time.

Open Public Hearing

Ken Kuramoto

MR. KURAMOTO: Thank you very much for letting me speak here at the BPAC meeting. I am here because Dr. Paul Holland was unfortunately unable to attend. He had other conflicting things in China.

I am the research coordinator at the Sacramento Blood Center, and I have been working with the PRISM since September of 1996. We were one of the clinical sites, and I am going to express some of my concerns or things with the PRISM instrument.

What we did was we tested the PRISM on-line with linked samples, so on my first slide what I am going to show is some of the things that Jim Stewart had already covered and some of the members of the FDA. What it is, is these are concerns that we have in our blood center and we think that the PRISM is going to improve on these facts.

[Slide.]

First of all, the PRISM controls everything from beginning to end, and we feel that because of the process control, this will eliminate most of the problems that we

have in our laboratory already, such as process control, process control because most of the errors, as I said, are caused by technical problems, people extending the incubation too long, adding the wrong reagent, as Jim had previously said.

So, therefore, the human mistakes are rare because of the instrument, the instrument controls every process, every step, and again, Jim didn't say this, but all reaction trays are the same for all assays, so therefore, there is no mistake of putting the wrong tray with the wrong assay, so therefore, you don't have a problem with that.

[Slide.]

Now, what happens is we were able to run this instrument, and we noticed right away many of the GMP factors that are involved. As Jim said, it reads bar codes, it reads codabars and it has ability to read ISBT 128, which everyone is trying to strive for, but if there is problems with your bar codes, you can double enter as a safeguard. You enter it once, then, the instrument asks you to enter it again, so therefore, you have to enter it twice.

[Slide.]

And, as Jim said again, the event log documents every step of everything that the operator does on the instrument. All the errors are documented. We found that because the errors were documented on the instrument, that

we were able to find possible problems with the instrument, such as one of the pumps may have a little bit of crusting over of the dispense module. We reported that. It was a rare event, it was like 1 in 500 samples or so, but it was something that we reported it.

There was a little bit of crusting on one of the dispense things. It made it so that one of the dispensers was not quite proper in some of the cases, so therefore, we reported, they found it. It was easy to fix. So, therefore, due to the error logs, we were able to capture and find this problem before it really became a major problem.

[Slide.]

Although I don't have a slide for this, all of the reagents were bar coded, as Jim said, and on the video, from the reagent bottle and the line that you put it on, so there was no mistakes. We did not have a problem. We had two errors on the system the entire time we were running it, and I have been running it for about a year and a half, two errors because of control problems, and the CD was a little bit off, or one of the pumps was acting up. We could have kept running if we were able to use the backup channel, but since we were in clinical trials, we were unable to continue until the instrument was fixed, but like I said, we could have continued.

We noticed that the precision and accuracy of added reagents were very, very good. They were much better than most clinical laboratories. Most clinical laboratories, it is like 5 percent. The precision of 1.5 percent is generally better than what laboratories can do, so therefore, the process, the controls, the actual running of the instrument is at a much better proficient rate. Since it works on a discrete channel system, it makes it so that the whole system, all the reagents are added at the exact same time. The process continues.

You do not have a problem of taking your tray, you say your first well incubates actually longer than your last well, well, in this instrument it does not happen because, as the system moves through every 40 seconds, if you calculate 40 seconds, you say, well, that doesn't come out to 160 samples like Jim said, but it adds it in duplicate, so therefore it adds 2 samples at a time. When it does that, it goes through and every 40 seconds it moves and adds the reagents at the same step for every assay. So, therefore, it is well controlled, the process is controlled by the instrument. We have no problems because the incubation was too long or too short or whatever.

[Slide.]

Along with that, the instrument checks everything. The instrument goes through its checks as you are starting

up the instrument, as Jim said, the reagent usage. What we really liked was the fact that each reagent bottle can test up to 5,000 samples from one bottle, which means for a center of our size, we may have to only change the reagents once every two weeks, which makes this much better for us.

The trays are checked by the instrument. If you run low, it alarms you, it tells you that you are running low. If you are running low on reagents, it will let you know at the time that you are running low on reagents as you start the instrument up, which makes it much better for us.

For CLIA regulations, we do external checks, external checks to verify the volume, and I tell you that volume was well within the accuracy of what they had stated. In fact, they had to slightly open it up because we couldn't hardly validate it sometimes because our pipetters from our Central Laboratory, even though they were within CLIA standards, was beyond the 3 percent, it may have been 4 percent, so therefore, they had to open it up a little bit and now it is much easier to check. So, therefore, the instrument is much better than what we had really anticipated when we first began.

[Slide.]

This is very complicated here, but the members of the Blood Products Advisory Committee do have this in their packet, I believe. We do give the GMP concerns that we have

had, that I have discussed here.

Down here, we give specificity. The specificity was equal to or better than the standard ELISA technique that we were using at the present time. Upon further investigations, we found that the sensitivity is also slightly better, as previously discussed. We have been working with the instrument on a research basis, analyzing other samples that we have been able to obtain for analysis on the instrument, so we have been finding that the sensitivity is better.

So, in conclusion, we feel that this instrument is going to be of big value to blood centers because of the process control, because of the GMP issues, and we are looking forward to the time that this instrument gets licensed, so that we can implement it because of these tight controls that the instrument has.

Also, it means that we will use less personnel -- not that we are going to fire anyone -- we will be utilizing the people in other ways to be able to conform with more compliance issues, being able to utilize them so that we can meet standards within other departments other than just the processing laboratory.

Thank you very much.

DR. HOLLINGER: Thank you.

The next speaker will be from DiaSorin, Dr. Anne

Bodner.

Dr. Anne Bodner

DR. BODNER: My purpose in presenting today is to stimulate some interest, I hope, in looking at alternative ways of confirming HIV antibodies.

[Slide.]

Right now western blot technology dominates confirmatory assays for HIV. There is a licensed IFA assay, but it is not widely used. There is no licensed confirmatory assay for HIV-2, for type O HIV-1, for HTLV-I and II, and there is no objective automated confirmation assay. I want to present some data on an assay made with the technology that could possibly meet all these confirmatory needs.

[Slide.]

The instrument system is a flow particle analyzer that uses light scattering to discriminate particle size. The bottom chart here shows four particle sizes. The one on the left in the top of the bottom chart is an internal reference particular that checks optics and fluidics and also sample specificity.

The three particles there can be separate assays run simultaneously. There can be a fourth particle there. You can see that is a latex agglutination test that is automated, and you see on the bottom where there is a

positive sample that the histograms or the test go down relative to the internal records particle.

[Slide.]

We put together a research HIV assay. We don't have any plans to commercialize this now because right now we don't have the rights to use the sequences, but we are interested in developing such an assay. This is how the test results I am going to talk about were configured.

The particle on the left is a reference particle, and we have three particle sizes - one coated with HIV-1, gp41, one with a peptide for type O, and one with gp35 for HIV-2.

[Slide.]

We first looked at the results on the BBI HIV-1/2 panel. We were able to correctly identify each of the 15 samples, and we were able to discriminate HIV-1 and HIV-2.

[Slide.]

We then went on to test samples that we obtained from Dr. Susan Stramer of the American Red Cross, and all of the samples that we tested were HIV western blot confirmed samples.

[Slide.]

We tested a subset of 472 samples that the Red Cross had confirmed as HIV-positive during 1997 using western blot. We used the research Copalis HIV assay, and

all the samples we used were archived, unlinked samples.

Because there is some interest in how specific western blot assays are for p31-negative samples, we divided the sample groups into two, p31-negative and p31-positive. We tested 42 samples of the p31-positive, and the 42 samples we tested were the ones with the lowest reactivity in the screening assay, and the two very lowest samples in the screening assay were RNA negative in a licensed competitive HIV PCR assay.

We tested all 22 of the p31-negative samples, and of those 22 samples, 11 were RNA-positive and 11 were RNA-negative.

[Slide.]

The results on the 42 samples that were p31-positive, we detected 41 out of 42, and the sample that the Copalis assay missed was RNA-negative. On the 11 samples that were p31-negative, but HIV RNA positive, we detected all 11.

On the 11 samples that were negative for both p31 and RNA, 8 were negative and 3 were positive, and 1 of the 3 positive samples was right at the cutoff.

[Slide.]

This shows the 8 lowest of the 42 lowest screening activity samples, and you will see that sample 24 and 30 were RNA-negative. Thirty is the one the Copalis assay

missed. The next highest reactivity was No. 24, which Copalis did detect.

[Slide.]

Interpretation of these results. It looked as though the Copalis HIV assay correlated better with RNA presence than those of the western blot. If you believe that in the 22 difficult-to-interpret samples, because they were faint on the western blot and because they were p31-negative, and some of them were HIV-negative, HIV RNA negative, if you believe that the RNA results are more or less a gold standard, then, the western blot correctly identified 11 out of those 22 samples, while the Copalis assay correctly identified 19 out of 22.

[Slide.]

So, I want to float a proposal that there could be a place for alternative technology in the retrovirus confirmation area. We could configure an assay with the Capolis system that would be objective and automated, you could assay for up to 4 antigens simultaneously. This could be multiple antigens for a single virus or it could be antigens for more than one virus. There could even be p24 antigen detection at the same time.

We could detect total antibodies for IgG, IgM, and IgA. The sensitivity will be equal at least to western blot, and it would be a rapid assay, less than 30 minutes.

Thank you very much.

DR. HOLLINGER: Thank you.

The next speaker will be Dr. Louis Katz from America's Blood Center.

Dr. Louis Katz

DR. KATZ: I would like to thank the committee on the part of ABC's 70 independent blood centers for the opportunity to comment on the pending consideration of licensure for the PRISM blood testing system developed by Abbott Labs. This committee may know that last year, ABC filed a brief with FDA urging expedited review of the "prison" system -- PRISM system.

[Laughter.]

DR. KATZ: Freudian slip. Part of the reason for our request is that PRISM offers improved specificity and sensitivity over current tests on our review of the clinicals that you have now seen. This will allow us to catch more infected donors while rejecting far fewer safe donors that are currently being deferred with available testing.

The far more important reason for our request for expedited review is that major improvement in total automation and cGMP compliance featured by the PRISM system. The tests in use today are already highly specific and sensitive, but their performance remains operator-dependent,

an error means that at best we must invalidate many test results, at worst we defer safe donors by the thousands, throw out many safe blood components, and conceivably release units that may be unsafe.

Further, today's tests are not tamper-proof and as we know, related to prisons, two individuals have gone to jail recently, and one major blood provider is under consent decree for trying to speed production by falsifying test results.

From our review of available data, PRISM is nearly tamper-proof and for that reason alone, PRISM should be considered as quickly as possible by the FDA. PRISM is an example of technology developed in the United States that is employed first overseas because of the regulatory requirements of FDA's thorough approval process.

Over the last two years, PRISM has swept Europe and Pacific rim countries as the next generation in blood testing. Despite the pace of FDA's process, we appreciate that FDA has also viewed PRISM as a priority. ABC looks forward to the approval of the PRISM system early next year.

Thank you for your attention.

DR. HOLLINGER: Thank you, Louis. You tempered I guess the letter of Jim's a little bit.

The next speaker is Sally Caglioti from the Blood Systems Laboratory.

Sally Caglioti

MS. CAGLIOTI: Thank you.

[Slide.]

I would like to make a few comments about the enhanced process control from our perspective at the Blood Systems Laboratory. Our laboratory currently tests about 1.1 million annualized donations from 20 blood centers throughout the United States.

We currently use for our test of record three bead tests and three microtiter well tests that have been briefly described today as being semi-automated, yet highly manual. We have been a participant in the clinical trials for the PRISM in three different trials where we have looked at the reagent, the instrument, and the software.

Over that time frame, we have had seven months of experience performing over 40,000 tests on the PRISM. I have three slides that are more practical in nature that illustrate the impact that the PRISM would have if we were to use it in place of our current technologies to perform our blood donor screening.

[Slide.]

This first slide shows you all of the manual events that happen on a daily basis in our laboratory, and we test on an average day about 3,800 donations. So, that is the number that we are looking at.

If you look here at the top, you can see the number of manual documentation events that happen in one day, over 11,000 events, also, over 11,000 manual reviews that are required, over 3,000 test processes, things like moving trays around, adding reagents, and over 37,000 visual level checks, fluid level checks in our trays, totalling about 62,000 manual events per day in comparison with our estimate for PRISM for the same value, less than 60, obviously, a couple of fold reduction in the number of potential errors due to manual event.

[Slide.]

The second thing we looked at is the reduction in complexity that we would see in our laboratory should we use the PRISM analyzer. We looked at three critical process control points here that we feel are very important in quality laboratory testing, one being routine standard operating procedures and training programs.

We currently have 12 that just give your technicians the instructions for how to do the tests themselves. With PRISM, we would estimate we would have one. Each one of these routine SOPs and training programs has to be developed, validated, and subject to change control.

We currently have 143 instruments by which we perform our infectious disease testing. We estimate that we

would need a total of four PRISMs to test our volume of about 1.1 donations. Each one of these instruments has to be calibrated, validated, subject to preventive maintenance and quality control.

In addition, we have 38 technicians performing this testing on a daily basis. We estimate -- this is a conservative estimate -- that it would take about 16. Each one of these technicians has to be trained and tested for competency and proficiency.

All of this, of course, leads to a reduction in error potential.

[Slide.]

My last slide shows what we project as deviations for unannualized data with the use of the Abbott PRISM analyzer in place of our current systems.

You can see here that we filed deviation reports for all of these different things that happened in our laboratory, one being failed runs. We see a failed run rate of about 3.5 percent, most of which are due to control failures, operator error, and equipment malfunction.

We feel that 90 percent of these failed runs would be eliminated should we use the PRISM analyzer. We have seen in one year's period, over 750 equipment malfunctions. We have had over 230 equipment service calls by our vendors, and have generated 237 quality assurance reports that could

be eliminated with the use of the PRISM, totaling over 2,400 deviation reports that we feel could be eliminated with the use of a single analyzer.

[Slide.]

In conclusion, we highly support the licensure of the Abbott PRISM. We feel that its use in a high-volume testing laboratory, such as ours, will have a positive impact on process control and ultimately the safety of the blood supply by reducing potential for deviations, through reduction in numbers of manual steps, instruments, procedures, and technicians.

Thank you.

DR. HOLLINGER: Thank you.

DR. SMALLWOOD: Before the next two speakers are called, I would just like to announce that these speakers, their travel was supported by Abbott Laboratories to participate at this advisory committee meeting. Thank you.

DR. HOLLINGER: The next speaker is Dr. Molijn from the Blood Bank Rotterdam in the Netherlands.

Dr. Hans J. Molijn

DR. MOLIJN: Members of the Committee, ladies and gentlemen, we use the PRISM now since June 1996, and I want to show you in a very brief presentation what we did with the system.

[Slide.]

We started off with a validation period of a few months, and we were very happy to see that the system was a complete system, fully automated, and took some of our worries away, and one of the worries was the extended incubations using disk plates, that could be either micro disk plates or the Abbott plates, large plates.

We were very happy to see that there was a logging system. Our technicians did write a lot during the day, and it is totally eliminated at this moment. The only thing they do is at the end of the run, they look up what was printed, and they check and set their sign under it. As I will show you later on, we have a minimum of staff at this moment.

[Slide.]

What we wanted to do with the system, and we stated that in our validation protocol, the system should be at least as sensitive as the test we used at that moment. For that, we bought some panels, Pedicheck panel coming from the laboratory of [Nicolady] of the Central Laboratory in Amsterdam, and we can show very easily that all the assays used in our country are at least as sensitive as this agent we used at that moment.

You can see there is no anticore assay, but that is due to the fact that we do not test in our country for anticore.

[Slide.]

Those are the results of our testing. We started on routine in June 1996, and up to now we have tested almost 200,000 samples, and you can see that the repeat reactivity of the assays is very low. We are very happy with it because it means that we have very small number of confirmation tests, and we do not test the confirmation ourselves, it is all done by the Central Laboratory in Amsterdam.

[Slide.]

Well, this was the situation up to June of this year, and because like other countries in Europe, our country is reorganizing, that means that our blood bank grew twice as big. That means that up to June of this year, we had one PRISM and one technician, and after June we had two PRISMs and two technicians, and the second technician is added, not because of the second PRISM, but of the enormous load for syphilis testing, ABD testing, and all the other things done. So, I think that two PRISMs can be operated by one technician.

[Slide.]

A lot has changed in our laboratory. Introducing the PRISM meant for us that because we decreased the number of staff, we started to do our testing during night hours, and that meant for our blood bank that when early in the

morning, the staff from our Blood Components Department came in, the splits in [counting] products already could be made, and that meant that the primary production in this department could immediately be done, and they could make any product out of the whole blood what they wanted.

[Slide.]

I have this last slide for you to show in time, this is 500 controls started from June 1996. You can see that the bands in measurements is very narrow, and you can see that the two [subchannels] in the assay nicely cover each other.

Thank you very much.

DR. HOLLINGER: The next speaker is Brian Gibbs from the Blood Transfusion Service in South Africa.

Brian Gibbs

MR. GIBBS: Good afternoon, everyone.

[Slide.]

The South African Transfusion Services are controlled by volunteer donors through an elected board, and the services operate as nonprofit companies.

[Slide.]

Our service collects about 135,000 donations each year from a large geographic area, and all of those donations are transferred to Cape Town for processing. Obviously, then, we are in a situation when an instrument

such as PRISM is of significant value.

[Slide.]

The instrument arrived in November 1995. We carried out extensive evaluations that included seroconversion panels, as well as parallel testing of donations, and those seroconversion panel results are in the handout that I have handed to the committee.

[Slide.]

We made a decision to adopt the instrument in 1996. We went live in June, and since that time, on one occasion, we have resorted to using the Commander system. I should add that we resorted to using the Commander because of problems with our computer software, not due to problems with the PRISM instrument or with the PRISM software.

[Slide.]

Our conclusions after two years regarding the software are that it is very user-friendly, it works very well. We have developed a reciprocal backup with another service in the country who also have a PRISM. You can imagine being at the southern tip of Africa, it is very hard sometimes to get backups.

The slides I am going to show you now will show the results of the two years of operation.

[Slide.]

First, perhaps that just demonstrates the extent

of the problem that we are faced with in that part of the world. You can see there are huge increases over the last 12 years or so in the positivity, and perhaps even more frightening, the last four years, a significant increase in the rate of positivity amongst repeat donors.

[Slide.]

Hepatitis B statistics. We don't really have any comment. We find it an excellent test. There is a peak there which was due to some contamination in the system, not in the PRISM system, probably in the tubes. We are very, very happy with the reproducibility and the way the system works.

[Slide.]

HCV, always a problem in our hands mainly due to high levels of initial reactives. We believe that some of the local African viruses may share some of the proteins with the HCV, and consequently, we might be picking up some forced reactives for those sort of reasons.

[Slide.]

HIV, well, we had a problem with tubes, which you can see there, but the confirmed reactive rate, if you exclude those few peaks towards the end, mirrors very nicely the actual repeat reactive rate.

[Slide.]

So, our general comments. Once a test sequence

starts, interference invalidates the results, because the control measures built into the system are violated. We really like that feature, and I think everybody else you have heard speak here today has echoed that feeling.

[Slide.]

We find that the system diminishes operator error, it improves productivity. By making the people less likely to make errors, it increases their confidence, and we believe that these factors all combine to improve the efficiency of the system.

[Slide.]

Really, simply to show you all, this is the sort of response I got from the staff, and there are four of them involved, and they don't all work all day, every day, in fact, two of them would operate the machine on any one day.

A score out of 10 for how they felt about the various aspects of the system, you can see that they really like it, and it has taken away the opportunities for them to make mistakes.

Thank you very much indeed.

DR. HOLLINGER: Thank you.

The next speaker is from Organon-Teknika, Tom Clemons.

[No response.]

DR. HOLLINGER: The last speaker we have listed

for the open public hearing is Kay Gregory from the American Association of Blood Banks.

Kay Gregory

MS. GREGORY: Thank you. I am pleased to be here to speak on behalf of the AABB, which as you know is a professional society for 2,200 institutional members including the American Red Cross and community blood centers and hospital blood banks, as well as 8,500 individual members.

Our highest priority is the safety of the blood supply. We have no personal experience to tell you about dealing with the PRISM blood screening instrument, but the AABB supports the application of cGMPs in blood screening and the FDA's efforts to regulate using cGMPs.

Any technology advance that can be demonstrated to automate and enhance compliance with cGMPs is of value and is supported by the AABB, and we urge the FDA to consider this aspect when reviewing applications involving new technologies, such as the PRISM.

Thank you.

DR. HOLLINGER: Thank you, Kay.

Is there anyone else that did not get on the list? I think that is everybody in the room now, but just in case. If not, we are going to take a half-hour break until 4:30. We will reconvene at 4:30 for the open committee discussion,

presentation of the questions to the committee, and discussion.

Thank you.

[Recess.]

Committee Discussion and Recommendations

DR. HOLLINGER: If we could have the questions presented or the questions presented to the committee, who has the questions?

MS. CLAGGETT: We have two questions for the committee. Does the committee agree that based on the information reviewed to date and presented, PRISM technology can be considered by the FDA for use in viral marker testing of donor blood?

The other question is, on the basis of information reviewed to date and presented, does the committee note any additional concerns that need to be addressed before licensure?

DR. HOLLINGER: With that in mind, I would like to open this up for discussion among the committee members.

Yes, Norig.

DR. ELLISON: Firstly, the questions are sort of backwards because if the answer to the second is yes, then, you don't even have to answer No. 1, but all the information that I heard presented was overwhelmingly positive. The only possible negative is that it might create unemployment

among blood bank technicians, but I think that automation is a great way to go forward for removing the human variability in processing blood.

DR. HOLLINGER: Yes, Katherine.

MS. KNOWLES: I was wondering, I mean it really sounded very wonderful, but was there anything that people, the people that were in the clinical trials, was there one there one thing, just one thing that was a problem?

MS. CAGLIOTI: We expected to find some things that we didn't like about it, we thought it would be down a lot. We did not find one thing that we didn't like about it.

MS. KNOWLES : Good.

DR. HOLLINGER: You had the machine how long?

MS. CAGLIOTI: We had it a total of about seven months. We still have it today.

DR. HOLLINGER: Dr. Verter.

DR. VERTER: Actually, all I would like to do is expand the question that was asked and ask the Abbott people, the implication or the data you presented were that there were about 88 machines or people using this for somewhere around two or more years. You have asked a number of those to come here, less than 10 percent of the users.

Do you have any data on any adverse experiences with the machine?

DR. HOLLINGER: Jim, I mean that would be an important question for everyone here. Obviously, people are going to call you and ask you questions about problems that are coming up with the machine, and so on, and when you have, like they have there, when they have four machines, if you have 143 machines, I think you said initially, and four go out, big deal. If you have got four machines, and one or two go out, that is a real big deal if it is not working and in terms of backups, and how quickly can you get backups to companies and things like that.

DR. STEWART: Let me treat that in two parts. First of all, we have pretty much annually, since PRISM was introduced, had rest-of-world user meetings where we have gotten input from customers and current PRISM users on what they like about the instrument and the things that they want to see improved.

I would characterize that the major feedback that we have gotten has really been in the vein of how can you take an instrument that they believe is solid and make it better. An example of that, for example, I mentioned in development was an HIV-I p24 antigen test. Clearly, antigen testing is a very important part of the U.S. blood testing scenario. They would like to see the PRISM instrument have these assays added, and this is the type of thing that gives us the input to know for future development efforts exactly

what we need to do.

Another example of that would be as you get multiple PRISM instruments within a laboratory, can they have the ability to communicate with one another, and if you have a retest sample on one PRISM, another PRISM know that that retest is being managed appropriately, so, we have viewed these in the vein of improvements and ways we can make the product better over time.

With respect to the chairman's question regarding reliability and the feedback we have gotten there, I would guess basically say that as the program director over two years, a lot of that type of feedback from rest-of-world can or would come back through in terms of a laboratory's ability or inability to get test results out.

One of the metrics we track very carefully is something that we call tests completed, that is, the ability of the user to put a test on the PRISM instrument and know that they will get a result on the other side, and to benchmark that metric versus what current tests do today.

Basically, what our data tell us is that the PRISM instrument is delivering the same level of test completed results or slightly better than what the combined test of records are doing.

So, issues that have resolved, and with any piece of equipment you can have issues of pumps malfunctioning and

the like, for the large part are being handled by the laboratories themselves, they take on a large amount of the responsibility for doing preventive maintenance, as well as interfacing then with the Abbott service staff, so there has been no single issue or major issue that has dominated our intention in terms of instrument reliability. Maybe the clinical sites would care to comment on that, as well.

DR. HOLLINGER: Yes, Dr. McCurdy.

DR. McCURDY: I am not sure that the question was really completely answered. I would phrase it that there is nothing better than automated piece of equipment that works, and there is nothing worse than one that doesn't, and the only thing I saw that even dealt with that -- I may have missed something -- but the users in South Africa had a note on service which they rated very highly, but that didn't tell us the frequency of service required, and so forth.

I have had experience some years ago as being the only one in the country who had trouble with a particular instrument, only to find out that a number of my friends had also had similar trouble, so I think reliability is an important issue that needs to be well addressed.

DR. STEWART: It is a very important issue and an excellent question. One of the things I mentioned in my presentation that the PRISM offers is the sixth channel which has the capability of running any of the assays and is

capable to be used as a backup. So, in the event that a particular assay channel has a problem, the laboratory has an immediate opportunity to go to a backup channel for completing their testing, so they are not stopped immediately from proceeding. In that respect, I think that helps with those types of issues.

Overall, when we look at reliability, on average, for a piece of equipment that is this complex, we average less than one service call to an account per month, and that type of data has been very good for us to receive in showing the high level of reliability that the instrument has had so far.

So, again, my fundamental answer would be that the users have been able to work through any issues that they have had instrument problems that have come up as they have gone through, sometimes with assistance of the Abbott service staff.

DR. MOLIJN: Maybe I can comment on this issue. As we started in 1996, we had a big concern what happens when the complete PRISM stops, and it is a disaster. One of the blood banks had an idea to keep a complete Commander line and waiting until the PRISM would break down.

We thought that was not the solution because before you start this whole thing, you clean it, you prime it, you validate it, it's already repaired, so what we did,

we looked for another blood bank with a PRISM backup side, and that was Leige in Belgium. Dr. Sontag, he was very cooperative and we validated with all our tubes going to Belgium, putting it there on the PRISM, testing it, and send it on-line over to our blood bank system.

That was in July 1996 we agreed to do that. We validated it, and we never needed it up to now.

DR. HOLLINGER: While you are still there, let me just ask you a question. I mentioned this once before to you, but in one of your sets of slides you showed, the anti-HCV really did not look very good, and yet the IMX did very well, which has the similar microparticles, monoclonal antibody, and so on, and this is always an issue.

If you have, though, if you are set into one company where you are having a whole bunch of different assays and one of them is not doing very well, either because of false positives or a variety of other things, you are sort of stuck. You can't go out and get another manufacturer's product to use in place of, say, all the others.

Would you deal with that a little bit, particularly with the anti-HCV, and then perhaps Jim or somebody else can respond about the other issues I just mentioned?

DR. MOLIJN: When we started, we thought, well,

how to deal with this problem, how do we see whether this is sensitive enough or not. At that time, we had no sensitivity panels, and seroconversion panels, you need them really to see how sensitive the test is.

So, what we did again, we went to Frau Sontag and we asked her whether she did it or not, and she had an enormous amount of seroconversion panels, and it showed that the HCV was equal on either Abbott's test in seroconversion. There was even one seroconversion panel that was one step better than the others.

So, then, we started with the Pedicheck and we saw that it was almost the same as the ELISA in use at that time. What we see now is we got once in the three months a sample from the National Office, and we have to test it, and it is a sample just above the cutoff, and we never, ever tested negative, and compared with other companies and the ELISA tests, it could differ from time to time very much, and that is why we just went with the system.

DR. STEWART: I am sorry. Could you indicate the second part of the question again?

DR. HOLLINGER: The issue is if one assay, say, has more false positives than, say, some other assay that is out on the market, you pretty much have to sit within all the assays that Abbott has. I mean that would be correct obviously, in the system, whereas, right now if I wanted to

use one assay, but then use a series of others for testing, I could do that, I could choose.

DR. STEWART: Clearly, our goal in designing the PRISM instrument was to have a complete menu of assays that, in terms of sensitivity and specificity performance, match or exceed the test of record. As you saw in the specificity data, some represent a significant improvement, some were comparable.

The choice that the laboratories face in that situation is in terms of the cumulative repeat reactives that they get across a system in that standpoint, and, of course, in the beginning, with PRISM as the assay menu will be limited the first couple of tests, there will be multiple technologies within the laboratories remaining as not all of the markers that have to be tested would be approved on PRISM initially.

DR. HOLLINGER: Dr. Tuazon.

DR. TUAZON: How do you envision the use of implementation of this instrument? Are you just thinking that the reference laboratories or blood banks will be using this, and how much does the instrument cost, the equipment?

DR. STEWART: In terms of size, from a design standpoint, PRISM was in general designed for laboratories that were at or above about 50,000 donations per year. We have laboratories that are smaller than that, that use

PRISM, use it effectively, but in terms of the design concept and the amount of throughput and the efficiency you would get from kits, it was with that idea in mind.

So blood bank laboratories or reference laboratories that would be at or above that threshold could very effectively use the PRISM instrument. At this point because the pricing for the PRISM is not established in the U.S., I will defer any discussion of that particular topic.

DR. HOLLINGER: Dr. Holmberg.

DR. HOLMBERG: Dr. Stewart, in your presentation on page 30, you talk about the failed run analysis for the U.S. clinical sites, but again going back to that reliability question, how many failed runs can we attribute to the PRISM?

DR. STEWART: Excellent question. During that same time frame, we actually evaluated the PRISM performance comparably, and if I may, I think we have a foil of that and answer that directly.

DR. HOLMBERG: I have another question, too, that maybe the gentleman from the Netherlands can answer. In your evaluation from your technicians, I noticed that there were I think two or three 7's up on the top. I think the first reliability one was a 7. Now, that is evaluation from your technical staff.

DR. MOLIJN: That is my colleague from South

Africa.

DR. HOLMBERG: I am sorry.

DR. MOLIJN: Almost in the neighborhood.

[Laughter.]

MR. GIBBS: I actually find it quite hard to comment on this. I could have a look at the slide. I don't suppose we could find it.

DR. KAGAN: I was wondering if any of the supportive blood banking operations do testing for OPOs for transplant cases where results are required on a STAT basis. I know in Cincinnati that we have actually had to go to outside laboratories because they like to batch everything and it is very difficult to get tests run for tissue donors or organ donors when you are pretty much down to just a few minutes time or a few hours time.

I am wondering whether or not any of you have any experience with this and whether or not it is easy to just pop in a sample and run it immediately or whether there is added expense and whether that is reasonable or not.

DR. MOLIJN: We normally do not test for organ transplants, no, but in our cancer hospital in Rotterdam, they ask for the testing in other laboratories within their university, and I know for sure that I do it around about 10 to 20 times a year that he calls me and asks me to test for him because I am very fast. When your equipment is running,

you tell it you have a STAT sample, you put it in, and with in an hour you have gotten a result.

DR. KAGAN: Does anybody else have the experience in the States?

MR. GIBBS: If I may comment on those two 7's, our problem as I mentioned during my presentation is that the software within our blood center gives us major problems, and these results were given by the staff when they were asked to comment on the overall reliability of the system and the overall consistency of the system.

That would include the relationship between the PRISM and our in-house computer, which in fact leaves a lot to be desired and which is continually being upgraded. If you look at the other marks on the other scores, you will see that most of those scores relate specifically to PRISM, and there are no outside influences, so those two relate to the problem issues that we have been identifying with our in-house computer.

DR. HOLLINGER: Mr. Dubin.

MR. DUBIN: Most of the discussion we have heard is about the blood banking side of the equation. I am not sure anyone is prepared to answer this, but do you see this in the commercial plasma end of things, have you had requests there, are you working in that area, as well?

DR. STEWART: One of our clinical sites was a U.S.

plasmapheresis center, and we actively see PRISM as being an instrumentation or technology that would be utilized in that industry.

DR. HOLLINGER: Dr. Nelson.

DR. NELSON: As you know, the agents that can be transmitted by transfusion geographically vary somewhat, and I wondered in areas, for instance, where Chagas disease was important, could add that or in areas where HTLV-I and II are not a problem, could you subtract, how flexible is it to adding new antigens or not using the whole panel?

DR. STEWART: In terms of new markers, clearly, we observe with great interest those markers that appear to be future candidates as blood screening markers, and Chagas is, in fact, a marker although I didn't indicate it on my slide, that is funded for development as far as PRISM assay. Abbott has other technologies that screen for Chagas, but Chagas is funded for implementation into PRISM.

In terms of the flexibility of being able to dissect, if you will, certain of the markers, PRISM doesn't give us that immediate capability. The HTLV-I/HTLV-II test, in fact, is formatted to screen for both markers. However, we have had the experience in certain areas of the world, taking Japan as an example, that there are unique markers there that they were interested in with PRISM. Surface antibody is obviously screened for there, and we developed a

specific procedure for surface antibody that is used only in Japan.

So, clearly depending upon the need of an area of the world, or if new variants or viruses came up, this is an important part of how we look at working with PRISM in the future.

DR. HOLLINGER: Again, Jim, I want to be sure. Right now it can only do an X number of assays. You can't add additional assays to it the way it is configured now, is that correct?

DR. STEWART: You can run on a single PRISM instrument, since there are six channels, you can run up to six different assays, and if you had a second PRISM instrument you could be running different markers on those. The critical piece is that any single channel, once it is dedicated to that assay, is for that assay.

So, if in the future we had eight or nine different virological markers, they may be split across different PRISM instruments, but you would be able to run on dedicated channels doing that.

DR. HOLLINGER: Yes.

MR. DUBIN: Could you run two of them in series?

DR. STEWART: Could you be more specific on that?

MR. DUBIN: For instance -- and I am coming from a sound background, so understand that I am looking at this

more like I would look at a studio -- if you had a whole series of markers, would you want to just run different machines or could you actually tie two of them together?

MS. KNOWLES: Like a network?

MR. DUBIN: Yes. Can you network them?

DR. STEWART: One of the improvements that I mentioned at the start was the idea of being able to have multiple PRISMs communicate to one another what test results had been done, and that is a type of improvement that we are looking at for the future for users, and particularly in the U.S., that is an important element because of the number of multi-PRISM sites that are more likely to be here.

MR. DUBIN: So, you guys are working on being able to network them?

DR. STEWART: Being able to link them together, yes.

MR. DUBIN: Yes, that is what I meant.

DR. HOLLINGER: Dr. Koerper.

DR. KOERPER: I assume that you have taken into account the Y2K problems, and we don't have to discuss this, but what I would like to know is does this machine interface readily with various software systems that are already in place in the various blood banks, or are the blood banks going to have to rewrite software or buy new computer systems, has that been a problem?

DR. STEWART: Obviously, given the range of systems that are available in the marketplace, it is something that we, as we went through our launch of PRISM outside the United States, had to work specifically with laboratories to understand what their unique needs were.

Even if there was a sort of common platform within a laboratory that they were using, their particular parameters might be unique, and it is part of the implementation process that we would go through in going in and checking out a laboratory in a facility for what they would need to support PRISM in terms of size, electricity, temperature, and everything.

A key part of that is the host interface issue and the communication of data from the PRISM.

DR. OGAMDI: The gentleman from South Africa indicated some outliers, possible cross-contamination or something. Have you done anything to resolve that, we know what that was?

DR. STEWART: I think the reference was specifically to anti-HCV samples, and at this point the exact reason behind the rates that they are seeing is unknown. It is one possibility that they are looking at that it could be related to something that gives a cross-reactivity in the test, but that requires further investigation.

DR. OGAMDI: Is anyone doing anything about it now?

MR. GIBBS: The universities in our area are working on those problem cases, and we are continuing referring such problem cases to the Abbott organization, but we believe it is related to the distribution of perhaps normal viruses within the population which is very different to the distribution of various viruses in the American population.

We have seen in the initial report back in 1996, i believe. There was some similarities between some of the initial things that we were seeing and some of the initial findings from the Spanish group, but as far as pinpointing exactly what they are, no, we have not done that.

What we have shown is that the specificity of the system in repeat testing mode with our population is not as high as it is in other populations.

DR. HOLLINGER: Jim, can you tell me, how is the calibration of the pipettes done? You showed some good data about how it detects if a clot comes in the system or something blocks it or there is too much flow going out, and other things with dispensing and washing, and so forth, but the calibration of the pipettes themselves to deliver X amount, 200 microliters, whatever, how is that done?

DR. STEWART: Well, that functions at a number of

different levels. One is in the assembly of the instrument or in the subassemblies demonstrating the precision at which those various pumps and/or syringes are calibrated to, so that is the first step in knowing what they are capable of delivering.

And then on an ongoing basis, in addition to the sensors that exist on the instrument, there is an independent external tool that can be used to independently validate what the volume dispensing is on the instrument as it goes through. That is part of the GMP aspect of bringing it forward separate from the instrument checks itself, an external tool that can be used. The same type of thing exists for temperature and the like, means to independently validate the dispense volume.

DR. HOLLINGER: You also mentioned, and I was impressed with the detection of the glycine to arginine substitutions on the mutants, which was interesting, but just as a matter of fact, though, were all of these anti-HBC positive anyway?

DR. STEWART: I am sorry, say that again.

DR. HOLLINGER: Were all of these anti-HBC positive?

DR. STEWART: In the case of the serological samples that they originated from, I am not sure.

DR. HOLLINGER: I would assume they would have all

been picked up anyway by anti-H, but my point is that they would all have been picked up by anti-HBC anyway as part of the screening procedure.

DR. STEWART: This was more looking as you saw from an analytical standpoint of what is the detectability or limits of detectability.

DR. HOLLINGER: But it would resolve a lot of some of the anti-HBC solitary responses that you sometimes see maybe.

DR. STEWART: Coming back, if I could, just for a moment to Dr. Holmberg's question, there is a foil I think on the projector, if you could turn it on.

[Slide.]

This is the same format as what I showed in the presentation, but it is looking at it from the other side. These are the PRISM assays that were being run and integrated. If you recall from the data on test of record, it was an overall run failure percentage of 4.5 percent.

During that same time frame, there were a total of 490 PRISM runs, that are nine-tailed runs, for a total of 1.8 percent, so we have seen overall a reduction in failed runs, which also translates into a reduction in failed tests or an increase in the tests completed rate that you get.

So, despite the fact that PRISM has a large number of these controls and specific types of statuses or error

conditions that it can identify, the overall throughput of samples is remaining the same or maybe even slightly improving as we move for NC screening on PRISM.

DR. HOLMBERG: So, when you say failed run, is that one tray of samples or is it just one individual donor?

DR. STEWART: A failed run in this case would be defined as an entire run failing. Can you put the second foil up there? The detail behind those nine will give you a sense of exactly how that happened.

I mentioned some of the errors that can occur on PRISM that essentially stopped an entire run, and this will give you an idea. These are the nine occurrences. Now, there was one case where the calibrator was not successful because bubbles were detected in the pipetting. Since it could not adequately calibrate the entire run, it shut down.

There was a shroud failure. A shroud refers to the device that is used to create the light seal that you can then read the chemiluminescent result from. There was one case of that. When the instrument detected that error, it stopped the run entirely.

Calibration failures are probably a more common mode that you would find, if something happens with calibrators in the run or a run control, which was that end of batch release control, those accounted for the other seven instances.

DR. HOLMBERG: One other question in regards to Sacramento. I noticed on your sheet there it gave statistics for all six parameters. Are you using it for all of your viral marker testing?

MR. KURAMOTO: That was for the clinical trials, and during the initial clinical trials we did one assay at a time and then at the very end, we performed five assays at a time, and there are six assays. They want to evaluate or compare one with reductant versus one without reductant, and Jim Stewart could probably tell you more about that.

So, really, it is five assays.

DR. HOLLINGER: Dr. Boyle.

DR. BOYLE: I want to be sure if I understand something. I believe you said that at the end of each run, there is a test against a positive sample to make sure that you are picking up positive samples. My question is, is that positive sample across all the assays, or does it only represent one?

DR. STEWART: It is a multi-constituent control, so it is pipetted by the system across all the assays, and for the five primary markers that I described it has HIV-I, HTLV-I, anti-HBC, HCV surface antigen all titered in there at a low level. It is not a master lot of kit component, it is provided separately, so it functions much in the way that a run control, independent run control would function, but

it has a special purpose here in that the system looks for it, it must be there, it must be positive or the entire batch of data is not released. No results are there for the technician to interpret.

DR. OHENE-FREMPONG: Just a comment. I believe I have seen in the literature something about its own backup system.

DR. STEWART: Yes, I didn't allude to that on the slide, but there is an ininterruptable power supply that is provided with the instrument. This is for those occurrences if the laboratory should experience a brownout or a blackout condition, it can provide power to keep the instrument running for up to 10 minutes, which based on the data that we had seen, greater than 99 percent of the occurrence where power is interrupted, it is for that short a time period, so that is standard part of the PRISM.

DR. HOLLINGER: And it has a refrigerated component, is that correct, for the reagents?

DR. STEWART: Yes, the biological reagents are stored in an on-board refrigerator, and that is where the bar codes for the bottles are matched up to the fluidics lines, and they are maintained in there, so you don't have to pull the reagents out until it is time to replenish a kit.

DR. HOLLINGER: But in case of what was just

mentioned, they could take them out and put them in a refrigerator, it is not something that is locked in that they can't remove?

DR. STEWART: No, if it was going to be a condition that the power was going to be down for a longer period of time and the reagents needed to be removed, they could be removed. When they put back on the PRISM, the PRISM will remember how many test reagents were left and pick up essentially where it left off. In fact, many laboratories will do this if they have a 5,000 test kit and test 4,500 or 4,800, they may pull that test kit out for just doing retests, you know, at some point, but the number of tests is part of what is managed by the system.

DR. HOLMBERG: How do you detect the end of the bottle, is it a pressure, a light source?

DR. STEWART: In terms of pipetting?

DR. HOLMBERG: No, all the reagents that are stored in the refrigerated section.

DR. STEWART: Actually, that is a test counting mechanism, so the PRISM keeps track of the tests as they are processed and factors in for when primes are purchased or being done and extra fluid is being utilized.

DR. HOLLINGER: Dr. Hamilton.

DR. HAMILTON: I have a couple of comments and questions that probably are best addressed by you, Dr.

Stewart, if I may.

They revolve around the handling of the specimens or the nonhandling, I think as you like to phrase it, such that there is a minimum level of contact of the technician with the specimen itself, thus reducing the option for error, but if we take this maybe from the start here, this specimen gets collected somewhere, and this specimen gets collected in some kind of tube that then arrives at your facility wherever that might be for testing.

Is that tube the one from which the pipetting is done, or does that tube then have to be re-entered in some kind of log, some kind of something, thereby not failing to avoid what we found in a survey done some years ago now that the major reason for a false positive test for hepatitis surface antigen was testing the wrong blood. It simply was an error in documentation.

So, I have always wondered why those tubes can't be the same thing, but they are not, are they, in your case? Blood comes in a standard vacutainer.

DR. STEWART: In most cases, they should be the same, a primary collection tube is what would be loaded onto the instrument directly with an appropriate bar code. Clearly, there are handling issues from the time a tube is collected until it is brought into the laboratory and whatever bar code label is assigned on it.

PRISM obviously is designed from the standpoint once that tube is entered with a particular bar code to control the process from there, that that tube and that bar code, however they are matched up, then are carried through the process.

DR. HAMILTON: So, the needle goes in the arm, the blood comes out the tube into the container that goes in that machine.

DR. STEWART: Typically, that is correct, yes.

DR. KATZ: Those systems are all developed in essentially all blood centers now to maintain positive I.D. throughout the process. The donor history card, which documents answers to questions, the pilot tubes are contained, those samples that will go directly into the machine, and the blood bag containing the transfusible components all have unique bar code identifiers that allow positive I.D. through the entire system.

DR. HAMILTON: So, you are seeing my perspective here. I don't work in a blood bank, I work in a hospital. In the hospital you get a glass tube and a vacutainer, and you have to do something with it, and between there and the test, there is all kinds of opportunity for error, and if this kind of thing were applied in a hospital that does, let's say, 50,000 tests per year, it would be very helpful if that potential lesion could be corrected. I don't know

if it can be, but it would be useful, I think.

DR. STRAMER: The analogy that you just made to collection and the complexity from collection to the initiation of testing is what we all face in the blood centers during testing.

The whole process of testing including all the documentation that all the proper reviews were performed, the testing results that are transmitted to release the blood components are all done properly, those include multiple review steps and today, in any blood testing environment, there are more manual review steps and more manual documentation, in fact, than actual testing.

I would also like to remind the committee that yesterday we had a workshop on HCV nucleic acid testing that the blood centers will be forced, if you will, to initiate in the beginning of 1999, and that testing in its first phases will be very, very complex.

So, I think the issues revolving around lack of employment for blood center staff will easily be compensated by the reproduction of pooled testing and testing for RNA for HCV and HIV. So, the complexity that we will introduce into that system, it would be nice if we could include the improved cGMP features, process control that automated systems afford to us, so we can concentrate on doing things that improve the safety of the blood supply rather than

redundant reviews, manually making errors on hand entry of records, that then cause recalls and errors.

So, just again, the primary focus of the system is the improved process control.

DR. HAMILTON: Could I just carry on with that? Did you have a related question? I just want to carry on this theme, if I can.

So, we now have vials that are definitely from so-and-so, and they are marching down the line, and it is the original specimen, and there is no other specimen, is that also correct, or somebody says there is three other specimens.

DR. STRAMER: We have three tubes currently. They go to different areas in the blood center for different testing. In fact, when we initiated the RNA testing I referenced earlier, we will have yet a fourth tube, and each tube will go to a dedicated area currently, and even in the future, when automated assays come out. So, each tube has its destined location of where to flow in the blood center.

DR. HOLLINGER: They have a bar code on those, each tube has the exact same --

DR. STRAMER: Yes.

DR. HAMILTON: At the end of the line it gets dumped someplace, it goes into a waste container.

DR. STRAMER: The tubes.

DR. HAMILTON: The tubes. So, my concern was it has gone down the line, a test has been done, you are going to get a report that it's invalid, and the test is in the wastebasket, the test sample is in the wastebasket.

DR. STRAMER: No, we retain the tubes for a given period of time, and we do that currently, so that processes probably wouldn't change. We would just have less documentation to perform, but all the tubes would be retained, guaranteeing validity of test results and issuance of components, retrospective reviews, so the tubes would be there for a certain period of time if, God forbid, retesting needed to occur.

DR. HAMILTON: My final point and then I will shut up here. So, you didn't emphasize it, but I think it is not irrelevant that there are additional benefits for having the technician separated from this specimen in the form of that technician's health. It is not nearly as prevalent these days, but in times past, one of the major risk groups to acquire any of these diseases in the hospital were laboratory technicians, and the less they have to handle specimens, the better. I would recommend you put that somewhere on your prospectus as an advantage.

DR. OGAMDI: Just a little question for the small laboratories, medium laboratory. Have you done any evaluation on the reagent shelf life?

DR. STEWART: Reagent shelf life or stability is a fundamental part of the data that we submit in our regulatory submissions including in the U.S. Are you referring to reagent shelf life more so within the laboratory?

DR. OGAMDI: That is, how stable it would be if it is not running 50,000 tests, how stable will it be for small laboratories to continue to run?

DR. STEWART: As long as the kit reagents are utilized within the dating period assigned and the PRISM verifies that, then, the results identified from those are accepted regardless of whether they were a high-volume site, low-volume site, or the rate at which the tests in the kit are being utilized.

In either circumstance, if they exceed the expiration date, then, the instrument would not allow you to proceed.

DR. HOLLINGER: I think we will go ahead and look at the question and vote on the question.

The question posed by the FDA is does the committee agree that based on the information reviewed to date and presented, PRISM technology can be considered by the FDA for use in viral marker testing of donor blood?

All those that agree with that statement, raise your hand.

[Show of hands.]

DR. HOLLINGER: All those opposed?

[No response.]

DR. HOLLINGER: And we will ask the industry representative, I saw you put your hand up anyway, but we will ask the industry representative and consumer representative.

DR. BUCHHOLZ: Yes.

MS. KNOWLES: Yes.

DR. SMALLWOOD: Results of voting, there are 16 voting members here. There were 16 yes votes. There were no no votes, no abstentions. Both the consumer and industry representatives agreed with the yes votes.

DR. HOLLINGER: Thank you. I think the second part of that question has been answered, and I think it was discussed. I don't think there needs to be a vote on it. Is that correct, Jay? Okay.

I think this completes the session today. It has been a long day. Tomorrow, the session is on leukocyte reduction. That will start at 8:00 in the morning.

[Whereupon, the proceedings were recessed at 5:15 p.m., to be resumed at 8:00 a.m., Friday, September 18, 1998.]

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CERTIFICATE

I, **ALICE TOIGO,** the Official Court Reporter for Miller Reporting Company, Inc., hereby certify that I recorded the foregoing proceedings; that the proceedings have been reduced to typewriting by me, or under my direction and that the foregoing transcript is a correct and accurate record of the proceedings to the best of my knowledge, ability and belief.

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