DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

WORKSHOP ON DEVELOPMENT OF DONOR SCREENING ASSAYS FOR WEST NILE VIRUS

Tuesday, November 5, 2002

8:00 a.m.

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P R O C E E D I N G S

DR. NAKHASI: Let's get started. Happy Election Day to you all. We need to get out today quickly because we have Election Day today so we all need to vote, especially in Montgomery County. As you know, it is a very close race so we need to make sure every vote counts.

Anyway, we will try to be on time as we did yesterday. I would like to welcome you all today again. We have a full agenda today which includes starting with the viral inactivation process, pathogen inactivation of target West Nile Virus, then proposed studies on prevalence in donors.

We then will talk about the regulatory issues which a lot of you may have questions about and implementation issues on blood and tissue, and then, finally, a panel discussion. That will be a free-for-all because we need to make a definite outcome of this meeting.

Before I pass on my podium to my esteemed colleague, Dr. Mahmood Farshid, there is a change in the setting here. Joe Wilczek tells me that, around 10:00, when the first break is there, we have to vacate this room

because there is going to be a marriage ceremony going on here. We are all welcome to stay here if you want to, on a lighter side.

But, anyway, seriously, we have to leave this room at 10:30 at the first break, go upstairs one flight. The meeting will be in Cabinet Judiciary Suites. If there are extra people there--I was told that it only holds 225, but I guess yesterday we had 300 people here--there is a room next to it where we can put the spillover and there is also a big screen like this so we can have that, also.

See you then and I guess I will pass it on to Mahmood. Thank you.

V. Pathogen Inactivation Targeted at WNV

Chair: Mahmood Farshid, FDA

Luiz Barbosa, NIH

DR. FARSHID: Thank you, Hira.

My name is Mahmood Farshid. I am with the Division of Hematology in the Office of Blood at CBER, FDA.

The first session for this morning is pathogen inactivation methodologies as applied to West Nile Virus. I and Luiz Barbosa of NIH will be moderating this session. The session is divided into two parts. The first one

applies to the methodologies which are applicable to the labile blood components, and the second part will be the established methodologies currently used in the fractionated plasma-derived product.

We have a packed schedule and without further ado, it is my pleasure to introduce our first speaker, Dr. Steve Wagner, who is the Director of Cell Therapy and Blood Cell Therapy Development at American Red Cross, Holland Laboratory.

Steve.

Viral Inactivation Methods in Blood Components

Overview of Different Methodologies

DR. WAGNER: I would like to thank the organizers very much for inviting me to the meeting. I would also like to say that I heard that yesterday's meeting was very good. More than a half-dozen people mentioned that they didn't see me yesterday. I was hard at work at the office.

I am going to talk today about different challenges and a broad overview for inactivation or pathogen reduction of West Nile Virus.

[Slide.]

There is a number of rationale for inactivation. The first rationale is to deal with residual infectivity that might be in the blood supply as a result of screening tests that are already in place.

In addition, we all know that pooled products transmit a threat of infection if any member of the pool were to be contaminated with virus. Pathogen reduction also constitutes an additional layer of safety in addition to donor questioning and screening with respect to West Nile Virus.

West Nile Virus is currently acknowledged, it has been around for a number of years, since the '50s, it has been described, but currently, there is no test that is available in a licensed form for blood centers to use, and I understand that you heard yesterday about some potential development of tests that are currently underway.

In addition, pathogen reduction might be able to deal with variant agents, HCV, HIV, a number of the viruses that have the capacity to mutate to other viruses at a frequency that might be a bit higher than some of the DNA viruses, particularly appropriate when discussing variant agents, and then there might be new agents that come about.

Of course, the public and the folks in Congress are very concerned about the safety of the blood supply, and any risk is of great concern.

[Slide.]

So, West Nile Virus, you have probably heard this, is a flavivirus. It is an enveloped, single stranded, positive stranded virus. Some very early studies in the 1950s in infected patients indicated that these patients, when samples of their blood were taken, could be diluted between 100-fold and 100,000-fold and inoculated in the susceptible animal in a bioassay, so titers are considerable although they are certainly not more than 10^6.

The CDC has estimated that levels in asymptomatic donors may be between 10^3 and 10^4 genome equivalents. With that said, however, the relationship between the genome equivalents and the plaque-forming units hasn't been completely characterized yet and I imagine a lot of this work will go on in the next few months as we get more information about West Nile Virus.

So, the actual log reduction right now necessary to prevent transmission from the blood of asymptomatic

donors is really not known at this time, and this is some very important information that needs to be collected.

[Slide.]

With respect to taxonomy, West Nile Virus is related in many ways to other viruses that have been used for pathogen reduction experiments. We have used Sindbis in our laboratory with some experiments a number of years ago. Most of the companies and we now have looked at BVDV, however, perhaps hepatitis C is a more closely related virus than even BVDV.

Of course, West Nile Virus itself is the best model, if you will, or the best virus to test for its inactivation, and this is somewhat hampered by the fact that it is classified as a BL3 virus at this point, and so that is going to limit the availability of different laboratories for working on the virus.

[Slide.]

The good news is that both flavivirus and togaviruses should be susceptible to pathogen inactivation agents. Just looking at some classic books by Block indicates that it is susceptible by UV light, gamma irradiation, a number of disinfectants including

glutaraldehyde, hydrogen peroxide, chlorine-containing compounds, bleach, for example, and alcohol, as well as iodine, so it is a very susceptible virus.

[Slide.]

There is a number of approaches to inactivation. Almost all of these are led by company approaches. For platelets, there are psoralens S-59, also called now amotosalen. There is a group working with riboflavin and red cells. There are some alkylating agents. One is FRALE, which is called S-303, and then there is another agent called INACTINE.

I am not going to be really going over plasma today, but plasma, as you know, dealt with solvent detergent, and there are other approaches, phenothiazine dyes, as well as a psoralen that is being investigated for plasma.

[Slide.]

S59 is a planar molecule, psoralen or a fucocumarin. Its status is that it has completed Phase III clinical trials in the U.S. and Europe. It is licensed in Europe with a CE mark. The buffy coat method has been

licensed in Canada, and the plasma work is in Phase III trials in the United States.

[Slide.]

Psoralen is a photochemical. The first step is for the drug to intercalate between the bases of double stranded regions of DNA and RNA. Even RNA has double stranded regions. Upon the absorption of ultraviolet A light, psoralens make mono- and di-adducts with pyrimidine bases in nucleic acid.

Diadducts and monoadducts can prevent the subsequent nucleic acid replication of the pathogen, and because pathogens contain nucleic acid, and platelets and red cells do not need nucleic acid for their storage and viability, this is an approach that all the companies take.

[Slide.]

There is a number of steps that are used for pathogen reduction for S-59. They use, at least the Phase III trials in the United States and Europe, have looked at apheresis platelets.

S-59 is added to the platelets and then the mixture is transferred to a UV-permeable plastic container where it is illuminated with light, and then after

illumination, the platelets containing S-59 are added to a bag that contains a resin that absorbs a lot of the free S-59. It stays in the resin for several hours before it is transferred to a container for storage.

[Slide.]

I am not going to go over any West Nile Virus data, I presume the companies will do that. I am just going to be talking about the published data on viruses that are close relatives to West Nile.

There has been identification of inactivation of HCV in a chimpanzee model with a platelet suspension treated with S-59, as well as inactivation of bovine viral diarrhea virus in a platelet suspension.

[Slide.]

The other method for inactivating viruses and particular potentially West Nile Virus in platelets is riboflavin, some molecule riboflavin. It is a vitamin. Its status right now is in preclinical.

[Slide.]

It works also by a nucleic acid method. It binds to DNA by intercalation. Upon absorption of either visible

or near UV light, the complex induces guanine oxidation, single strand breaks, and the formation of covalent bonds.

[Slide.]

There are some published data for pathogen reduction of viruses related to West Nile Virus in this respect, bovine viral diarrhea virus of more than 5 logs with riboflavin and light. Some of these data was presented just at the recent AABB conference in Orlando.

For red cells, as I mentioned, there are some alkylating agents that are being used. One of the companies is working on a method using S-303. S-303 has a very similar structure to a compound called quinacrine mustard. These are acridine nitrogen mustard compounds, and one of the major differences between quinacrine mustard and S-303 is S-303 has this ester bond in the middle.

Its status right now is in Phase III in the United States. The mechanism by which FRALES work is that the anchor, which is the acridine moiety of FRALES, intercalates between the bases of double-stranded regions of DNA and RNA, and the nitrogen mustard moiety or the effector of the FRALES makes adducts with nucleic acid bases.

Diadducts, for example, form a cross-link between nucleic acid strands and again, like the psoralens, that prevents subsequent nucleic acid replication.

The ester moiety in FRALES, which I pointed out before in the alkyl region of the compound, is the frangible linker region, and it hydrolyzes forming negatively charged acridine compound that doesn't further interact with nucleic acid, and the rate of reaction of the ester linkage is slower than the nitrogen mustard, and that is how that compound works.

The reactants produced by the FRALE that are free in solution, and not alkylated, can be potentially depleted by a removal device.

[Slide.]

S-303 pathogen reduction, they published on bovine viral diarrhea virus, and they see more than 5.6 logs inactivation.

[Slide.]

The other compound that is being studied in red cells, the company calls INACTINEs. This is a cartoon, a picture of INACTINEs. It has got a 3-cycled ring, which is a covalent modifying group. It has a cationic alkyl tail

to it. The alkyl tail is positively charged, which confers DNA binding to nucleic acid by electrostatic interactions.

It is said to stabilize molecule, and this molecule has a much smaller size than the others, so it can inactivate viruses whose capsid structure proteins are tightly interdigitated, that are somewhat resistant to inactivation by other agents.

The INACTINEs have this azito [?] moiety at the end of the compound. This is an example. I believe the actual compound that is being studied is PEN110, so this is not it. This is ethylamine. So, it has a zerodino [?] moiety at one end, and then it has two or more nitrogens in the compound, separated by hydrocarbons.

[Slide.]

INACTINE reacts with the N7 bond of guanine, producing a monoadduct. This can serve as a stop signal to replication. Also, repair enzymes can recognize this and cause the loss of a base or a basic site, and once this occurs, there is a potential for strand breakage.

[Slide.]

Some work was done where people did typical sequencing of a template, of a normal template, as well as

a template that has been treated with INACTINE, and as you can see here, at high salt concentrations, you can get some replication of the template, but in particular notice that in the C residues of the primer, that there is considerable stops, and this indicates that at the G residues of the template, replication has stopped probably because of adduct formation.

[Slide.]

Their process is adding the compound to red cells with an incubation period. I believe this is at room temperature. Then, the compound is removed by extensive automated washing.

[Slide.]

They have seen more than 6 logs of bovine viral diarrhea virus in red cell units.

[Slide.]

There are a number of challenges for pathogen reduction techniques. First of all, there is potentially a lot of transfers between bags. Every time you transfer a component from one bag to another, there is some loss, so there may be some unwanted reduction in cellular yields.

Although some agents may be specific for nucleic acids, that is not universally true. There are going to be some side reactions that occur. The side reactions may be reactions to lipids with the compounds, reactions to proteins with the compound.

In both of these circumstances, whether or not a particular method has a removal technology, they are not going to be able to remove the compound when it has reacted to lipids. If it has reacted with a cellular protein also, that is not going to be able to be removed, that is going to be transfused to the recipient.

In addition, the photochemicals have the potential, even though they may be adduct-forming, to generate reactive oxygen species which can be harmful to cellular membranes, and that is of a concern, as well.

These side reactions may be responsible for the loss of survival or function of blood components. Some of the loss of survival, for example, have been observed in clinical trials with some of the agents, so these things need special consideration and thought.

[Slide.]

There are some other challenges. The side reactions could be responsible for unwanted low-frequency adverse events. The search of things that might be important to look for, for these low-frequency adverse events might be immunological reactions, they might be allergies, but they could go to anaphylaxis. This could be 1 in 100,000 units, we don't know, but until a method is used extensively, that information won't be available.

In addition, there may be increased sensitivity of blood cells to other pharmaceuticals. For example, if there is some singlet oxygen damage or oxidizing damage, the blood cells may be sensitive to oxidizing drugs.

If a chemical interacts with glutathione, it also might be sensitive to oxidizing drugs. So, these drug-drug interactions may be of importance to patients when enough of them are investigated and more people are treated with these agents.

In some agents, an unexpected, accidental exposure to people who are manufacturing the drugs or transporting the drugs or blood center staff could lead to increased genotoxic risk, and that obviously is of some concern.

[Slide.]

So, in evaluating pathogen reduction methods, it is important to pay attention to the potential for low frequency adverse events, so you really need to implement before you will able to see some of these effects if they are to occur.

Without implementation and long-term study, it might be difficult to predict the risk to blood bank workers or recipients by accidental exposure or by residual drug.

Without implementation and surveillance, it may be difficult to assess a risk of allergic or hypersensitivity or anaphylactic reactions in susceptible recipients caused by alkylations to proteins or by drug metabolites.

Without implementation and long-term surveillance, it may be impossible to determine if the risk of a fatal outcome from an inactivation process is greater than the current risk of fatalities from infectious disease transmission.

So, there has to be a good surveillance system put in place if these methods are introduced, so that these sorts of things can be measured.

[Slide.]

In conclusion, all methods that I have discussed today I believe target nucleic acid. These methods can reduce the infectious titer of extracellular and intracellular enveloped viruses.

All available preliminary information suggests that West Nile Virus should be susceptible to all the mentioned pathogen reduction techniques. It is not known what level of West Nile Virus reduction will be necessary to prevent transmission from asymptomatic donors.

Implementation and surveillance may be required to assess low frequency risks. In the low-frequency risk assessment, it is essential for establishing that fatalities from the pathogen reduction process are less than current fatalities from infectious disease transmission, and non-nucleic acid side reactions, as I call them, may be important to understanding some recipient reactions, as well as to explain any loss of cellular function, recovery, or survival.

Thank you very much.

[Applause.]

DR. FARSHID: We will have a question and answer session at the conclusion of this session. Thank you very much, Steve, for an insightful overview.

Next, we move to industry presentation and we hear from industry representatives, which currently they are working on this technology, and we are promised a adduct-driven presentation relevant to West Nile Virus.

Our next speaker is Lily Lin from Cerus/Baxter.

Industry Representatives

Cerus/Baxter

DR. LIN: I would like to thank the organizer for inviting Baxter and Cerus here today to present the data.

[Slide.]

In my talk, I would like to cover three areas, the first on the Helinx technology developed by a sponsorship between Baxter and Cerus, and secondly, I would like to discuss the inactivation of pathogens in general, and then move to the last part of inactivation of flaviviruses, and I would present data on inactivation of

hepatitis C virus, the bovine viral diarrhea virus, BVDV, and inactivation of the West Nile Virus.

[Slide.]

The Helinx technology is based on the following. As Dr. Wagner already pointed out, that pathogens, leukocytes require nucleic acid for replication, and in contrast, blood components do not require nucleic acid for therapeutic function. So, Helinx technology relies on small chemical compounds that target and modify nucleic acids to prevent replication of viruses, bacteria, protozoa, and leukocytes.

The compounds I am going to talk about today, the first one is amotosalen, also known as S-59, is developed to treat platelet product. The same compound is used to treat plasma product. A second compound was developed to treat red cell products called S-303.

[Slide.]

Both compounds operate under the same mechanism of action. These compounds are represented here. Each of them has two reactive sites, and because of the small size, they intercalate effectively and reversibly into the helico regions of both DNA and RNA. Only when activated, these

compounds will react and form covalent bonds with bases of the nucleic acid.

Amotosalen is activated by a long wavelength ultraviolet light UVA. S-303, on the other hand, is inactivated by a pH shift. After activation, because of the two reactive sites, it forms a permanent cross-link between the two nucleic acid strands, and these cross-linking products effectively prevent a replication of the nucleic acid.

[Slide.]

Now, the systems developed for treatment of platelet concentrates contains two components. The first one is a UVA illuminator that delivers the required dose of UVA, and the second component is an integrated container set that allows the addition of the amotosalen and treatment of the platelet concentrate in a closed system.

[Slide.]

The system developed to treat plasma also uses the same UVA illuminator, the same amotosalen compound, but the integral disposable set is slightly different from what is used with the platelet concentrate.

[Slide.]

The system under development for treatment of red cells will use a series of disposable containers, but all of these systems are designed to treat single unit products, a single unit platelet, plasma, or red cells.

[Slide.]

To validate pathogen inactivation using these systems, we have used full-sized therapeutic units of platelet concentrate, plasma, or red cells. Each unit was spiked with approximately 10^6 infectious unit of a pathogen per ml of product, all with the highest titer stock available.

The contaminated platelet and plasma product were treated with 150 micromolar amatosalen and 3 joules of UVA light. Contaminated red cell units were treated with 200 micromolars of S-303. In many cases, inactivation kinetics were measured.

The infectivity of each pathogen was measured using either culture methods or, in cases that culture methods were not available, we used animal models.

[Slide.]

The results demonstrate that Helinx technology inactivates high levels of a broad spectrum of viruses,

bacteria, leukocytes, and protozoas. Here, I have only summarized a subset of the data.

This table has a list of all the pathogens being tested today in blood centers including HIV-1/2, hepatitis B, hepatitis C viruses, HTLV-I/II, and a Treponema pallidum that causes the syphilis.

The inactivation levels were expressed as log reduction, and the greater than sign demonstrate inactivation to below the level of detection.

[Slide.]

Flaviviruses, as you heard today and yesterday, they are enveloped, single-stranded RNA viruses. Examples include yellow fever, Japanese and St. Louis encephalitis viruses, border disease virus, hog cholera virus, the Dengue fever virus, and Usutu virus.

Those three viruses highlighted here, the hepatitis C and BVDV virus, and West Nile Virus are flaviviruses, and I am here today to present you the inactivation data for those three viruses.

[Slide.]

The study design used for HCV inactivation in platelets is the following. We have spiked 4 1/2 logs of

chimp infectious dose of the well characterized, the Hutchinson strain of HCV into three, full-sized units of apheresis platelet concentrate, and treated with 150 micromolar S-59 or amotosalen, and 3 joules of UVA.

After treatment, the entire unit, approximately 300 ml was infused into a seronegative chimpanzee, which was followed for six months for development of hepatitis, as well as molecular and biological markers of HCV infection.

As you know, infection of this Hutchinson's strain of HCV in chimpanzees has been shown to be uniform and consistent.

[Slide.]

Results of one of the chimps shown in this graph here, the arrow indicates the time zero for transfusing or infusing the entire unit of treated and spiked platelet concentrate. This animal showed normal liver histology both before infusion and six months after infusion, and throughout the evaluation period, there was no antibody developed against HCV virus or by an RT-PCR methodology, there was no HCV viral RNA detected.

Throughout the evaluation period, the liver enzyme ALT and AST were normal, and they were consistently at the background level. So, these results clearly demonstrate inactivation of 4 1/2 logs of the HCV virus as measured by an infectivity assay in chimps.

[Slide.]

Inactivation of BVDV uses the following design. Approximately 10^5 to 10^6 PFU per ml of BVDV was spiked into full size units of platelet plasma and red cells. The contaminated platelet plasma units were treated with 150 micromolar amotosalen and 3 joules of UVA light. Contaminated red cells were treated with 200 micromolars of S-303, and the viral titer in the sample was measured using a plaque assay bovine terminate cells.

[Slide.]

The results are summarized here, and as you can see, we have achieved consistently very high levels of inactivation. These are updated results, the number may look slightly different from what Dr. Wagner presented.

For the platelet and plasma product, we have achieved a greater than 6 logs of inactivation, and in red

cell product, we have achieved a greater than 7.3 logs of inactivation.

[Slide.]

Now, just to evaluate how sensitive BVDV is to Helinx treatment, we have done a kinetic analysis. As you heard, that would be 150 micromolar amotosalen combined with 3 joules of UVA is the process developed for treatment of platelets. Using that condition, we have achieved a more than 6 1/2 logs of inactivation.

We have also taken out 30 ml aliquots after only half a joule of elimination, and demonstrated no recoverable viruses in the aliquot in four out of four experiments. These results demonstrate inactivation of more than 6.3 logs of BVDV.

So, to borrow a phrase that is coined by Dr. Bernie Horowitz, these results demonstrate that the Helinx technology or the system has plenty of reserve capacity to inactivate BVDV.

[Slide.]

To look at the sensitivity of this virus to Helinx treatment, we have also looked at the dose-response curve. The results shown here demonstrate that we can lower

the concentration of amotosalen from 150 micromolars to as low as 3 micromolars. With a combination of 1 joule over UVA light, we have inactivated more than 5 logs of the virus.

[Slide.]

So, finally, inactivation of the West Nile Virus. The study was conducted in collaboration with Dr. Kristin Bernard of the New York State Department of Health. The viral inoculum used in the study was prepared from the BHK cells infected with a full-length infectious clone of the West Nile Virus.

The parental strain of West Nile Virus lineage 1 was isolated from the epicenter of New York City during the year 2000 outbreak. The infectivity and virulence of the cloned virus and the parental virus are similar. The plaque morphology of the cloned West Nile Virus is also indistinguishable from the parental virus, and we used the working stock has a titer of 10^8 PFU per ml.

[Slide.]

We have spiked approximately 10^6 PFU/ml of the cloned West Nile Virus into full size units of platelet concentrate or red cells. The spiked platelet units were

treated with 150 micromolar amotosalen and 3 joules of UVA light, and the spiked result units were treated with 200 micromolars of S-303.

The titer of the West Nile Virus in the sample was measured using a plaque assay on Vero cells.

[Slide.]

These are preliminary results and they are summarized in this table. For platelet units, the treatment volume was approximately 300 ml. An aliquot of the pretreatment sample confirmed the level of the inoculum at 5.4 times 10^5 PFU/ml.

After treatment, we have seen no recoverable virus in 1 ml samples in two of two experiments, demonstrating inactivation of more than 5.7 logs of West Nile Virus in platelet concentrate.

Similarly, for red cell units, the treatment volume was approximately 300 ml, and the pretreatment aliquot demonstrated the infectivity of the inoculum at 9.1 times 10^5 PFU/ml.

After treatment, no recoverable virus was detected in 1 ml samples in two of two experiments. These

results demonstrated inactivation of more than 6 logs of West Nile Virus in red cells.

So, these preliminary results confirm our prior expectation that the Helinx technology inactivates West Nile Virus very effectively.

[Slide.]

In conclusion, Helinx technology inactivates a broad spectrum of viruses, bacteria, protozoa, and leukocytes in the three components of the blood, platelets, plasma, and red cells, and our preliminary results demonstrate inactivation of high levels of West Nile Virus in platelet concentrate and red cell components.

Both the amotosalen and S-303 are effective against West Nile Virus.

Thank you very much.

[Applause.]

DR. FARSHID: Thank you, Dr. Lin.

Next, we hear about INACTINE technology by Vitex. Dr. Bernadette Alford will present the data.

Vitex

DR. ALFORD: Thank you very much. It is our pleasure to speak today about another pathogen reduction

technology, and that is the INACTINE technology. I will focus most of my efforts specifically on West Nile Virus.

[Slide.]

What I would like to do first is just briefly introduce you to Vitex, if you are not aware of who Vitex is. Our goal at Vitex is to introduce a new safety barrier for red cell concentrates beyond donor selection serologic screening.

What we would like to do is to build a safety into the manufacturing process rather to inspect safety into the product. Our focus is not to restrict the donor pool, but rather use the single step to address a wide range of viruses, as well as eukaryotic and prokaryotic pathogens.

Our approach is chemical inactivation by a compound called INACTINE PEN110, which is combined with red cell purification, and the regulatory approach is through IND BLA, and we are currently in Phase III clinical trials.

[Slide.]

This is just quickly to depict our INACTINE-automated processes that we have just recently developed. We have INACTINE PEN110 delivery to the red blood cell,

which is a completely automated system both for formulating our working solution from a concentrate involved with the delivery of PEN110.

Then, there is an incubation step at room temperature and in washing, as was described by Steve Wagner, and after washing, we have a unit that is what we term "pathogen inactivated" and ready for transfusion.

[Slide.]

What this process does is really produce two particular steps. The process combines chemical inactivation of pathogens, and that is viral inactivation of both enveloped and non-enveloped viruses, as well as cell-associated and latent viruses.

We also have shown studies to prevent bacterial outgrowth during storage, protozoan inactivation, as well as leukocyte inactivation. The contaminant removal by washing was developed to address PEN110 and potentially PEN110 adducts, but in addition, it is able to remove soluble prion proteins. We have demonstrated removal of immunoglobulins, cytokines, and other plasma proteins.

[Slide.]

This depicts a slide of some of the non-enveloped viruses that we are able to inactivate. It encompasses everything from human B-19 through various different size, genome sizes of non-enveloped viruses.

In each case, if you notice on the far right column, there is a reduction in infectivity as a log of 2 CID50/ml, and this represents the highest possible spike that is able to able to be added to a full red cell unit, and this is to the limit of detection of that virus, so we can't add any more virus than what is shown here. At the end of our kinetic studies, we see no infectious particles.

In addition, I should tell you that all of the viral studies are done without washing, so that we are really quenching right at the end, so what we are looking at is really the capacity and the capability of the INACTINE PEN110 to do inactivation.

This is our focus of today, of course, is more on the enveloped viruses. This has listed some of the enveloped viruses that we are able to inactivate. Clearly, we are able to inactivate HCV, BVDV, as Steve showed us. We have also used Sindbis as a model, but the focus today is specifically on West Nile Virus.

I am not going to go through the mechanisms of action. Steve Wagner now does a better job than I on describing the mechanisms, so I will leave it up to Steve to do that, but what I would like to do is focus more on the inactivation itself of West Nile.

[Slide.]

Before I do that, I think it is very important to call attention to the cell-associated viruses that we have studied. We have looked at both HIV and CMV in their latent and active forms, and this is a very important point to take as we move forward to look at what some of the preliminary results we have uncovered through studies on West Nile Virus.

So, our research approach started with establishing collaborations and methodologies, and we established a collaboration with Dr. Fred Brown at the USDA Plum Island Animal Disease Center, with Tom Mather at University of Rhode Island, and Robert Tesh at the University of Texas.

We also have an internal focused virology team looking at West Nile Virus, and we, of course, continue our

close interaction with the FDA since we are in Phase III pivotal trials on our technology.

[Slide.]

This was the first inactivation study that we did earlier this summer and was presented at the workshop in August. This was inactivation of an isolate from a crow from New York. It was done in conjunction with Dr. Fred Brown at the Plum Island Institute, and we added about 7 logs of spiked to full units. We saw complete inactivation within about 15 minutes, and that was to the limit of the detection. We did this on two separate experiments.

[Slide.]

After we completed this experiment, we realized there is some fundamental questions that we have to really start to address to have a better understanding of both the virus and what the capabilities of our technology is.

So, the questions included can the West Nile Virus survive in blood under blood bank storage conditions, can the virus exist in different blood compartments, is it cell associated, is it free, can the West Nile Virus then infect human leukocytes.

Of course, we are very interested in the fact that can the technology effectively inactivate the West Nile Virus in blood, and then finally, a comparison of pathogen inactivation versus diagnostic testing.

[Slide.]

So, the first experiment we did following that initial study was to confirm the initial kinetics, and this was done with Tom Mather at the University of Rhode Island. In this case we used isolates from mosquitoes from New Jersey and a crow from Rhode Island.

They were done in duplicate with two different isolates in both cases, and you will see the kinetics here. In all cases we had inactivation to limit of detection in samples collected 24 hours post-treatment, which is part of our treatment procedure.

[Slide.]

We then moved to study survival, and that was survival in the virus in human red blood cell concentrates because this is the focus of our technology, and this was using a high titer viral spike. "High titer," I mean a titer that is near a log of 7.

[Slide.]

That is what you should see here, and this is storage time in days up to 35 days. Of course, these are pilot studies, I want to remind you, so if you were to call on Thursday, we would have the 42-day data. This is as of last Thursday.

You will notice in the supernatant, there really isn't any change at all in the survival of the West Nile and red blood cells. As you will note, it appears to be a decrease in the red blood cell concentrates, and this is suggested to be a consequence of cell association, but the experiment that was done at this point, which is one of our earlier experiments, wasn't done really to address cell-associated, but I will show you that data in just a moment.

[Slide.]

We similarly used a low titer viral spike, this is a spike of around 4 logs, and we got very similar results, and this experiment has gone up to now about three weeks or 21 days.

[Slide.]

We then wanted to study more in a natural setting, so we did move to an in-vivo model, and this in-vivo model was looking at the virus in golden hamsters. I

am just depicting for you here the day after infection, and you could see what the highest level of viremia, which occurs about day two-three in this particular model.

[Slide.]

The West Nile levels and blood fractions then from naturally infected hamsters, what I showed you on the previous slide, was carried out, and this was using a West Nile isolate from Snowy Owls that were isolated from the Bronx Zoo in 1999, which was the onset of this virus in the United States specifically in the New York area.

[Slide.]

If you notice here that we looked at each of the blood fractions, both whole blood, plasma, PBMCs, and red cells, and it was day three post-infection based on the model that I just showed you with either one-day storage or four days storage, and the hampster data indicate that there is a cell-associated and a virus-free form, but the duration of the viremia in the cell-associated form is currently unknown.

[Slide.]

We went further to look at the kinetics of inactivation of whole blood to confirm the initial cell-

associated virus that I was speaking to, so we used a couple--this is a very similar model to what I described before--it is two groups of hamsters that were infected with 10^4 TCID50.

One group, the blood was collected three days post-infection CPD and stored for four days at 4 degrees, The other, similarly collected, but after storage for just one day. Our standard inactivation technology involves 0.1 percent volume to volume with PEN110. We incubate up to 24 hours and then we did infectivity of TCID50 in Vero cells.

[Slide.]

This is the results of the initial kinetics. I do apologize, there is only a couple points here. I do want to remind you that it is early pilot studies, and we will be confirming the full kinetics of these studies, but you could see that within 24 hours, both groups had complete inactivation--and this is of whole blood--to the level of sensitivity of the assay, and the assay now is becoming much more sensitive.

[Slide.]

The next question, one of the last questions we want to answer was can West Nile Virus infect human

leukocytes, and we did a study obviously with media control, non-simulated PBMC, an IL-2 simulated, but the most important fraction that we studied here was a monocytic cell called THP-1.

We collected samples once a week and fed fresh media new cells if it was necessary to retain the cell level consistent throughout the samples, and then we tested for infectivity of the cultured cells after trypsinization and washing, and that is a very important point.

The trypsinization is very necessary here to remove any free viruses, so specifically we are looking at what is infected in the leukocyte.

[Slide.]

This is the infection of West Nile Virus in human leukocytes, and the important point that I really want to draw you to is obviously in the non-stimulated cells. This is just PBMCs in the media. We see a reduction in titer, just what you would expect, but this is that monocytic cell line that I was referring to, and there is no reduction, so clearly, there is a potential through this model that one can see that West Nile infection does occur in human leukocytes.

[Slide.]

So, what about this transmission by the mosquito versus blood? Well, the mosquitoes, as we know, can transmit West Nile to humans, and, in fact, the mosquito contains about 10^3 to 10^4 PFUs, and this was by communication with Dr. Tom Mather at Rhode Island, who we are collaborating with.

Therefore, one could envision that about 10^4 PFUs would be enough to infect an individual. So, a blood unit that is contaminated with 10 PFUs/ml, and it is about a 350 ml unit, would likely transmit the virus.

[Slide.]

So, the conclusions from these studies, and these are the questions that I started out with, is does the West Nile Virus survive in blood under blood bank storage conditions, and the answer is yes, the West Nile survives in storage conditions at least for 35 days, and the study, as I said, is ongoing.

Where in the blood does the West Nile Virus exist? Can the virus infect human leukocytes, and not only is the virus harbored in red cells, plasma, and platelets,

but from our studies, it also appears that it is in leukocytes.

Since the West Nile is present in plasma, the leukocyte filtration will not address the health risk of West Nile Virus. Further INACTINE PEN110 inactivation of West Nile is not sensitive to the presence of these leukocytes.

[Slide.]

Can our technology effectively inactivate the West Nile Virus in blood? The answer is definitively yes, INACTINE PEN110 can effectively inactivate even 1,000 to 100,000-fold of the amount of West Nile Virus that has been reported during human infections in the literature.

Insofar as I have demonstrated to you, we have tested four different isolates, and as Steve Wagner told us earlier, we don't know yet what the log reduction is necessary. That is why it is very important for us to have a tremendous fold inactivation above what we think could be occurring.

[Slide.]

Obviously, there is additional studies that are ongoing, but for a moment I would like to compare for you

pathogen inactivation versus diagnostic testing. This is a paradigm scenario that one would consider.

Obviously, there is a donor, and the concern is donor to a recipient. Right now the donor questionnaire, as we heard yesterday, there is about 80 percent that are asymptomatic or there is no specific risk factor, so it is very hard to identify who that particular donor would be and how to exclude that donor from the pool.

We talked yesterday about West Nile donor screening and there is going to be a lot more discussion. It was a very good presentation yesterday and a lot of wonderful interaction, and we were pleased to be party to that, but some of the questions that we have seen come up and are addressed as we move forward in the future, is there is very low viremia.

There were questions raised yesterday about that, so there is a potential for false negative. There is a high infectivity, which means there is a very low infectious dose that is potentially required.

It appears from our data that the virus is cell associated, can NAT address a cell-associated virus? What

about the persistence of cell-associated virus? It is very different.

Then, finally, of course, the IgM antibody, how do you address a window period with a test of that nature? One would offer an alternative. I believe that alternative appropriately could be pathogen reduction, and not only does it inactivate West Nile Virus, but in addition, it offers a broad spectrum inactivation of other viruses, parasites, bacteria, and even leukocytes.

Our concern, of course, together is that recipient, that recipient who potentially is a very high risk. We understand about 20 percent of the blood transfusions occur in immunocompromised patients and about 70 percent are in patients that are 65 years or older, so it is very important to address this specific population.

I offer that just as a point of interest and discussion, and it is based on a lot of the interaction in the discussions we heard yesterday.

I thank you very much for your time.

[Applause.]

DR. FARSHID: Thank you, Dr. Alford.

The last speaker for this session is from Gambro. Dr. Ray Goodrich was supposed to give the presentation, but he called in sick, and Robert Antwiler will substitute for him, and they talk about their technology using riboflavin.

Gambro

DR. ANTWILER: Thank you. I am going to be speaking today on the reduction of the West Nile Virus in packed red cells, single donor platelets, and plasma using the riboflavin and light technology.

[Slide.]

As Steve Wagner showed earlier, this is a picture of the riboflavin molecule. Riboflavin is commonly known as vitamin B2. It is an essential nutrient and it is a dry powder in the solid state. We use it packed as a liquid in a saline-riboflavin solution.

[Slide.]

Riboflavin has the following absorption curve. It absorbs in both the UV and in the visible region. In both regions, it activates in the same mechanism. We use the UV region for treating platelet and plasma products, and we use the visible region for treating the red cell products.

[Slide.]

I have included a picture of our illuminator. The illuminator is this white box that has a drawer that you simply pull out. You place your units to be inactivated on the shelf. The shelf oscillates back and forth to provide mixing of the product.

In use, of course, you close the door, you turn the switch on. It starts the process, monitors the amount of light delivered, and then stops automatically when the proper light delivery has been achieved.

We do have a system for recording the data and logging all of the parameters during the inactivation process.

[Slide.]

Today, I am going to focus on the West Nile Virus study that we just recently did. This study used the TCID50 method using Vero cells. There is some additional analysis in process using the TaqMan PCR testing. That is underway and I do not currently have the data to share with you on that. All of the procedures and the assays were performed at the CDC laboratories in Fort Collins, Colorado.

[Slide.]

On the West Nile Virus plasma study protocol, we used the UV process. We did a kinetic study. The energy points that were chosen for this study were based upon our previous experience with BVDV. I am going to show you one slide just to illustrate where and how we pick those energy points.

We did an N of 3 for plasma. The riboflavin concentration was our standard 50 micromolar, and the product volume was 250 ml of plasma.

[Slide.]

This is the curve I said I would show you. Plotted here is data from previous viral inactivation experiments that we have done, plotted as the log virus, and this is log/ml reduction versus the energy delivered. The purpose of this graph is simply to show you the BVDV, which is where we ended up picking our energy points from, fairly linear kinetic down to the limit of detection and then it becomes flat.

[Slide.]

This is the data using the same energy points that we had chosen from the previous BVDV data. This is

the N of 3 for the log reduction of West Nile Virus in the plasma product. Again, this is plotted logs reduction/ml.

The open symbols represent data at the limit of detection. You can see that we do reach the limit of detection, around 6 joules/cm^2.

[Slide.]

We followed that with a study looking at the kill of West Nile Virus in platelet products. Again, the platelet product uses the UV process. We did a kinetic study. Again, the energy points were based upon our previous experience with BVDV. Similar conditions as with the plasma, N of 3, 50 micromolar, riboflavin concentration and a product volume of 250 ml of platelets.

[Slide.]

The kinetics for the platelets is very similar to that with the plasma, reaching the limit of detection around 8 joules per cm^2. Again, log reduction/ml is the expressed reduction factor.

[Slide.]

We then did a study of the West Nile Virus reduction in our RBC products. That uses the visible light

process, similar experimental design, kinetic study, energy points chosen based upon the BVDV experience, N of 3.

The riboflavin concentration with our RBC product is 500 micromolar, and the product volume was 266 ml at a hematocrit of 30 crit.

[Slide.]

Shown here is the data for those three experiments. You can see that we achieve log reductions in the 4 1/2 to approaching 7 again depending upon the limit of detection here.

[Slide.]

In conclusion, we have shown that the West Nile Virus is reduced by the Gambro inactivation system, greater than 5.1 log/ml inactivation in plasma products, greater than 4.8 log/ml inactivation in a single donor platelet product, and greater than 4.0 log/ml inactivation in packed RBC products.

We would like to acknowledge the cooperation of the people at the CDC and Fort Collins, and thank you very much.

[Applause.]

DR. BARBOSA: The morning session on pathogen inactivation targeted at West Nile Virus was predominantly focusing on cellular components of blood. Now, we are going to switch to plasma, viral clearance methods in plasma and plasma derivative products.

Before we start I wanted to make a request of the speakers to limit their time to 10 minutes, no more than 10 minutes.

We are going to start with Dr. Dominique Pifat from Bayer Corporation. She will be talking about the role of model viruses in current inactivation studies.

Viral Clearance Methods in Plasma

and Plasma-Derived Products

Role of Model Viruses in Current

Inactivation Studies

DR. PIFAT: Thank you.

Good morning. I am going to be speaking to you this morning on behalf of the Plasma Protein Therapeutic Association, and I would like to thank the FDA for giving us an opportunity to speak this morning.

[Slide.]

I am going to be talking about manufacturing processes for plasma-derived products. We have the opportunity and the obligation to demonstrate that these manufacturing processes can provide effective inactivation or removal of both enveloped and non-enveloped viruses.

[Slide.]

Now, when we evaluate our manufacturing processes, and I am going to concentrate this morning on enveloped viruses because obviously, West Nile Virus is an enveloped virus, and when we develop manufacturing processes and we evaluate them for their ability to inactivate or remove viruses, we choose a panel of viruses that are very varied in terms of their genome, RNA, DNA viruses, their size, their shape, and this is deliberately done to address not only the removal of inactivation of known pathogens, but also to potentially address unknown or emerging viruses.

So, the enveloped viruses, of course, that we choose in our studies are different, but they have, of course, their envelope in common.

[Slide.]

One of the reasons that the envelope is an interesting target, of course, is the lipids from the envelope are not derived or not coated for by the viruses themselves, so they are less susceptible to antigenic variations, so the envelope is usually the target of inactivation.

We have shown that actually, inactivating the envelope is very robust and effective for a whole variety of viruses. Again, the inactivation processes are not influenced by subtle antigenic variations.

[Slide.]

When we look at inactivation of envelope viruses we have a number of tools at our disposal. The most common tools that are used throughout the industry are solvent detergent, heat pasteurization, caprylate, low pH.

Now, most of the manufacturing processes include at least two significant inactivation steps for envelope viruses, and by "significant," we mean that they provide at least 4 logs reduction and overall our manufacturing process provide at least 10 logs reduction for envelope viruses.

[Slide.]

I am going to address now the use of model viruses to evaluate the safety of the products.

This is a compilation of published data with a whole variety of envelope viruses, clearly of different families. Some of them are Flaviviridae, but there are other families represented here clearly. The three processes that are evaluated in these data are pasteurization, solvent detergent, and caprylate.

You can see that these three different methodologies provide reduction in all these different viruses from all these different families to the limit of detection in all of these experiments, so a priori there would be no reason to believe that West Nile Virus would behave significantly differently from all of these other envelope viruses. In another talk, my colleague from Baxter will actually confirm that that is the case, that West Nile Virus behaves very similarly to all the viruses described here.

[Slide.]

We have seen this slide more than once already during these two days. Of course, Flaviviridae are broken down into three different genera. One of the viruses of

interest in the industry is clearly hepatitis C virus, and what is commonly used in industry is bovine viral diarrhea virus as a model to look at the inactivation or removal of hepatitis C virus because hepatitis C is not easily grown in tissue culture.

So, by this reasoning, if you use a pestivirus as a model for hepatitis C virus, there is some logic to using bovine viral diarrhea virus as a model for West Nile Virus, which, of course, belongs to another genus.

[Slide.]

These are some of the data that are compiled just for comparison purposes for the inactivation or removal of HCV, so since we can't cultivate HCV, various viruses have been used as models. BVDV, of course, I already mentioned, yellow fever virus, Sindbis virus, tick-borne encephalitis virus.

If you look at this table and look at all these Flaviviridae, you can see that again pasteurization, solvent detergent, and caprylate provide inactivation to the limit of detection for all these different Flaviviridae.

[Slide.]

Justification for using BVDV as a model for West Nile Virus, clearly, BVDV closely resembles flaviviruses including West Nile Virus. BVDV has been successfully used as a model for HCV inactivation.

There is a very large body of inactivation data that exists for BVDV, and these data should provide assurance that West Nile Virus can be inactivated during plasma-derived manufacturing processes. Again, we will show that there is some evidence that that is actually correct.

The second speaker in this series is also going to present a compilation of the data with various model viruses that have been obtained throughout the industry, so all of the members of the Plasma Protein Therapeutic Association have provided data to show the effectiveness of our processes.

[Slide.]

There is always potential limitations to using model viruses, but actually, the safety of our products is really the evidence that the use of BVDV as a model for HCV, for instance, is a valid thing to do because of no seroconversions to HCV.

Again, it is the safety record of our products that validates the clearance studies using model viruses.

[Slide.]

The safety of our biological products today is assured because we can demonstrate clearance with model viruses when it is not possible to use some of the viruses of interest.

[Slide.]

I have told you that we have done preliminary, we are going to show preliminary data on West Nile Virus itself, but the PPTA members will continue to conduct studies on the specific viral inactivation and removal steps in the manufacturing processes for plasma derivatives with West Nile Virus itself to confirm the reliability of the predictions that are based on model virus studies.

Thank you.

[Applause.]

DR. BARBOSA: Additional data on the model viruses will be presented now by Dr. Albrecht Groner from Aventis.

Dr. Groner.

Robustness Data from Model Viruses

DR. GRONER: Thank you very much for the opportunity to discuss with you the effort of the PPTA member companies to demonstrate the effective virus inactivation and the removal capacity by selected steps.

[Slide.]

The virus validation studies are essential to document the effective virus inactivation capacity and therefore we have to evaluate a wide range of viruses with known physical/chemical properties.

There will be, of course, a quantitative estimate of the overall virus reduction capacity of the process, and that will also have an indirect evidence that the process will inactivate or remove also novel or emerging viruses.

[Slide.]

In these viral validation studies we are performing, we are using, of course, known viruses, and these known viruses should resemble the viruses of interest. They should represent the widest range of physical/chemical properties of viruses, and they should include, of course, laboratory strains which can be easily assessed and used.

We have to consider that, of course, these laboratory strains have or mainly have different properties from the natural occurring viruses and therefore we conclude that all viruses used in various validation studies are in principle model viruses.

[Slide.]

As I already said, we have to use in our validation studies, viruses which can be reliably assayed in an infectivity assay. Therefore, we have to have the appropriate system in place. This system is, of course, reliable, sensitive infectivity assay, and the virus we are using should go to high titers to document high virus inactivation capacity of the manufacturing process.

If there are two similar viruses available which could be used because they represent, say, the target virus, then, the more robust virus should be used, the more resistant one. There is also no question about we are looking for the lowest human hesset [ph] virus to avoid some negative impact on our stuff.

[Slide.]

The viruses which are used within the different companies are, of course, viruses HIV-1 and HIV, and the

specific model viruses - BVDV and sometimes Sindbis virus as a model for HCV, as well as the parvoviruses from porcine and canine as a model for parvovirus B-19 and as a nonspecific model virus, often the herpesviruses are used in here especially the rabies [?] virus.

[Slide.]

Now, I would like to discuss with you the compiled data from PPTA member companies on the different products and to document the robust virus inactivation capacity after flaviviruses.

The albumin, as you can see here, is used in the pasteurization at 60 degrees centigrade, and there were different concentrations from 3.5 to 25 percent protein, and under these production conditions, BVDV, as well as tick-borne encephalitis virus, as well as Sindbis virus are removed below the detection limit.

The range you can see here is the range which are supplied by the different bumper companies, and that is just due to the fact that the amount of viruses added in the process is different when you have a higher titer to spike this definitely the virus reduction capacity will be higher in that respect.

We are looking now for out of specification temperature and an intrastabilizer concentration, nevertheless, BVDV will be inactivated below detection limit.

[Slide.]

Similar data are true also for the SD treatment of the factor VIII product. It is also inactivated below detection limit, the BVDV, as well as the Sindbis virus using robust conditions, with only 50 percent of the SD concentration.

We also have definitely a very effective BVDV inactivation.

[Slide.]

That is just to demonstrate the BVDV as well as the Sindbis virus inactivation capacity within a very short period of time we are reaching the limit of detection of the assay.

[Slide.]

Now, when we are looking for very robust concentration, I just showed you that 50 percent of the SD concentration will have no impact on the virus inactivation

capacity. Here, in this slide, you see that even one-third has no impact on the excellent virus inactivation capacity.

When we have 1 to 9 and higher dilution of the SD concentration, then, we see an effect at that concentration which is more than out of specification will be not inactivating the BVDV as expected.

[Slide.]

When we are now going for factor VIII and pasteurization, we have again BVDV, yellow fever virus, Sindbis virus, as well as Semliki Forest virus, inactivated below detection limit under all conditions, and that is also true for robustness when we are using temperature below the specification and stabilizer concentration above specification, as well as different protein concentrations.

[Slide.]

That is demonstrated here. In a graph, you see within fairly short period of time, the virus is inactivated below detection limit, and you can see from the graph that there is room enough to further inactivate an even larger amount of virus.

[Slide.]

When we are now going for factor VIII and by heat treatment, you see also under all conditions excellent virus inactivation capacity.

[Slide.]

That is also true for factor IX solvent detergent treatment, BVDV, as well as Sindbis virus.

[Slide.]

And in pasteurization of factor IX also excellent virus inactivation capacity for BVDV, as well as tick-borne encephalitis virus.

[Slide.]

In immunoglobulin preparations, we see again the same data. The solvent detergent treatment is inactivating the flavivirus model and the togavirus, which is used, under all production conditions, which differ, but they are always inactivating the virus below detection limit.

[Slide.]

We are now going for immunoglobulin and pasteurization, again, we see an excellent virus inactivation capacity at what action conditions, as well as be on the specification of the production.

Here is just a graph showing the different parameters which were tested. That means standard stabilizer concentration, increased stabilizer concentration, decreased temperature, different pH values, as well as different concentrations. In all conditions, these virus inactivation capacity is very effective for BVDV.

Now, we have, despite virus inactivation, we have also virus removal, dedicated virus removal steps in our production process, and there is, for instance, the nanofiltration.

[Slide.]

You see here different products from different companies. You will see different filter devices, and you will see here again an excellent virus removal capacity for BVDV, always below the limit of detection of the assay.

[Slide.]

When we are now looking for further manufacturing steps, which are used to purify and concentrate the protein, as caprylate and octatonic acid treatment as acetone suspension and chromatography, whether it is affinity chromatography or hydrophobic interaction

chromatography, as well as the cold ethanol precipitation steps, we have always very good virus removal capacity of these selected steps.

They may not be effective as the dedicated virus inactivation and removal steps, but they certainly contribute to the virus safety.

[Slide.]

I now would like to conclude that BVDV, tick-borne encephalitis virus, as well as yellow fever virus and Sindbis belong to the Togaviridae, are model viruses closely related to the West Nile Virus, and these are inactivated as we documented in our virus validation studies, very effectively, and we could demonstrate in the robust inactivation and removal of these enveloped viruses by the plasma derivative manufacturing processes.

Thank you.

[Applause.]

DR. BARBOSA: Thank you, Dr. Groner.

The last presentation on model viruses for validation and evaluation of inactivation procedures will be given by Dr. Thomas Kreil from Baxter Bioscience.

I invite all the speakers to come to the podium immediately after this last presentation for the general discussion. Thank you.

Equality of Model Viruses and Current Data for WNV

DR. KREIL: Good morning, ladies and gentlemen.

[Slide.]

I would like to use the next 10 minutes or so to share with you the results of an investigation that we have performed at Baxter Bioscience in verifying that West Nile Virus indeed is just one of the flaviviruses and particularly so with respect to its being inactivated through the major inactivation processes which have been implemented by the plasma products industry in their respective manufacturing procedures.

[Slide.]

As you have heard from the two previous speakers, this industry really knows a lot about flaviviruses in general, obviously driven by hepatitis C virus here, a virus of potential concern for transfusions, and a virus which is unfortunately not available to us experimentally.

That is why this industry has resorted to the use of model viruses, most notably the bovine viral diarrhea

virus here, but then also some similar viruses, such as tick-borne encephalitis virus here, which really is almost a twin brother of West Nile Virus.

[Slide.]

This is the result that we have generated at Baxter for these viruses. I think at first glance it is fair to say that all of these viruses are very susceptible to the virus inactivation processes that this industry typically uses, that being, for example, pasteurization here for human serum albumin, then solvent detergent for factor VIII, also solvent detergent for intravenous immunoglobulins, and then vapor heating for here, for example, are factor VIII inhibitor bypassing activity product.

It is also important to note that when you compare the inactivation of the different viruses used, that being flaviviruses--here are some related togavirus--they are all very similar one to the other.

[Slide.]

Now, we tried to verify that West Nile Virus indeed would not be behave any differently to what we knew about the other flaviviruses and therefore we have obtained

a West Nile isolate from the 1999 New York outbreak. The virus was isolated from the liver of Snowy Owl and provided to us by Dr. Robert Shope, and I want to thank him for that.

The virus was then characterized by sequencing the genome, and the spiked virus that we have used for subsequent studies was prepared as a supernatant of Vero cells, serum-free Vero cells in this instance.

The assay we have used is a Vero cell assay, and the cytopathic effect can already be read after three days.

[Slide.]

This is some results from the assay setup experiments. You can see that we have had eight operators titrate the virus on seven different days, and as you will be able to appreciate, there is no variation almost between days and also between operators.

The titer of the stock virus available to us at 8.9 log 10 or, in other words, a billion infectious units per ml is very significant and lends itself ideally to do some studies with that virus.

[Slide.]

This is the same table that you have seen before now including the data that we have obtained for West Nile Viruses in these processes here. You can see that West Nile Virus was inactivated to below the limit of detection in all instances, just as we would have predicted by the data known to us from other flaviviruses.

Well, obviously, the reduction factor is not everything you can investigate to make a side-by-side comparison of the different flaviviruses, and so we went into some further detail.

[Slide.]

You can see here we used a downscaled version of our large-scale manufacturing process for albumin. This is only the critical process parameters for pasteurization, that being the process temperature here, the treatment time, and then the protein concentration, the lowest and the highest end.

In our downscale, we have used a temperature just below the process temperature and the large scale, the shortest possible time, and then we have bracketed the protein concentrations.

[Slide.]

This is what the results look like for a number of different flaviviruses. While this is a pretty busy slide, I think the message is quite clear in that all the viruses that we have tested are very, very quickly inactivated to below the limit of detection, that being probably the most widely used model virus BVDV, then followed up by tick-borne encephalitis virus, a twin brother, as I have mentioned before, to West Nile Virus, now complemented by West Nile Virus data itself.

Here we have a togavirus in there, a Sindbis virus, but again no difference to be seen between all of these viruses.

[Slide.]

This now is another albumin preparation that we commercialize, a slightly different composition of intermediate, and therefore, we tried to also generate some data on this preparation again using the same downscale setup.

[Slide.]

As you can see here, again indistinguishable kinetics of inactivation between bovine viral diarrhea

virus and West Nile Virus itself, also not dependent on protein concentration in this specific process.

[Slide.]

This is another product of ours, the anti-inhibitor product FEIBA, which is subjected to vapor heat treatment process, and I need to walk you through the manufacturing process just briefly here.

The product is heated at 60 degrees Celsius for 515 minutes. Then, it is brought up to 80 degrees Celsius for another 65 minutes, and all that with the lyophilized product at between 7 and 8 percent residual moisture content.

The downscale has used temperatures just below that specified for manufacturing, the shortest possible incubation times, and then at a residual moisture content of the lyophilized product similar to the manufacturing process.

[Slide.]

This is what the data looked like. This is again the process. You take the product up to 60 degrees Celsius, treat it in this instance for 510 minutes. Then,

we bring it up to 80 degrees Celsius for another hour, and then we cool it down.

The first thing I want to mention is that all of the viruses tested in this downscaled model were inactivated to below the limit of detection already within the 60 degrees Celsius treatment phase. In other words, the additional 80 degrees Celsius phase here only provides additional safety margins to this product.

Also, you should be able to see that all the viruses were inactivated with very parallel slopes of inactivation, which indicates that they have a very similar sensitivity to this particular heat treatment.

The reason why they are only parallel is that for tick-borne encephalitis virus here, in orange, and then West Nile Virus here, in red, we have higher spiking titers here in the range of 8 logs for the spiked product, which is some 2 logs higher than what we were able to use for bovine viral diarrhea virus earlier.

[Slide.]

Solvent detergent, yet another inactivation procedure widely used throughout the industry, the key process parameters being the temperature at which the

treatment is performed, the time obviously, and then the concentration of SD chemicals.

This is really an important point to notice because if we use the nominal concentration, such as those given for manufacturing, then, what we get is instantaneous virus inactivation, so you are not able to demonstrate the kinetics of inactivation.

That is why in the downscale, we resort to using a drastically reduced SD concentration, here, only one-tenth of the nominal concentrations of the components for this SD treatment.

[Slide.]

What you can see is that West Nile Virus still, despite the fact that we are using only one-tenth of SD chemicals in this instance, is completely inactivated instantaneously. The "B" here indicates that we have used bog titrations, so that meaning 10-fold bigger sample sizes to determine whether there was any residual infectivity, and there was not.

In this instance, really, BVDV is somewhat more resistant to that treatment although I should reemphasize that at the nominal concentrations, BVDV follows a course

of kinetics just like this, so this is 10-fold reduced SD chemicals.

[Slide.]

Here, we have another SD treatment as applied to our intravenous immunoglobulin product Gammagard. Again, you have given here temperature and time of treatment, which are similar between downscale and manufacturing procedure.

Here are the SD chemicals and again we have to use a drastically reduced concentration of SD chemicals to be able at all to demonstrate kinetics. In this instance, we have reduced it by 20-fold from the nominal concentration, and the reason why we went further down even here is that this is a tricomponent SD treatment using two detergents and one solvent here, which is even more effective than the SD treatment that I have shown to you before.

[Slide.]

This is what the result of this experiment is. You get instantaneous inactivation almost for both West Nile Virus and BVDV even at a 5 percent only SD chemicals, so I think very nicely supporting what it is thought to be

the code case anyway, that this is one of the most effective inactivation procedures really.

[Slide.]

This is the results now from a study which has been performed by Alpha Therapeutic Corporation on their alpha-1 proteinase inhibitor product. The step investigated here is a 15 Asahi nanofiltration. You can see a comparison here between manufacturing parameters and then here the equivalent downscale parameters, and again the two mimic one and the other very nicely.

This is the results obtained in a run using, as an assay system, the NGI SuperQuant PCR system that has been introduced to you yesterday, and as you can see, from the spiked greater than 9 log genome copies per ml, and that is log tens, I need to emphasize, the filtered alpha-1 product is below zero log, 10 copy numbers, giving you a very high reduction factor of greater than 9.

[Slide.]

As of the end of last month, the FDA has provided guidance to the industry in relation to the blood product safety, and I would like to quote from that. "The FDA has reviewed the viral reduction processes in place for all

plasma derivatives. The methods in place have been validated to inactivate flaviviruses related to West Nile Virus."

[Slide.]

I guess it is fair to say that the West Nile data presented here support the FDA's conclusion and that it verifies that West Nile Virus does not behave differently than other flaviviruses.

Also, the concept of using a range of physicochemically diverse model viruses for the validation of virus reduction steps has been verified in that the behavior of a virus of interest, which here obviously is West Nile Virus, has been adequately predicted.

Thank you very much.

[Applause.]

DR. FARSHID: Thank you. Now, we open the floor for questions and I invite all the speakers to please come up.

General Discussion

DR. FARSHID: While people are making their way to the microphone, I would like to start with a question

that is in reference to use of the model virus for performing inactivation studies.

The principle that we apply in FDA in evaluating viral validation studies is that if the relevant virus is available and is feasible to use, i.e., if there is a high-titer stock and there is a quantitative infectivity assay present, the relevant pathogen must be included in validation studies.

Use of specific model viruses should be justified, for example, in case of HCV or HBV, because the culture is not available, therefore, we basically have no choice but to use the specific model viruses which resemble HCV or HBV.

Therefore, we encourage the manufacturer to validate their process and show its capacity to inactivate, and one other reason for that is because these viruses, they behave differently, and this has been shown even if you use one virus, for example, in the case of hepatitis A, it has been shown one virus with different strain may behave differently.

Therefore, to come out with high degree of assurance that the manufacturing process or the

inactivation process can inactivate the virus, that specific virus needs to be included in validation studies.

Dr. Tabor.

DR. TABOR: Ed Tabor from FDA.

To underline what Dr. Farshid just said, I would just like to comment on a statement made by Dr. Pifat. Dr. Pifat said that the safety of her company's product proves that BVDV is a good model for showing the inactivation of hepatitis C virus.

Now, whereas, BVDV has been used as a model for the inactivation of hepatitis C virus because hepatitis C virus cannot be grown in cell culture, the fact that her company's product is safe does not prove that BVDV is a good model. It only proves that her company's inactivation procedures are effective for eliminating the hepatitis C virus.

DR. NAKHASI: Hira Nakhasi from FDA.

I think I would like to congratulate all the speakers this morning. We heard very excellent presentations from how the industry has really gone and done excellent work on inactivation process, and I think I would like to thank you, congratulate you all.

The question I have is well and done, you know, the activation processes work very well. How does it affect the product itself, because many of these are, as some of you alluded to the fact that yes, it intercalates the DNA, you know, many of them are very dangerous stuff, and I guess Dr. Wagner alluded to the fact that there may be some adverse effects.

So, the question to the panel is have you looked at any adverse effects or anything like that of the products after treating with this stuff.

DR. ALFORD: What we have done are several things. First, we have conducted two, a Phase I and a Phase II clinical trial, and we are in pivotal Phase III clinical trials to address any related safety issues as it applies to man. We have not seen any safety issues at all or adverse effects in the studies we have done to date.

Then, in addition to address what you are talking about in the quality of the red cells itself, we have done survival studies and additionally to that, we have done a series of studies on the red cells from osmotic fragility to ectocytometric assessments, even we have subjected it to sheer force under simulated extracorporeal circuitry, so we

have looked at it as much as we can right now prior to completing our Phase III trials to see what the quality of that red cell is, as well as any adverse effects.

Of course, related to that is an extensive toxicology program which we have published on in part and presented, and which we are completing, so those are I think the three major focuses that we are relying on to answer the question that you are speaking to.

DR. ANTWILER: I can speak to the case of riboflavin. It, of course, is a natural vitamin that we all have. The photo byproducts of riboflavin are in each and every one of our bodies right now and it is produced every time we walk out into the sunlight, lumichrome being the photo byproduct, so we have currently natural mechanisms of dealing with its photo byproducts.

We have done extensive toxicology, genotox mutagenicity testing in addition, and all of that has shown negative. So, I hope that is at least a partial answer to your question.

DR. LIN: I would like to make a comment on the Baxter-Ceres system. We have completed the Phase III clinical trials for both the platelet and the plasma

product in the Phase III clinical trials for the red cell system is ongoing, and much of the data for platelet and plasma have been published.

As far as the efficacy of treated platelets and plasma, we have demonstrated almost equivalence in terms of stop bleeding for the platelet product although a secondary endpoint platelet count increment demonstrates slightly lower for the treated products.

My second comment on the toxicology study is that we have conducted a very comprehensive list of studies for both the amotosalen and S-303 that includes the acute toxicity, genotoxicity, total toxicity for amotosalen and carcinogenicity, and also all the absorption distribution, metabolism, and excretions in both animal studies and in clinical trials, and we have not seen relevant results in the treated.

DR. GOLDING: I just would like to continue on the same vein as Dr. Hira Nakhasi. Clearly, for these products to be approved, one would have to do some kind of risk-benefit analysis. On the one side, we are talking about removing West Nile Virus or other viruses, and on the

other side, we are talking about what happens to the products in terms of safety and efficacy.

The third part of the equation that we haven't discussed yet, it is on the agenda, is what is the prevalence of West Nile Virus in these products in the first place. So, I think somewhere along the line, maybe not today, we are going to have to take all this information and look at it in a comprehensive way and make a risk-benefit analysis.

The other point I would like to make, one of the speakers on pathogen inactivation referred to the fact that while if you can remove the pathogen from the blood, maybe you do not have to worry about other things. I am not sure if that was really the implication, but that is how it came across to me.

I think that the FDA's approach to it and I think part of the industry's approach to it, as well, has been that we have to look at multiple levels, we cannot just choose one approach when it comes to viruses especially life-threatening viruses, that we have to look at the donor screening and maybe pathogen inactivation and viral inactivation removal steps, testing of minipools and plasma

pools and maybe final product, and not look at just one part of the process and say that is sufficient to provide safety in this arena.

DR. DHAWAN: I would like to make two comments actually that relate to the questions by Dr. Nakhasi and Dr. Golding, again, the safety issues. I can see these cross linkers, 303, INACTINE and PEN110, and while they can be used to inactivate West Nile Virus and other pathogens, in the systems like plasma and serum, I don't see how it can be used to inactivate whole blood. In red blood cells, okay, but if they intercalate the nucleic acids, when you feed the whole blood with these inhibitors or intercalator agent, I don't see how you can use the whole blood for transfusion. That is one.

Number two, have any of you studied the effect of all these agents on differentiation of bone marrow, progenitor cells or other cell types?

DR. ALFORD: Maybe I could start. I presented some preclinical data in the hamster on whole blood. I am not suggesting at all that INACTINE PEN110 is the mechanism to inactivate pathogens in whole blood. That product was specifically designed to address pathogens in red cells.

That was just a preclinical experimental design, so if I was unclear, I apologize. The technology is only for red cells as an example.

The second part of your question, we have not yet addressed a bone marrow in the manner you are speaking, but it is a very good question.

DR. DHAWAN: One of you, I don't remember who mentioned about the infection of leukocytes.

DR. ALFORD: That was me.

DR. DHAWAN: And you said, well, the filtration of blood or centrifugation and filtration will not be an effective way of eliminating viral components or virus, whole viruses, then, how would you eliminate cell-associated virus with any of these agents?

DR. ALFORD: I don't think I was addressing filtration or centrifugation. What I was suggesting is that if the virus, and there is further confirmation that would be necessary that it is cell-associated, my questioning is if we do a diagnostic screening, will that be able to, as we move forward, address the cell-associated virus.

It may be that there is more than one barrier that is necessary, and maybe screening is one barrier, and a pathogen reduction may be an additional barrier, to have both address the safety issue. That is what my suggestion is.

DR. LIN: I did not have time to present all our data. Maybe results have been published or presented elsewhere. The Helinx technology has been shown to inactivate not only cell-free viruses and cell-associated viruses, as well as a provirus.

My second comment, the question of whether any of these technologies works for whole blood, initially, we are developing a separate technology for platelet products and red cells. Our company is working on a methodology to treat whole blood, and as soon as the results are available, you will hear about it.

DR. PETERSEN: Lyle Petersen. I have two questions. One question is, is what is the anticipated cost of implementing these technologies, and the second question is, do these technologies produce a toxic waste problem?

DR. ANTWILER: I can speak for the riboflavin technology. I cannot tell you what the cost is on that, but it does not produce any toxic waste products.

DR. ALFORD: Speaking on INACTINE, we are developing right now a pharmacoeconomic analysis to determine what the costs are as we are finishing our development of our process. As soon as we have that ready, we will be glad to share it with you.

On a waste disposal, if you will, we do have disposables. I believe all the technologies would have a disposable involved at some time, and I guess I would consider that a waste, because it is a disposable that has seen blood.

We do washing and, as you know, we have some liters of wash solution, and what we are addressing is quenching that solution, so we could go directly to drain, but otherwise, it is really a disposable that you would still be looking at for a potential waste product.

DR. LIN: The comment I would like to make is that we have MSDS information for amotosalen, and since it is treated in a closed system, the way to treat the waste is no different from your biological waste.

DR. FARSHID: Dr. Wagner, would you like to comment on that question?

DR. WAGNER: I think this is an issue that needs to be looked at pretty carefully by parts of the government that are involved in environmental safety, both with respect to workers, as well as what goes down the drain.

I think these are issues that are probably being grappled at to some extent now, but a lot more thought and work needs to be placed on these issues.

DR. WILKERMAN: I would like to come back with a short remark and a question to the comparison of the properties of the behavior of some viruses and the question of model viruses.

I think that Thomas Kreil presented very nice data I think demonstrating that West Nile Fever Virus is as sensitive as we thought or as we predicted from other studies and that we can assume that the manufacturing processes which are in place for inactivation are really factors.

I think this comparison of the kinetic even at conditions which are a little bit strange to these procedures give excellent information about it.

I have two questions. First, can we expect that such data are soon published, and the second question is what is with the other methods which are working according to another mechanism? I mean INACTINE and psoralen and riboflavin.

If you look directly on the kinetic, do you see then differences between West Nile Fever Virus and BVDV or the other viruses which have been used, and I mean mostly BVDV has been used, do you see differences if you look really in detail on the kinetic?

DR. ALFORD: I will answer your second question first. We see a little bit of difference between the kinetics of BVDV, Sindbis, as examples, HCV if you are going to look at it, but specifically BVDV and the different isolates, in fact, on the West Nile Virus that we are using. We have used four different isolates to date.

As I hope I portrayed, our information is pilot, we have just started. We hope to get much more information in the next few months.

On a publication perspective, one of our goals is to publish it as much as possible. In fact, in the most

recent issue of Transfusion, we have three publications in that.

One of our three collaborators is struggling very quickly because he wants to be the primary author, and we are going to get that paper out as soon as possible, so that we are sharing not just the results, but the methodology that is used.

So, if there is comments on the methodology or critique on our methodology, we would be glad to discuss that further. Thank you.

DR. WILKERMAN: Thank you. And PPTA, will PPTA publish it, the data of PPTA?

DR. KREIL: I can obviously not comment for PPTA, but the data that we have seen from Baxter Bioscience, they will be published very soon.

DR. FARSHID: Dr. Lynch.

DR. LYNCH: Tom Lynch, Clearant.

I had an editorial comment actually on the use of model versus relevant viruses. Your statement that relevant viruses must be used when they exist and when they are available as laboratory strains is I think an accurate reflection for the most part, but not entirely true, a

little bit oversimplistic. There are some viruses that are simply considered non-issues for plasma derivatives even though they exist in the source material or blood itself.

Some are simply not considered important enough to assess and are not looked at. There are many, many viruses that one could isolate from a human blood or plasma donation if one wished to, and the idea that each and every one of those, where they are available for cultivation in the laboratory, must be evaluated, is I think too broad a statement, but the principle derives from a time when the tools that were available to assess these methods were far less sophisticated than they are now.

The focus 20 years ago on these very specific risks has shifted today to a much broader assessment of the capabilities of these technologies. This idea of robustness that came out of Europe to assess the breadth of effectiveness, not just the effectiveness to one, or two, or three or four specific viruses has proven to be very valuable, and the data that I have seen today on West Nile confirms the value of that approach.

The fact that these techniques have been evaluated for their robustness in this sense of being

broadly effective, and then the confirmatory tests that says this virus does, in fact, behave as we expect it to, would suggest to me the need to assess whether or not all the resources to revalidate all these products and all these processes is putting the resources where they matter the most.

There are other problems, there are other viruses that may be more important to focus on if West Nile does, in fact, prove to be representative similar to other flaviviruses.

DR. FARSHID: If I may say that I did not imply that every relevant virus and every virus was confined in plasma should be included in validation study, but if we determine a virus is relevant and pathogenic, then, and is available to do the experimentation, I don't see any reason not to use it.

So, if we are faced with an application and the manufacturer comes and say we want to do the validation studies, why not ask them to include West Nile Virus basically, as I indicated, to increase the degree of assurance in regard to capacity of the end process to

inactivate the virus. It is pathogenic and a virus of concern, and should be included.

If it is not feasible to do that technically, that is a different question.

DR. WILKERMAN: I completely agree with your position in general. I think in the case of West Nile Fever Virus, it is I think relatively difficult to give the general or to require in general that all methods are revalidated toward this virus. I think it lasts relatively long.

I mean if it is possible to compare those viruses and to demonstrate by, of course, not only with one method, to demonstrate on a broader basis that the behavior of this virus is really sufficiently reflected from the model viruses which we have used already, then, I think this is also an acceptable approach.

West Nile Fever Virus, we could ask the question is it possible to replace maybe BVDV by West Nile Fever Virus because, of course, if West Nile Fever Virus would have a similar behavior, maybe that is similar to HCV, we cannot check it because we have HCV not in our hand.

But I think we should also consider that this virus is not so easy to handle. It is a virus which requires laboratory Level 3 for the general use, and so I think we have to be a little bit cautious with such recommendation.

On the other side, we are in a status or we are in the situation that we have already I think a lot of data which demonstrates the robustness of manufacturing processes which are in place, of course.

I think if it can be demonstrated that for these processes, which we use already a longer time, and for which we have some really good databases, if it is possible to demonstrate that West Nile Fever Virus fits very well into this databases and confirmed what we know already, then, from my perspective, this would be sufficient, or sufficient maybe is not a good term, maybe this would convince me at least.

So, it is a little bit different if you are looking on other methods, so it should be demonstrated again, I mean if we look on other mechanisms of action, then, this should be investigated I think.

But on the other side, it is impossible. I think we have to trust a little bit on some robustness studies which we have seen already if it is equivalent with the viruses, to the other virus, and can be demonstrated by basic experiments. That is my present opinion.

DR. FARSHID: Dr. Busch.

DR. BUSCH: Focusing on the cellular products, I think if we were three or four years down the road where we had inactivation of both the platelet and the red cell products, as well as the FFP, which I think is where we have to be to be relaxed now and not consider testing, it is interesting to think about whether we would be here today and whether West Nile would cause still a concern.

I think it would. I think we have all discussed and I think at the FDA Workshop on Pathogen Reduction, there was pretty much of a consensus that once these methods are introduced, we will still need to serologically screen, we will still need to NAT screen, because your methods, although very impressive, they still may have problems with very high titer viremia, so it is really an additive safety issue.

We are hearing today on the derivative side, enormous kill, and yet there is still concern, there is still debate. We are still hearing the consideration of testing of plasma for fractionation. We saw NGI's presentation yesterday, they seem to be moving toward introducing West Nile Virus NAT even though their focus is strictly derivative manufacturing.

So, in just trying to think forward, I think we would still be here if all of your methods were in place and we still might need to test or at least do a lot of studies just as is being discussed for the derivative side.

DR. BULT: Jan Bult, PPTA.

I would like to add to the comments made by Dr. Lynch and Dr. Wilkerman. There is no way that I could have said it much better than they did, but I would like to remind FDA on the presentations that were made on behalf of the industry, where you can see that first efforts have been made to test for the actual virus, and as you have seen at the presentation of Dr. Pifat, there is a commitment of the PPTA member companies to perform additional studies, and if we add that together with comments made by Dr. Lynch and Dr. Wilkerman, then, I would

encourage FDA to look at this data before final decisions are being made about full validation studies.

DR. FARSHID: Actually, I need to indicate that the view I presented here is not FDA's views, they are mine, and we definitely would look at those data, and there is more deliberation needed to be done before the final conclusion can be reached whether the products currently on the market need to be validated and their validation study needed to be supplemented using West Nile Virus.

In my opinion, with this data, that may not be necessary. As I indicate again, this is my view, it is not FDA's position. We have not reached a decision at the agency level how to approach that.

However, the comment that I made is in regard to a new product. If a new submission is being sent to the FDA and we are asked to evaluate the model viruses which are used for doing the inactivation study, I think it is reasonable to request that West Nile Virus, which is pathogen, is of concern, and is available and can be tested, should be tested to assure that the method that is being used has the capacity to inactivate the virus.

This is simply for the new application and if someone wants to do the viral inactivation. I think it makes more sense to use the real virus rather than extrapolate. We have a large amount of data which shows that the viruses, they may behave differently, and we are dealing with a complex biological product, and the manufacturing processes are different, and these manufacturing processes have their own effect on how the inactivation methodology will be effective, and this has been shown.

Therefore, just to be assured that basically, that the relevant pathogen need to be included if possible.

DR. NAKHASI: I think I just want to say again I would like to compliment to the industry that they have done the studies. I think nobody denies the fact, and the processes are ongoing.

I think the very fact that this virus is dangerous, and somebody made a comment it is very difficult to get the BSL-3 facilities, and the very fact that this virus is dangerous and it requires BSL-3, begs the fact that it should be shown that it is inactivated in the process, and I think ensure the safety of the process even

though, you know, the data showed this morning that the inactivation process is the same as the other viruses, but since again I would like to reiterate the point. As long as there is a virus available and can be grown, I think it begs to the point that it should be shown that it is inactivated. Thanks.

DR. FARSHID: One more question, then we break.

DR. FITZPATRICK: I was just curious about the slide on washing and the statement that further reduction of viruses or prions was provided by the washing steps when we know from the past that we thought washing removed viruses, and Dr. Alter showed that we really didn't do that, and I was just curious as to how you were substantiating that washing was further reducing the load.

DR. ALFORD: I am sorry if you gather that from my presentation. I didn't speak to that washing had any effect on the viral load whatsoever. In fact, in the experiments that we do on viral inactivation, we stop the experiments by quenching, so it purely is the inactivation step. We have not studied the viral inactivation due to washing. I am purely representing it as chemical inactivation.

When I talked about prions, we have done some spiking experiments with some soluble prion proteins both platelet derived, as well as the alpha and the beta recombinant forms, and with those spiking experiments, we have just demonstrated a log removal of the soluble proteins, and they seem to track with other plasma proteins like albumin and serum.

So, what that demonstrates is that there are proteins that are removed, specifically, some serum proteins. We study IgG and albumin obviously also during that washing process.

DR. FITZPATRICK: Thank you.

DR. FARSHID: Thank you. Now we take a 15-minute break. Please don't forget to go upstairs for the next session.

VI. Proposed Studies on Prevalence in Donors

Chair: Mary Chamberland, CDC

Linda Harvath, NHLBI, NIH

DR. CHAMBERLAND: Thank you for your patience. In addition to a little rearrangement in the room accommodations, we have also rearranged this session a little bit at the request of the participants.

This is Session VI, Proposed Studies on Prevalence of West Nile Virus, and Liana Harvath from NHLBI will be co-moderating the session. The participants have requested that instead of three individual speakers, Mike Busch is going to speak about the project that is being proposed, and he will then be joined in a panel discussion by Steve Kleinman and Sue Stramer from the Red Cross.

Mike will be our first speaker, Mike Busch from the Blood Centers of the Pacific.

The NIH Collaborative Donor Prevalence Linked Study

DR. BUSCH: Thanks, Mary. I am happy to represent the study group, which actually formed essentially the day that the first potential transplant cases were reported by CDC, we began to call around, convene conference calls and begin the process that is still evolving as you will see to develop the best study we think we can to try to understand, not only the prevalence of West Nile viremia in the donor pool, but also scientific questions, transmission rate, cofactors that determine transmission, and disease outcome in donors and recipients.

As I think you will see, it is a struggle. I remember Indira yesterday talking about the virtual repository and in a sense this study is like a virtual study because we are really trying to chase an epidemic that is very regional and very temporal, and as I think we saw yesterday, by the time the first cases were reported, the epidemic had essentially already peaked, so the effort to try to capture specimens that are optimally relevant to determine prevalence has been a challenge.

I just want to first again acknowledge the work of a number of people. This effort is kind of co-sponsored by NHLBI and CDC, George Nemo and Liana Harvath from NIH and Mary and Lyle, who you know, but also Ken Clark, who is really the person at CDC who has been focused on developing it originally, as we will talk, probably later, there was a concept of a Phase I link study that Ken was driving, and then this larger link study, and now essentially these two have merged.

Within the REDS radar group, I particularly want to acknowledge Simone Glynn, who is really a wonderful physician and statistician, who has driven the Westat team to put together the protocol, and then, as you will see,

the study that we will be pursuing involves a combination of some existing ongoing donor/recipient cohort studies called the RADAR and TRIP study, and within that radar program, Steve Kleinman is the lead on that project for REDS and Dale Truzey [ph] who is not a primary REDS investigator, is leading the site in Pittsburgh that CDC is supporting, the Institute of Transfusion Medicine.

As you will see again, the TRIPS study is kind of a clustered study within this larger donor/recipient follow-up study to Harvey Alter is leading. It is essentially a continuation of Harvey's historical donor/recipient follow-up studies, but involves much more frequent samples and characterization of genetic markers of all the viruses in the serial samples.

But as we will get to, in addition to these historical repositories or these repositories that are being built prospectively, we realized quickly that the samples in those repositories would not be adequate, so we engaged Sue Stramer and Chyang Fang and Roger Dodd, and as you will see, Red Cross is contributing a large number of additional specimens for use in this study.

In addition, we have additional donations that we have identified from some of the ABC Centers, Sally Caglioti from UVS, Darrell, actually representing the Life Source Chicago collections, and Mike Strong who is a representative from the Roche Group and, as we will talk later, there is now an effort to capture specimens at Roche collection Sites

This really is a study in progress, and we are engaged very carefully with the NAT testing manufacturers to try to identify and employ the best test we can and the blood screening assays as they are being developed in the study.

The objectives of the study, first, we have an understanding that we really have the challenge of assessing the performance of the assays, particularly the RNA, but also the IgM assays, so really the first objective is to establish an analytic sensitivity panel that we can use to validate the RNA tests that will be the primary screening assays for the study, and our target is to have assays that achieve or exceed a less than 50 genome per ml detection limit.

There is now work in collaboration with the CDC Fort Collins Group to really get these panels put together and distributed, so we can really understand the relative and absolute sensitivity of these assays initially focusing on the assays, the candidate assays to support the link study, but then subsequently or in parallel, build larger panels, perhaps in collaboration with some of the commercial companies like BBI that are building these panels already, established panels for both RNA and IgM assay assessment that we can use as the blood industry to compare these assays and understand the relative performance and role of these assays, both the screening tests, as well as confirmatory tests, if donor screening moves forward.

The second set of objectives relate to defining the prevalence of viremia and the disease outcomes and the donors, so here, we will be testing archived donor samples for RNA using the most sensitive RNA tests we can identify and as single donation testing, so that we can identify as many viremic samples as may exist to the limit of detection of the assays.

There again, this study will exploit the specimens that are available regionally, as well as temporally, to maximize representation of hot zone specimens, but also with the inclusion of samples from other regions of the country, so we can generalize a national prevalence estimate, and that will be sort of linked with Lyle Petersen's estimates of the relative prevalence around the country of West Nile disease.

Once we find these viremic donation samples, we will characterize the viral load, the IgM status, and they will be subjected to culture to try to understand the determinates of transmission and also to guide the decision about what will be the advantage of trying to move to an individual versus bringing up West Nile on minipool NAT and is there ability of IgM to detect a subset, a significant subset of these viremic donations.

The donations that are found viremic, this is a linked study and the protocol and consents and follow-up materials are in place, so that we will recall the donors, verify the infection status through both IgM and RNA testing, and also to recall the donors administered

questionnaire as to whether they recall having developed symptoms shortly after the donation.

We will also look at the blood centers routine callback records to see if any of these donors in fact did call back with the enhanced FDA recommended callback system to find out, you know, again, was there disease in any significant fraction of these donors.

The other thing we realized we needed to understand was the background prevalence of infection in the donor pool, as well as in the recipients. Most of these samples we will be testing actually will not have pre-transfusion samples from the recipients, so we will have recipients we are recalling three, six months after they received a blood transfusion, and we will be assessing the transmission rate from viremic donors by determining the IgM rate in these recipients.

That needs to be viewed in the context of a background rate within a recipient population to determine the transmission rate. So, we have designed two sort of control populations.

The first will be to look at we are projecting about 10,000 representative allogeneic donors from

different West Nile activity regions, do IgM, and with that rate of IgM positivity, we can I think work with Lyle Petersen to feed his model to estimate the incidence rate based on the crude idea of reactivity, and knowing the incidence rate, one can estimate the transfusion risk given the understanding of the duration of the viremic window period. So, that is one piece, the allogeneic donor piece.

But, secondly, rather than trying to enroll a large population of control recipients, recipients who did not get viremic donations, we are actually going to use autologous donors as a surrogate for recipients.

We have done work recently, Steve Kleinman presented at AABB an analysis. Autologous donors are essentially people who are coming in to get a transfusion in the next week or two, and they are just giving to themselves.

What we know is that the rates of all the markers are very similar in autologous donors in pre-transfusion samples from recipients and in the range of 8- to 10-fold higher than in allo donors, so we are using auto donors as a surrogate to get a background rate in recipients, and these other donors, the samples will be tested in an

anonymized context, non-linked, so we don't need to recall those people, and the samples will actually be collected, the samples that we will test will be samples that will have been collected around the same time as we are testing the recipients.

The next issue, and the critical really outcome of the study, in addition to donor viremia, is transmission rate to recipients. We really don't understand that particularly, the relationship if we do find a population of low level viremics, you know, are they infecting recipients, if IgM is present in a subset of viremics, does that influence transmission rate.

So, we will be recalling the recipients of the viremic donation, testing them for both IgM and RNA to determine did they become infected, looking then at the donor and recipient factors that may influence whether transmission occurred, so viral load in the unit, IgM status, infectivity in tissue culture, and the recipient sort of underlying disease considerations.

Also, then, recipients will be administered a symptom questionnaire to try to determine what proportion

of recipients of viremic units who became infected actually manifested West Nile related disease.

Now, to show you the kinds of samples that we have, there are two repositories I alluded to, one RADAR, the other TRIPS. Now, RADAR is an NHLBI-sponsored linked prospective donor recipient study. It began about two years ago at seven regional sites.

The goal of the study is to enroll 4,000 recipients having pre- and post-transfusion, about six months post-transfusion samples from these recipients. We are about almost at 3,000 at this point, so this is a study that is going on at the opportune time.

Now, these 4,000 recipients will have received about 15,000 donations, so we will have 15,000 donor exposures over this period of time in these regions. In order to be sure that the maximum number of donor units go into the recipients, as you will see, the blood centers actually over-collect donations and designate them for RADAR recipients.

So, we have about 8-fold the number of donations in the repository than actually went in to the recipients

who enrolled in the study, and that becomes a factor in a few minutes.

The strategy of this, it is a study that is really intended to test donation samples and have the specimens from the pre- and post-transfusion samples and the recipients in the freezer, so that as a new agent like West Nile now comes along, we can very quickly go to these samples and simply test them.

The recipients have consented to and the donors to storage of their specimens and to subsequent testing for new agents although we do have to go back and get IRB clearance that all the pieces are in place, but we do not have to reconsent the donors and recipients.

We have both frozen whole blood aliquots and plasma aliquots from donations and recipient samples. The general strategy is to test the recipient 6- to 12-month sample, and then if the recipient is positive, then, we test the 3 to discriminate a transmission event from a pre-existing infection in the recipients.

The TRIPS study stands for Transfusion Related Infections Prospectively Study. It is Harvey Alter-ism. This again is an ongoing study that essentially carries on

what Harvey has done for several decades, focused in the D.C. area. In addition to the NIH Clinical Center, the study actually includes a site at the Children's Hospital D.C. site.

This is somewhat similar although Harvey is really endeavoring to get all donation samples for all enrolled recipients frozen away. He is focused on highly transfused patients whereas the RADAR is particularly looking at orthopedic type in cardiac bypass.

Now, Harvey's, the big difference again is the RADAR only has pre-transfusion 6 months, whereas Harvey's collections include essentially weekly samples for the first month, then monthly, so it allows much more careful characterization of the time course of events in infected recipients.

In fact, in a prospective context, these recipient serial samples are being monitored for all the viruses shown here by both nucleic acid, as well as serologic methods.

So, these are, you know, wonderful resources and a large investment of both the CDC and the NIH to build these repositories, but as we have come to learn, when you

have got something as temporal and focal as West Nile, this repository is not the whole answer.

So, what you see here are the samples in the repositories from the participating centers by month, so it includes both the RADAR and TRIPS samples.

So, we have a total of about 15,000 donation samples of over this 4-month period of interest, but when we look then at the West Nile activity within the regions represented in the study, it turns out that only 3,000 or so of the 15,000 samples are from regions that have proven to be relatively high in terms of West Nile activity.

In addition, if we look at the curves that Lyle showed yesterday, and the fact that the epidemic really peaked in a very brief, you know, one- to two-month period, only a subset of these samples are really very high yield samples.

In addition, as I mentioned, we over-collect in order to have enough donations to support the enrolled recipients. We enroll and freeze away a large number of additional units.

What this slide shows is the percentage of the RADAR 15,000 donations in the repository during the period

of interest that are actually from recipients, the corresponding units that went into enrolled recipients, that were linked to enrolled recipients.

You can see that actually only 13 percent of these units have recipients for whom we already have pre-sample and post-sample in progress.

So, in fact, in this study, we are not only intending to test the link, but we will also test the donations that went into the recipients who did not enroll, and these recipients will be traced through lookback. We won't have a pre-transfusion sample, but these are important samples to contribute to a prevalence and estimate, and through lookback, will determine transmission rate.

Now, again realizing that the numbers from these formal studies were not sufficient, we continued to closely work with Sue and the Red Cross folks. Sue immediately on, you know, the appreciation this was going to be a problem beginning of September, initiated retrieving and freezing away plasma, actually freezing down the residual plasma in the PPT tubes, which are used at Red Cross for NAT testing.

This slide shows the number of specimens that the different sites have frozen from sites that, in conjunction with CDC, were determined to be potentially important sites for prevalence.

You see that she has got 27,000 donations from St. Louis, 23,000 from Detroit, 21,000 from Cleveland. These are collected over about a six-week period, and we are working now with Lyle to determine which of these samples are the highest probability given the temporal epidemic in these regions, and just continuing three additional sites of Gulf Coast Red Cross collection, Chicago, and Memphis, the numbers you see here.

But again if you focus on the periods of interest, we will probably end up focusing or prioritizing testing a subset of these specimens.

Actually, this then summarizes the available specimens and the subset that are likely to be of reasonable probability of yielding viremic donations based on the epidemic, so these are the numbers we just talked to, only 3,000 of the 14,000 or so in RADAR and TRIPS are likely to be informative for yield. Potentially as many as 45,000 at the Red Cross, 90,000.

We have also supplemented with 3,000 samples from both the Mississippi UVS collection site and the Life Source Chicago collection site, separate aliquots frozen away for the link study, and there is close discussion with Roche.

The Roche Net System creates an archive plate, which is a 1.5 MLD bottom microplate archive, and our hope is to work to use some of these samples in conjunction with Roche at either in an initial phase, perhaps at the CDC Fort Collins test lab, but subsequently, as Roche brings up their assay in the spring, we hope to work with them to employ the same protocol to expand the data on prevalence and transmission.

So, a total of over 100,000 specimens available of which potentially, more than 50,000 may be informative.

Again, just to step back, in terms of the study, we sort of defined two phases, a Phase I, which is really the period where we are now working to establish the performance of candidate assays, benchmarking these candidate assays that are being developed to get program Roche assays against the Fort Collins' assays, so really creating panels that can, head to head, define the relative

sensitivity and absolute sensitivity of these tests, also developing larger performance panels that would be available to anyone who would be interested to assess the performance of the RNA, as well as IDM tests.

Then, the major Phase II component, which will involve testing. The plan is to test at least 50,000 specimens over time, perhaps in a phase mode, prioritizing these samples that have the highest probability of viremia, again over this past summer/fall.

This will allow us to define the prevalence of viremia in the donors and to characterize the viremic samples, recall the recipients and testing of the recipient follow-up samples that are already in the RADAR/TRIPS repository will allow definition of transmission rate and lookback will be required, though, for the majority of samples because they are not enrolled in that study, and we will be able to look at correlates of transmission.

I mentioned that we will be getting background rates both in allogeneic donors to model the probability of window phase donations, as well as in autologous donors to assess the background rate of recipients for definition of

a transmission rate from the viremic donations by subtracting away the background rate.

Timing sort of summary. Again, fortunately, these RADAR/TRIPS samples have been collected, you know, for the last few years, and continue to be collected through the entire epidemic, but unfortunately, they are not in the hottest locations and temporally, the numbers are not sufficient, so this is why we kicked in these additional sites beginning in September.

We have also been working actively and have a protocol that is going through IRB clearance now.

We don't need to go through all of this, but the laboratory component of this is really a plan to launch in the beginning of next year with the major testing being in the first quarter of next year, so we would have data available to guide policy issues.

The recalling of the recipients and the donors will trail immediately after we find viremic units, we will immediately proceed with donor and recipient lookback, but will likely continue through mid-summer of next year.

Then, the analyses will phase in, in terms of the initial analysis of performance of the assays, will go on

over the next few months, but the major analysis of transmission rate will occur next summer.

Just to close, I just want to throw out sort of some power sort of analyses that ask the questions of, you know, how big does the study need to be, and given as big as we are able to do, you know, what is our confidence of being able to rule out a significant prevalence or define a transmission rate with precision.

The first thing to sort of think about is if we test a certain number of units, and we don't find any viremics, what is our confidence bound on the possible prevalence in the donor pool.

So, it turns out that sort of this so-called Rule of 3, if you get zero, the 95 percent upper bound is 3, and you divide 3 by the denominator, and you get the 95 percent confidence interval. It is an easy way to do it, but there is a computer that does it.

You get these kind of numbers. So, the bottom line is if, for example, we test 50,000 donations and find zero events, there is still a 5 percent probability that the prevalence could be as high as 6 per 100,000, which is not very reassuring for blood bankers.

So, this is why a study of 50 or 100,000 and a negative result doesn't give us the confidence we like to say there isn't a significant risk.

Small numbers here, but just the principle, and I will walk you through sort of one line kind of thing. The next question is how many viremic donations will we find and what is our confidence bound around the prevalence of viremia dependent on the true prevalence and the sample size.

So, if we take, for example, this first line, which is maybe realistic given Lyle's more updated models, where the prevalence may be as low as 5 per 100,000 or 1 in 20,000, and we test 50,000 donations, we predict picking up, you know, only 2 or 3 viremic donations, and the confidence bound around the prevalence of viremia would go from zero to 6.

If we can push these numbers way up, if the prevalence is the same and we had 200,000 samples representative of the areas of interest, then, we would pick up 10, and the confidence bound would be 4 to 16.

So, these are not very big numbers in terms of viremic donations to address the other questions of the

study, the transmission rate, determinants of transmission, disease outcome. So, it is all really dependent on the true prevalence and our ability to enrich for prevalence by focusing in on higher prevalence regions.

We can also ask in terms then of viremic donations and then recalling recipients and assuming, I forget the exact assumptions, but I think we assume we will get 1 recipient enrolled with outcome for every 2 viremic donations just due the realities of lookback tracing and testing.

So, with that, if we take, for example, this first line of .05 or 5 per 100,000, and only test 50,000, the likelihood is we will only pick up 1 or 2 enrolled recipients, and 1 or 2 donor recipient pairs, so we will get a very imprecise estimate of the transmission rate.

This is actually the confidence bound around the transmission rate at the two bounds of that.

So, the point here is, is that when the prevalence rates we are predicting, and the numbers of samples we have available, we will probably only end up enrolling a moderate number of recipients, and not get a real precise estimate around the transmission rate.

You can take it the next level and say, okay, well, I have given the same potential prevalence rates and sample sizes, and assuming transmission rates of 25, 50, or 75 percent, how precise can we be around a probability that these recipients will have developed West Nile disease.

So, these give us confidence bounds around those disease rates, and nothing specific here for you to take away other than that because of the low prevalence, and the limited availability of samples from the critical time periods, we don't think we are really very well powered to look at these secondary questions of transmission rate and disease penetrance. Nonetheless, we believe the study is critical to conduct and are proceeding.

The one sort of change that has evolved recently is a decision to prioritize the best samples from the study and probably have those tested over the next few months at the CDC Fort Collins lab.

They will only require a small fraction of the volume needed, so there will be ample volume to come back to be licensed or to be implemented assays, but the idea is to probably prioritize sort of the 5,000 best, but based on

the prevalence data, the 5,000 best, most likely viremic samples and test those first.

Thank you.

[Applause.]

DR. HARVATH: Thank you, Mike. We have about 15 minutes for the panel discussion. I would like to invite Steve, Sue, and Mike. If there are burning questions in the other room, please feel free to join us.

Discussion

DR. KLEINMAN: Liana, I would like to start off by making one comment, if I could, and that is, it is hard to know at this point how productive this study will be, as Mike mentioned, because we simply don't know the prevalence rate, but we have worked out very good, I think, donor and recipient materials having basically started with what CDC has, and protocols for donor and recipient recall.

I think one additional benefit from this study could be that we may be able to take the approaches that we worked out, and if we can make these available, perhaps manufacturers, as they submit INDs, can use for testing next year assuming that is going to happen, can use these fairly uniform approaches, so that if we don't get enough

transmission data based on this research study, and if we are in the circumstance of doing testing, at least we may have good donor follow-up data if everybody does the same sort of thing next year, and obviously, you won't have recipient data because those units wouldn't be transfused, but you could have lookback data.

Just a thought that came to me while Mike was presenting, that there is an additional benefit of having done all this early work.

DR. STRAMER: I would like to just add to what Steve said, and, in fact, the cases, the Red Cross and all the blood centers are investigating now, which use some of the donor follow-up materials, will only complement the study and we will be able to build a stronger database by what we have collected through case investigations through what the study yields.

DR. BIANCO: Celso Bianco, America's Blood Centers.

This is very nice, but somewhat frustrating because of the fear of not getting the results. One thing is that if the study group distributes those materials, it won't be bad at least at ABC to have all our centers have

access to those materials and try to be uniform, and I am sure that within the Red Cross, that is not an issue.

I just would like to hear your thoughts. You must have thought a lot about what if we don't get anything, what is that going to do to us next year, next year when the manufacturers come up with assays that are based on whatever samples are available, some spiked, which is we just go ahead?

DR. KLEINMAN: Well, I think it is an important question. In fact, when I present, you know, that is exactly the question that we have, you know, the AABB will have in its statement what is the threshold for deciding that that testing will proceed.

But in thinking about this, it seems to me that we would have two different--we have a scenario today that is different from one that we had a month ago when we planned the study, and that is we didn't have these well-documented case investigations a month or two months ago. We knew of the one organ donor early on, but that was it.

So, I think if we think back six weeks or so and we said negative results might have a significant influence on policy, because, in fact, we may not have a problem, but

I think in the face of six proven transmissions, you would have to say six proven transmissions, and more perhaps to come since cases are under investigation, that is going to be more meaningful data in terms of policy than the results of the study.

Now, the results of the study, if we find viremic donors, may be helpful in terms of knowing how well the assays will perform, whether minipool testing is sufficient, what the role of IgM is and all these kinds of things, but I think in terms of a policy decision, we have another source of data out there, which is the case investigations that I think is a more meaningful source of data than what will generate from this study.

You can still ask the question do we have enough transmission from the case investigations to justify screening, and I think hopefully, we will have a discussion of that this afternoon.

DR. BUSCH: I would just add that we are going to be testing with a more sensitive test than probably is viable for implementation next year. We will be doing single donations and the high sensitivity assays, and realistically, everyone here knows mass screening next

year, if done, will be done mostly in pools. We might be able to modify pool size, but there is no way to bring up single-unit testing on a national level.

So, the study is actually probably better than routine screening, but the numbers are too small. In thinking this through and actually talking with Jay Epstein last week, if we had, say, 100,000 reasonable prevalence samples and found none, then, I think even in light of the case reports, there might be a more balanced consideration of having capacity to test, but actually tracing the epidemic closely, and implementing potentially regionally, temporally, but prospectively, I mean what is not allowable according to FDA is to not have a test that could be implemented prospectively next year.

But then realistically, and Sue can speak to this, historically, we have never thought about regional testing, selective testing. It is almost impossible to implement systematically.

DR. NAKHASI: I just wanted to echo Celso's feeling again. Looking at the balanced rate, and from Lyle's data, and what you presented here, the question is obviously you will find, and the question is what is the

denominator, how large the study has to be to really find those samples.

The second question is are you planning to include next year, samples, because obviously, if there is an epidemic next year, are we planning in advance in the conjugation of this study, to take care of those samples and to involve the samples?

DR. STRAMER: I think the premise was by the next mosquito season, we would have screening in place. Now, if that doesn't happen, I think then we would have to look at whether it is expanding the numbers to the study or continuation.

I think the message that we got at the AABB meeting that I think we had on September 20th, was we have to move lightning speed ahead and get some type of screening method in place at least by the next mosquito season.

So, I think as part of the IND process, we will build in research questions, but I think those will change as we bring up the IND tests.

DR. NAKHASI: Also, I didn't hear you mention, are we taking care of both the serological as well as the

nucleic acid testing? Again, you said it can be minipool or NAT. Could you elaborate more on that, please?

DR. BUSCH: The concept was to primarily screen the full set of samples to be tested by RNA with single donation RNA. We could then reflex those samples both to a sort of dilutional or minipool type assessment to see would they have been detected by minipool.

We also intend to test those by IgM to determine how many of those viremic units actually could have been detected by an IGM test. In addition, the plan is to test approximately 10,000 representative samples for IgM that are not RNA positive to get a rate of IgM positivity across the regions of study.

DR. KLEINMAN: I think one additional comment, though, is the IgM aspect of the study is, of course, dependent upon having an assay, an IgM assay, that can easily test 10,000 samples. It is quite clear that CDC cannot test 10,000 samples by ELISA, and I don't think that there is yet another identified source for IgM testing, so that may be something that doesn't happen in as timely a frame as the RNA testing although if manufacturers work on

developing that assay, I think we will have to qualify a candidate IgM assay as it comes along.

DR. STRAMER: Conversely, if there is an IgM assay on a platform that can be done in a more automated fashion than the CDC assay, ideally, actually, all of the samples should be tested, and the IgM assays would through an immune complex disruption procedure, so if there is low level virus, we would be able to detect it in these samples.

Certainly, a study of 10,000 may not identify enough IgM positives that have low level viremia, and in order to really identify the utility of IgM, the numbers may be increased, but as Steve just said, I think it is dependent on the availability of an assay.

DR. BUSCH: Two comments. One is in terms of next year, actually assuming we are screening minipool, one thing, the study could actually continue and freeze down the samples from regions that are high West Nile activity, and retest them singly as part of a research protocol, and then we will have recipients of those products and could get more information on transmission rate.

The other thing that I think came up last night in continued discussions is perhaps the most important aspect of the study is really to give us better confidence in the accuracy of the model, because that is really, you know, we always are modeling everything, everybody believes those things, but it is important to have data.

DR. NAKHASI: Absolutely.

DR. BUSCH: And the IgM data, you know, the samples we have are actually great for IgM testing because they are on the heel of the epidemic. I think, like Sue says, maybe expanding the IgM test component and understanding the rate of recent infections will allow us to get a better sense of Lyle's models appropriately.

DR. STRAMER: In some of what we learned yesterday, looking at the titer of IgM, at least in a research mode, ratios of IgM to IgG, we may be able to plot a time course to when IgM positives would have been infected.

DR. KLEINMAN: I think the goal at this point is to build the research protocol to be as flexible as possible, because we are trying to research something that is a moving target obviously, and given the administrative

requirements of putting a research study in place, that is, drawing up the protocol, getting IRB approval, getting funding, you know, that obviously takes a number of months to do.

So, I think we want to have all those pieces in place, so that we can start the study, actual testing early next year, but I think we also want to build it in a way so that it is easy to go back to IRBs and say, well, we have amended the protocol and we can get a quick turnaround time, you know, presumably within a month to approve these amendments.

So, I think you are seeing, as Mike said, a work in progress and depending on where we are, as long as we have the sample sources and the procedures in place, we should be able to change the testing to reflect the current state of the art, and as Sue said, even keep the study going longer even in the face of screening, we will not have answered all the questions, and there is room to do additional either RNA testing or IgM testing even if we have something in place for routine screening next year.

DR. NAKHASI: Along the same lines, are there plans just not only focusing on West Nile, you know,

yesterday's discussion was could we expand it to other, you know, Japanese encephalitis, for related families of viruses, are you planning to look at that?

DR. BUSCH: No.

DR. STRAMER: I think that would be just kind of nonavailability of our agents to do that. We have samples now in the repository, we are not rate limited, I mean that can be done, but reagents would obviously be needed.

DR. KLEINMAN: There are two issues. With regard to RADAR and TRIPS, we do have permission to test for new agents that might be threats, so I think it would not be difficult to extend the study to that if the reagents existed.

With regard to the Red Cross samples, these were not obtained in advance with consent of the donors to test for West Nile because that wasn't really a consideration. So, I think because of the problem with West Nile, the IRBs won't have any problem with testing for West Nile. Whether they would also extend that to testing for other flaviviruses that are not acute public health problems, using those same sample sources, I think remains to be seen. I guess we just don't know at this point.

DR. STRAMER: No, we don't know. You would have to take that up with the IRB, but I wouldn't anticipate it is a problem if you are looking at emerging threats.

DR. BIANCO: I would like just to reinforce what actually Mike touched on first. I think that if you could, you should start planning to continue the study next year in face of the fact that most of us will be testing in minipools. In certain regions, as soon as the birds start falling off the trees, you could focus on those populations because in minipool testing, we will not know at that point what the sensitivity of that testing will be.

Since you already are applying for IRBs and all that, it would be extended a little bit, and I am sure you can convince NIH and others to continue, and CDC, to continue funding.

DR. BUSCH: I am not so sure about that one, but if you look at the dynamics, both what we saw yesterday in the hamster data, which I thought was very nice from the Vitex people, you know, this ramp-up is so fast. The difference of a pool size of 10 and 24 is trivial, I think, so I really don't think that the issue--I mean we all know the debate over ID versus minipool for HIV and HCV, and we

are comfortable with minipool at least on an interim basis, and I think we should be very comfortable with minipool for this virus.

The dynamics are so brisk that even though there is relatively low viremia, it is not that low, and it comes up fast, so I agree with you that we should do that, but I don't expect we will find much that was missed.

DR. TABOR: That was a nice summary of the planned studies. I don't know whether I missed it, but I don't think I heard you say which tests, whose tests, whose methods you were going to be using.

I want to use that to ask a question. Clearly, we are in a situation where the test methods are going to be evolving. We heard yesterday that some of the companies are using the same platforms that they use for NAT testing for HCV and HIV, but we also heard about IgM assays.

We heard that IgM assays are rarely positive in the same sample as PCR-based assays because we have an evolution of the serologic picture, the serologic and virologic viral load picture that we don't fully understand yet.

I also got the sense from the presentations and things I heard on the break that the people working on IgM assays are going to be--they must have something in the works because they are people who think that IgM assays are going to be used next year, and clearly, there are problems with the IgM assays because of their long period of positivity after infection, and maybe the fact that they are aren't caught early enough.

So, I guess my question is how are you going to take into consideration the fact that these tests are going to be perhaps a lot better, some of them are going to be a lot better, say, next spring than they are today, are you going to retest things, are we going to have to relook at the data?

DR. BUSCH: I think on the IgM front, we really don't I think anticipate--again, the primary screen strategy will be RNA because I think most people believe that is the strategy, to interdict by infectious units.

The IgM testing isn't planned until next spring. The major initial focus of the study, actually Chyang Fang is leading a working group that is building these panels, and we are now going to work closely with Rob Lanciotti in

CDC to integrate their performance panels that we heard about yesterday, along with additional samples, and really compare these assays, both the RNA assays and the IgM, so we hope to have data to decide which test is the best available at the time we need the data.

I think the discussion this afternoon and now needs to be is the data from this study going to be a determinant of a policy of do we screen or not and how do we screen, and if so, and we think it should, and therefore, that is why we are actually planning an accelerated testing of the highest probability specimens using the CDC Lab, because that is really the only assay that is available now.

DR. TABOR: We are obviously going to be, as in many of these situations, if you and Sue Stramer and a few others weren't generating this data, we wouldn't have the data to base policy decisions on. We are all very grateful for these studies, and there may be a few other sources particularly from testing plasma, other sources of field information.

But in the case of these studies, whose methods are you planning to use, or it that not yet known?

DR. BUSCH: Obviously, you know, the two tests, I mean there are other possibilities, but the two tests that are able to be brought up for RNA screening next year are GenProbe, Chiron, and Roche.

Because NHLBI has a support relationship with GenProbe for their TMA development, we have a resource there that is available to us for the testing. We are in discussions with Roche about them participating by testing under a similar protocol, the samples that they have through their archived plate retention work.

I mean we would like the study to help enable these companies to move forward, and the study will include the comparative performance of these assays relative to CDC.

DR. TABOR: And whose IgM assay?

DR. KLEINMAN: As I said, we don't know. We have to qualify assays as they come along and determine by criteria, I don't think we have totally worked out, but they are dependent on panels, you know, that we have a sufficient sensitivity assay.

This reminds me of the early days of HIV and the early days of hepatitis C, when you are both trying to

generate data and recognizing that the tools, if you take your valuable specimens and test them today, your tools are not as good as if you waited six months, and yet if you don't test them, you don't have the preliminary information that is useful.

I think we have encountered the problem before, and I think we just have to--it is sort of unusual, if you will, for a very large study like this, we have got to fly by the seat of our pants a little bit and know that we will look at the information. We have got good people on the steering committee and will presumably make the best decisions, but I don't think we have them worked out yet.

DR. STRAMER: To continue the response, we won't be using these reagents until they are under IND, so sensitivity criteria will already have been worked out at least in good enough measures to label reagents IUO.

In addition to that, we have study criteria, the protocol, to say less than 50 copies per ml, which is what Mike did review in the protocol, and to address the critical samples that will be tested first with the CDC assay.

Those samples will be returned and then retested with assays having improved sensitivity. So, we will try to optimize the samples of greatest interest to be tested with the assays of greatest sensitivity.

DR. KLEINMAN: Certainly, any positive samples that we find I think hopefully will have enough sample volume to then run them through other candidate assays. It is obvious we have to choose the right screening assay to get positive assays, but I think the intention would be to look at all available resources and subject those samples to testing by multiple tests as they become available.

DR. BUSCH: And I think the data we are seeing from GenProbe and Roche, the screening assays, they are building, what is now tested are head to head as good as what we have got for HIV and HCV, and with 0.5 ml input, I don't think we are going to see any increased sensitivity beyond what they have already begun to show us.

MR. DAWSON: Dawson from Abbott. Just a comment. We will be looking at IgM tests for potential for blood screening. We are looking at different antigens and different assay formats, but, you know, I have heard a lot of more discouraging type of discussion at this meeting

than encouraging for IgM screening, but still we have an open mind to look at what we can do with IgM. We have built IgM tests for 30 years at Abbott, have got some experience, so we like to look.

You know, for some viruses, for HCV/HIV, an IgM test really didn't help very much and you only find it sporadically here. It looks like it is a little more hopeful that a lot of people do make IgM. Whether they make it early enough is another question, but we really need the help of various individuals here, many of whom I have spoken to.

The real key and the heart of the matter with IgM is those samples that transmit the virus, are they IgM-positive. Most of the data we have seen to date, they have been negative, but we would like to take a shot with our assay format, with perhaps different antigens, you know, can we detect IgM in these samples.

This will probably be our decision process will depend on how well we do on these viremic samples that transmit virus.

DR. BUSCH: We could discuss including an assay like yours in the study in a way that these allogeneic

donors that we test for IgM, it may be justified. Obviously, those, we might want to retest for RNA if they were initially negative to make sure, but in addition, maybe on a subset of IgM positives that have particular high-titer characteristics, we could retrieve recipients and see is there any transmission linked to IgM positives.

MR. DAWSON: Sure, yes, and to maybe even look, follow up on individuals who are persistently high level IgM, is this some indication of continuing viral presence. You might not find it in the blood. It could be compartmentalized in organs or something else.

But we are in the game at least for the time being, and we would want to look for those samples that are going to really help us make our mind up, you know, what is Abbott going to do in the blood screening arena.

DR. NAKHASI: I just really want to, you know, sort of focus attention one more time again. As Mike said earlier, we don't have to keep our mind closed about the seasonal variation, geographical distributions of this virus, and on one virus is present, and all those things.

Looking at Lyle's epidemic, you know, prevalence charts, and modeling, and I will be very much looking

forward to what this kind of study comes out, I think we need to keep our mind open whether it needs, the implementations need seasonal or non-geographical.

That is my personal feeling, I am not speaking for FDA at the moment, but what I am saying is having a scientific study and the outcome of that study coming out of that study will be very important, as you said, for decisional processes.

DR. HARVATH: On behalf of the group here, I would like to thank everyone for their participation and especially, Mike, Sue, and Steve for the great discussion.

I understand we have another session immediately in this room, and I have been instructed as to which key will pull up their presentation, so whoever the moderator is, if they would step forward. Thank you very much.

[Applause.]

VII. Regulatory Issues

Chair: Paul Mied, FDA

DR. MIED: Thank you, Liana.

My name is Paul Mied. I am from the Division of Emerging and Transfusion Transmitted Diseases in the Office of Blood at CBER.

Welcome to the session on Regulatory Issues. Quite a few regulatory questions have been raised during the sessions yesterday, during the coffee breaks yesterday and today, and I hope we can address all of those in this session.

We will have a question and answer period for our two speakers after their presentations, so I hope you will take that opportunity to ask all of your questions.

Our first speaker in this session is Dr. Robin Biswas from the Division of Emerging and Transfusion Transmitted Diseases in the Office of Blood at CBER. Robin will address considerations in the development of assays for testing blood donors for West Nile Virus.

Robin.

Considerations in Developing Assays for Testing Donors for West Nile Virus

DR. BISWAS: Thanks, Paul.

After the one and a half days and particularly after the discussion that we just had now, I find myself in a pretty difficult position to give this talk.

I would like to say that pretty much everything that I will cover has already been covered and we had a lot of interesting discussions.

Now, these considerations, we are very, very flexible on this. We are dealing, of course, with a moving target, we have some data, there is more data to be gathered, and so I can't be here and set policy.

I would also like to say that in regard to the assays, I would like to say upfront that there is a need for FDA to talk to the individual manufacturers together with the blood organizations to set exactly what the studies should be, what the clinical trials should be for the individual assays.

[Slide.]

Now, what types of donations should be tested for West Nile Virus? I think our colleagues at CDC have done an enormous amount of work with a lot of stress, and they have shown really that donations of blood components for transfusion really needs to be considered.

They have likely transmitted West Nile Virus associated with morbidity and mortality, and at the present

time, blood components for transfusion do not undergo viral inactivation.

[Slide.]

Now, in regards to plasma, well, we had a very spirited time in the other room. I should just like to say that West Nile Virus transmissions by plasma derivatives have not been reported so far, at least as far as I know, and I see Mary Chamberland nodding her head, thank you, and plasma for derivatives undergo effective validated viral inactivation removal of viruses similar to West Nile Virus, as we heard. Nevertheless, the question still exists should plasma for further manufacture be tested.

[Slide.]

Studies are needed to assure that West Nile Virus is cleared during manufacture of plasma derivatives. Should studies show that West Nile Virus itself is cleared, you heard the opinion of Dr. Mahmood Farshid earlier today.

[Slide.]

Here we go into the hot area. Direct detection of viral components probably--probably are most useful. We heard yesterday from Abbott and from Ortho Diagnostics, talks about possible antigen testing, nucleic acid tests,

NAT tests, definitely useful for identifying viremic asymptomatic donors early in the infection, and that is what we are interested in. We are interested in identifying viremic asymptomatic donors and also PCR--and the NAT assays I should have said really here--have been developed for research in clinical lab settings.

[Slide.]

Manufacturers and blood centers and FDA already have a lot of experience with NAT implementation.

[Slide.]

In regard to the current thinking of sensitivity of the assays, sensitivity of NAT, should be targeted to at least 100 copies per ml in the neat sample, i.e., capable of detecting at least this viral load in the individual donation to ensure 100 percent detection of 100 copies per ml in the individual donation.

In regard to the viral load in blood, these slides are pretty ancient now. I think that Dr. Wong yesterday told us that it can go up to 10^6 copies. These estimations I should say are just estimations, and we really need more work done on how the PFU relates to copy numbers, because we--when I say "we," I mean really the

manufacturers, the blood organizations, and FDA--we are really very used to working with copies per ml.

[Slide.]

Here again another hot topic, single sample NAT likely, I put here, but not necessarily inevitable for West Nile Virus donor detection. Mike just said quite eloquently, might not need to go to single unit. The suitable NAT pooled sample testing methods could be explored, e.g., increase the viral concentration by centrifugation, affinity capture, use of large volumes for processing.

[Slide.]

I just have up here different types of NAT assays for validation of investigational NAT if you have a positive in one test, you should use the other test to validate it. There are different ways that that can be done

Now, coming to antibody, now testing for antibody, I have said here antibody is unlikely to identify most viremic, asymptomatic donors early in the infection. While I think that is still true, but obviously, there are some more discussions going on.

What I would like to know, are IgM-positive, NAT-negative or NAT-positive units infectious. I think that CDC had said that they had four--was that right--four IgM positives, NAT positives?

DR. CHAMBERLAND: In terms of the confirmed donor?

DR. BISWAS: Right. There were a few anyway.

DR. CHAMBERLAND: Samples available at the time of donation were all VR-positive, IgM-negative.

DR. BISWAS: So, there are some samples like that, and are those units infectious, do they contain culturable virus. How long does IgM antibody remain in the blood? Yesterday, we heard that it can stay as long as 200 to 500 days, and the big question is are both NAT and IgM antibody donor testing needed.

[Slide.]

Another question. Should antibody-positive tests only, no nucleic acid, no symptoms, not be grounds for deferral or product retrieval? Can one use antibody as a supplemental or a confirmatory test?

[Slide.]

Now, even if NAT tests alone are selected for donor testing, during clinical trials, specific antibody assays will be needed for follow-up testing of investigational NAT-positive donors to validate the NAT-positive test results, and also antibody tests needed for continuing diagnostic and epidemiologic studies.

So, there is plenty of room for antibody tests here.

[Slide.]

Now, in regard to tissue and organ donors, there is a need to assess the effectiveness of West Nile Virus assays for cadaveric samples. I should say that solid organs and bone marrow is regulated by HRSA, as we heard yesterday, however, FDA approval is needed for screening and diagnostic tests.

Here, I just want to clarify something. Remember that for both tissues and solid organ donor testing, what we are talking about here is not actually testing the tissues or the organs. We are talking about testing a sample, a blood sample, a liquidy bit, because there seemed to be some confusion, I think, by many people whether we

were talking about testing the actual organ or the actual tissues.

[Slide.]

Donor screening and supplemental assays will be reviewed as a biologic product under the PHS Act, so it will require an investigational New Drug Application, and after the studies are done, then, a biological license application is needed. We will facilitate development and implementation of screening and supplemental assays, as I think we are doing right at this moment.

[Slide.]

I will go through this slide, things that do need to be considered for all screening for all blood donor tests, clinical sensitivity, clinical specificity, analytical sensitivity, analytical specificity, chemistry, manufacturing, and controls, reproducibility and proficiency, the stability instrument and instrument and software.

I have a slide, eight slides for all these and I am not going to go through them because of time, and I would just like to stop at that one and say that bottom bit in the brackets there, this discussion of doing correlated

viruses including, if possible, you know, if possible, those infections caused by related viruses, such as St. Louis encephalitis, et cetera.

What I want to say is that the thrust is really West Nile Virus, but if you have an opportunity, somehow you could do it, it would be a good thing to do.

[Slide.]

I just want to say that the instrument and software does require a separate 510(k). There are some additional guidances available, in particular this one, Guidance for Industry, the HIV one that many of you know.

[Slide.]

And this one is another good one, sort of an old one going back to 1989, but these draft Points to Consider should also be looked at by manufacturers.

[Slide.]

The way forward, FDA will continue to work together with NIH and CDC, and other components of the Department, and manufacturers and blood organizations to facilitate assay development.

If necessary, FDA would allow widespread study of appropriate tests under IND and how widespread that is going to be needs to be further discussed.

Sponsors are asked, that is, manufacturers are asked to seek FDA's guidance and to submit a pre-proposal before initiating studies to support an IND or BLA. If you can do that, if the sponsors, if the manufacturers can do that, the reviews will go so much quicker.

That is all I have to say.

[Applause.]

DR. MIED: Thank you, Robin, for that overview of where the different types of tests might fit in and what manufacturers need to address in the development of their assays and in the clinical trials, so that industry and FDA can successfully complete this effort with licensure of suitable tests.

Our next speaker is Dr. Martin Ruta. Martin is from the Office of Blood at CBER. Martin will discuss the role FDA is playing in West Nile Virus and the safety of the blood supply.

Guidance for Industry: Recommendations for

the Assessment of Donor Suitability and Blood and Blood Product Safety in Cases of Known or Suspected West Nile Virus Infection

DR. RUTA: Thanks very much. This has been quite an interesting meeting. I am glad to see some of the data coming out and hope to see more coming out in the future, but I think there has been a very good response by everybody, by the blood community, by the device manufacturers, and I want to thank everyone for coming and at least getting some of the studies started.

[Slide.]

What I want to talk about now is primarily guidance documents that we came out with on assessing donor suitability, but there are a couple of introductory slides, and I will try not to be too repetitive.

[Slide.]

As we have all gotten by now, it looks like blood transmission of West Nile Virus has been confirmed this past year. This is new. As we all know, it was not previously recognized in previous outbreaks in other countries, and I think we are going to learn a lot new

about West Nile Virus and West Nile Virus epidemiology over the course of the next year.

The magnitude of the risk in transfusion is unknown, but we think precautionary measures are indicated unless and until the epidemic is resolved. We have two approaches to assuring blood safety in the face of the risk of West Nile Virus, and one is a guidance from screening and there is guidance on management of donors and blood components, and the other is to facilitate the rapid development of donor screening tests.

[Slide.]

There are a number of assumptions that we base our guidance on, and some of it goes back to the literature of the 1950s, and I think we will be seeing over the next year how well those assumptions hold out.

The assumptions are that human infections are expected to occur seasonally during the periods of mosquito activity, but it may occur year-round in some parts of the country, that 80 percent of infected persons are asymptomatic and that mild symptoms are nonspecific, they are flu-like illness; that viremia occurs for up to two

weeks prior to symptoms, and that the duration of the viremia may be up to 29 days.

In addition, we think the virus titer in blood is low compared to other viruses, although we heard one data point yesterday that it may be 2 logs higher than what I have up here.

We think the viremia resolves rapidly after seroconversion to IgM, and that IgM positivity may persist for a very long time, and we are hearing up to two years and still going.

The final assumption is that there is no chronic carrier state.

[Slide.]

What have been our actions to date? Well, on August 17th, before there were any cases of transmission, when it was relatively quiet in the summer, we issued an Alert to blood establishments about the possibility of West Nile Virus transmission.

On October 3rd, we updated this Alert and we stated our interest in facilitating the development of donor screening and supplemental tests. Our guidance document issued on October 25th.

We have been cooperating with the CDC, with State and Public Health Departments, blood organizations, and HRSA, and I should add in here with the device manufacturers and with the device trade organizations, AdvaMed also has been very helpful.

There has been an ongoing epidemiological investigation led by CDC of possible cases of transfusion-transmitted West Nile Virus, that we have tried to assist, and the blood organizations have been assisting with, and we have tried to provide advice on the deferral of donors for all in-date products collected from suspect donors.

[Slide.]

Our additional actions have been to try and have open communication with the blood community, with health professionals, with the media, with the Congress and with consumers. We have tried to stimulate case reporting.

On the whole, I think that PHS is trying to put out a balanced message here of the risk of West Nile Virus, that your risk of getting West Nile is much higher from mosquitoes than it is from blood transfusion, and to properly weigh the risks and benefits of transfusion and

transplantation, where we are talking about life-saving medical interventions.

We also want to emphasize the uncertainty of the current knowledge base, we are in the learning phase, and there has been intense congressional interest. We had two hearings specific to West Nile Virus, on September 24th and October 3rd, and we had a third hearing on September 10th on the general blood supply and blood safety in which the issue of West Nile Virus also came up.

[Slide.]

Our current initiatives include the guidance document, which I am going to get to in a minute, and the second one is reflected in part by this meeting. We are trying to facilitate the development of screening and supplemental tests, and we have been cooperating again with CDC, NIH, blood organizations on rapid surveys of West Nile Virus in donors, and CDC is planning on conducting an unlinked study. We have heard this in the previous session about the link study conducted through REDS.

We are trying to identify the need for additional research. Some of the questioned that came up were about West Nile Virus inactivation by storage although the data I

saw yesterday didn't look too encouraging. It looked like the virus was pretty stable over 35 days.

We were concerned about West Nile Virus removal and inactivation during plasma fractionation and the need for more studies there although we saw one data point earlier today on specific inactivation of West Nile Virus.

The possibility of the need for surveys in frequent blood product recipients may be another area that may need further study.

[Slide.]

What I would like to do now is to take you through the guidance document. I hope everyone who needs to see this has seen this. This was posted on our web site on October 25th, and it contains our current thinking.

I will characterize it as our first Final Guidance on Recommendations for the Assessment of Donor Suitability and Blood and Blood Product Safety in Cases of Known or Suspected West Nile Virus Infection.

Our recommendations are--and I am sorry, most of this is in the guidance, but if you will bear with me, I am just going to read through it quickly--for donor deferral, we are recommending that a potential donor with a medical

diagnosis of West Nile Virus infection be deferred until 14 days after the condition is considered to be resolved and at least 28 days from the onset of symptoms or diagnosis whichever is the later date.

In addition, in the absence of current or recent symptoms, an IGM positive antibody test result alone should not be grounds for deferral. That relates to the prolonged IgM cause of antibody in infected individuals.

In addition, we are recommending that donors who report an otherwise unexplained post-donation febrile illness suggestive of West Nile Virus infection in the setting of active West Nile Virus transmission in the community should be deferred for 28 days from the onset of illness or 14 days after the condition is considered to be resolved whichever is the later date.

Donors whose blood or blood components were received by a patient with a possible case of transfusion related West Nile Virus should be deferred for 28 days from the date of potential transmission.

In addition, the FDA has been encouraging blood establishments to actively encourage donors to report post-donation illnesses potentially associated with West Nile

Virus, that is, flu-like symptoms that include a fever, occurring within two weeks of blood donation in the setting of active West Nile Virus transmission in the community.

Our recommendations for product quarantine and retrieval are that in-date components from current, prior, and subsequent collections should be quarantined and retrieved if a donor later reports a medical diagnosis of West Nile Virus. Product quarantine and retrieval should cover a time period dating back to 14 days prior to the onset of illness and 28 days subsequent to the onset of illness.

In the absence of symptoms, an IgM positive antibody test result should not be grounds for product quarantine and retrieval.

Medical directors should exercise judgment when an otherwise unexplained post-donation febrile illness occurs in the setting of active West Nile Virus transmission in the community.

Donors are considered to be potentially associated with transmission of West Nile Virus if the infected recipient received the donor's blood components

within 28 days before the onset of symptoms in the recipient.

Once a donor is identified as associated with a possible case of transmission through the transfusion, product quarantine and retrieval should be applied to in-date components that were collected in the period from 28 days prior to the suspect donation to 28 days after the suspect donation.

Finally, when blood establishments receive information that a donor has a medical diagnosis of West Nile Virus, blood establishments should consider notifying transfusion services to permit lookback recipient tracing and notification.

If a post-donation illness is not diagnosed as West Nile Virus infection, actions to identify prior recipients are not appropriate. When an epidemiological investigation--these are our friends at CDC--primarily suggests that a specific donor is the likely source of transmission of West Nile Virus to a transfusion recipient, that blood establishments should consider a lookback notification of other recipients as appropriate.

That is the end of my presentation. Again, I want to thank everyone, first of all, who participated in the development of the guidance. There has been a lot of hard work by FDA, CDC, NIH, and the PHS in general, so I want to thank everybody for that.

Again, I want to thank everybody for active participation in today's workshop. Thank you.

[Applause.]

DR. MIED: Thank you, Martin, for that summary of FDA's actions and initiatives pertaining to meeting the threat posed by West Nile and also for walking us through the guidance that issued on October 25th.

I would like to ask both Martin and Robin to come to the table behind me and take questions from the audience.

Discussion

DR. BIANCO: You are running a risk, you have this session before lunch. We are hungry.

Robin, you said in your slide that if necessary, FDA would allow widespread studies of the appropriate tests under IND. What would define it as necessary?

DR. BISWAS: Well, that is something that we have to discuss, and that is something that is a very good question, and I think that that is one of the questions that needs to be discussed this afternoon at the panel meeting.

It is very much defined regionally and temporally, and I guess widespread use--well, we would have to be very flexible. I just don't have an answer for you, which only increases your frustration, Celso.

MS. RICHARDS: Karen Richards from Chiron.

You mentioned traditional BLA, IND, and 510(k) processes. I have a specific question for you, so I am going to read it.

What specific least burdensome approaches will FDA use or establish to support new applications to well-established existing platforms, such as PMA and associated software?

DR. BISWAS: Well, there is a guidance out there, you know, a CDRH guidance of least burdensome, and I would suggest that all manufacturers read it. We certainly are complying with that guidance.

We will use least burdensome methods to review 510(k). Additionally, it does need to be, you know, our review will also be based on very sound scientific principles to see that it does work in a way that the manufacturer claims that it will.

DR. SAYERS: Merlyn Sayers, Carter Bloodcare and University of Texas Southwest.

Robin, you are off the hook because these are comments, not questions.

The comments are these. Under similar circumstances, similar meetings, we have spoken about alternative test sites before, and obviously, that issue came up when HIV antibody screening became an issue for blood programs.

I don't know the extent to which the next mosquito season is going to encourage individuals who have been bitten to be suddenly curious as to whether the critter that bit them was an albopictus or not. I would hate to think that they were going to have their curiosity satisfied by donating a pint of blood. We need to remind individuals that that is not the route to go.

As increasingly we test donors, so do we increasingly run the risk of giving them test results which they find either confusing or contradictory to their own sense of good health.

So, all I would say is that I hope that the last draft guidelines do, in fact, give us as much information as possible to relay to donors who are found to be positive in these screening tests.

The less sophisticated donors may have no questions at all, but the more sophisticated donors increasingly ask very difficult questions, and I suspect that one of them is going to be, well, what does long-term IgM presence represent for my continuing health. So, I hope the final guidelines do include as much information that we can give back to the donors as possible.

DR. RUTA: Thanks for the comment. Just to point out, this is actually a final guidance for implementation. As more knowledge becomes available, I left open the possibility that there may be a need to revisit it next year.

I take your point, but I think that your point on what is long-term IgM positivity mean, I think that is

something that the PHS will have to work out. I will defer if someone from CDC wants to get up and give their opinion since they are more expert than I am, but I take it that it is a general statement that the meaning of test results, whether they might mean that we don't think there is a consequence associated with it should be a message given to donors also.

DR. JAPOUR: Tony Japour. Two questions, actually one question based on two comments that you made. The first was that the risk of mosquito leading to infection is much higher than transfusion is very important and a real one.

The second point that you made was that in some areas of the country, is it an all year-round infection, not just seasonally. I think that is particularly relevant to a place like Florida where they just had their first case diagnosed, and the question that I have is what is the vaccine strategy that FDA may be trying to promote, because given the large numbers of elderly people, immunocompromised people in the State of Florida, that may be a risk all year-round once this virus establishes itself there.

Is there a vaccine strategy that may be used to prevent infection in people that are at high risk for mosquito-borne infection?

DR. RUTA: First, I will ask if there is anyone from Vaccines who cares to answer that. Hi, Jesse, go ahead, you would be a good person for that.

DR. GOODMAN: I think that really there has been little public divulged by vaccine manufacturers about their plans, so I am sort of limited to my comments to what has been divulged, but there are several potential vaccine strategies in the literature, and I think depending, you know, FDA and the Office of Vaccines, is certainly similar to the West Nile diagnostics, because West Nile Virus is a current acute public health threat, there is not treatment for the disease. We would consider this a very high priority for vaccine development and would do what we could to work with manufacturers to get studies going and potentially products licensed.

Having said that, I also think the appropriate public health strategy for use of a vaccine, what population, what locations would be used would depend on,

at that time, what is going on with the disease and also the safety and effectiveness of that specific vaccine.

So, I think it is premature to kind of make a policy comment, but one thing that is a little interesting here is that certainly, similar to in some ways influenza, the elderly people who perhaps have varying degrees of immunologic problems seem to be most susceptible to severe outcomes from this disease, and that seems very different than many of the childhood diseases we vaccinate against where children are highly susceptible to bad outcomes.

So, I think there is a lot of potential strategies. There is a lot of interest in a vaccine, and we are just going to have to see what these strategies come up with and what is going on with this disease.

DR. ROSSMAN: Susan Rossman, Gulf Coast Regional Blood Center in Houston.

I have a question relating to the guidance, the particular phrase "active West Nile Virus transmission in the community," whether that refers to positive mosquito pools as we move into flu season and we are talking about soliciting callbacks for very vague symptoms. It could be of concern to someone like us.

DR. RUTA: We actually mean human cases.

DR. ROSSMAN: Human cases, okay.

DR. RUTA: Human cases, right.

DR. LEPARC: German Leparc from Florida Blood Services, St. Petersburg, Florida.

I heard a couple of times this subject of regional testing or temporary testing, and as someone who has to deal with the donor as being on a cruise to Cancun, but then get off the boat with data, and then we have these donors who have been in Europe in a military base, but they ate only in the ship, I think, you know, I make a plea, if we are going to do the testing, this is a very mobile society.

I live in Florida. I have been in four states in the last four months, so I don't think that doing a temporary or regional testing will work.

DR. BISWAS: Thanks for those comments.

DR. RUTA: I think there are also practical considerations as to where we are with test development and where the epidemic is next year. So, it is going to also depend on what is feasible to do as we enter in the next season.

DR. BISWAS: I think that regional and temporal testing does create logistical sort of problems that need to be looked at.

DR. NAKHASI: I just want to respond to Celso's and Karen's comments. I think, not that Robin didn't do a very good job, Robin did a real good job in responding. I just want to clarify some of the points here.

One is that obviously, the outcome of the studies, which are being planned on being done, will also determine how we will be doing the studies. One has to keep that in mind.

Secondly, with regard to the least burdensome, obviously, least burdensome document says that you need to make it clear to people what the necessary documentations, what necessary data is needed.

It does not, you know, I should underscore it does not require that you have to underline the safety. I think one has to remember very carefully that even though the volume of this data, it has to be made sure that what is important for the safety data, and I think that will still remain standard.

DR. KLEINMAN: I was curious about the reporting of suspected cases in a recipient, and obviously, the CDC has encouraged that those cases be reported to either State public health departments or directly to the CDC.

Is there a provision that those should be reported directly from the hospital to the blood supplier? I mean you would think hospitals would do that, or in the case investigations, is the information now flowing from the public health department to the CDC, and then back to the blood supplier, which seems a little inefficient.

So, it is really a question more for CDC and the blood organizations, but it comes up based on your statement in the guidance.

DR. CHAMBERLAND: In the context of the investigations, we have actually received reports from any number of organizations, be it health departments, hospitals, the collectors. I think what we saw happening as the investigations started to increase in number is that actually, the first alerts that we were getting were often from the blood collection organization.

In talking with them about that, what seemed to be happening is patient in hospital, diagnosed with West

Nile Virus infection, the first thought, not mosquito, but transfusion, a quick review of medical records determined that transfusion happened, and hospital transfusion service, in turn, notified blood supplier.

So, that seemed to be how things were working at least in large part as time went on, but in point of fact, we have received reports from a variety of different sources.

DR. STRONG: Just a comment for Robin in terms of your liquid bits. For organ and tissue donation, it is quite common to recover lymph nodes, so I wouldn't rule out the other tissue bits.

DR. BISWAS: Thanks for that comment. I was just referring to the tests we were discussing, but thanks.

DR. MIED: I just want to take a moment and underscore some of the things that Robin said about what FDA is going to be doing, and in doing so, I want to respond to Glen Freiberg's question from yesterday, how can we approach a license more quickly, what can FDA do to help us get a test licensed.

As Robin said, we are interested in facilitating two things, first of all, the development of donor

screening tests and supplemental tests, and, secondly, the licensure of those tests.

With regard to the development of tests, we intend to facilitate that by defining the performance criteria that are needed, and you have heard some of that at this meeting already, also, through making panels and standards available.

Yesterday, Indira put out a call for positive plasmas and indicated that we are working with virus isolates and transcripts, and that we intend to assemble a CBER panel that we hope to make available to you to help you in your assay development.

With regard to facilitating licensure of donor screening tests and supplemental tests, I want to emphasize that we intend to do this through ongoing close cooperation between CBER and manufacturers by continuing the dialogue that we have started here and by doing this at each step of the way.

First of all, in the pre-IND stage, as Robin said, we would like to discuss with you the filing of pre-IND submissions. This will facilitate getting you started with your clinical trials. Now, we will review their

submissions, we will give you formal feedback on them, and this will help make the process of putting the test under IND and getting toward licensure go much more smoothly.

We would like to address with each manufacturer, the list of issues that Jim Gallarda raised yesterday, that he wanted to discuss with FDA. I think it is important for us to do that, talking about things such as the logistics of the multi-site IND, the scope of the IND, how many samples need to be run, what is the role of referee assays and supplemental test development.

I mean these are all issues that are on our minds also, and we would like to discuss these with you early on, so that your clinical trials will go smoothly and quickly, and will provide exactly the data that you need for licensure.

Then, we would like to continue that dialogue beyond the pre-IND process, but during the clinical trials that are ongoing under IND and also continue the dialogue during our review of your BLA.

I want to just end by saying that review of these submissions will be a top priority for CBER.

Any other questions for the panel?

[No response.]

DR. MIED: Let's get some lunch. When shall we be back? 1:15.

[Whereupon, at 12:15 p.m., the proceedings were recessed, to be resumed at 1:15 p.m.]

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

[1:15 p.m.]

VIII. Implementation Issues: Blood and Tissue Organizations

Chair: Alan Williams, Melissa Greenwald, FDA

DR. WILLIAMS: We will begin the session on Implementation Issues for Blood and Tissue Organizations related to preventative measures for West Nile Virus.

I am Alan Williams from the Division of Blood Applications in CBER, Office of Blood. The co-chair for this session is Dr. Melissa Greenwald with the Office of Cellular Tissue and Gene Therapies.

Obviously, this has been a packed schedule from start to finish. If you do the math, each of these speakers has a little over eight minutes, so I will ask you to please keep it between eight and 10, no longer than 10 minutes to leave us enough time for a little bit of discussion and questions related to implementation hurdles, which I think are a very, very important issue.

The first speaker is Dr. Steve Kleinman representing the American Association of Blood Banks.

DR. KLEINMAN: Welcome back after lunch. We have a lot of sleepy-looking people out there.

I have no slides. I am going to read a statement from the AABB. It raises a lot of issues that have already come up during the conference, so I apologize for being repetitive.

The first paragraph is the typical like nonsmoking disclaimer, but this is what the AABB is, so please bear with me.

The AABB is the professional society for over 8,000 individuals involved in blood banking and transfusion medicine and represents approximately 2,000 institutional members including blood collection centers, hospital-based blood banks, and transfusion services as they collect, process, distribute, and transfuse blood and blood components and hematopoietic stem cells.

Our members are responsible for virtually all of the blood collected and more than 80 percent of the blood transfused in this country. For over 50 years, the AABB's highest priority has been to maintain and enhance the safety and availability of the nation's blood supply.

The AABB commends the FDA and the entire PHS for their rapid response to the threat of transfusion-transmission of West Nile Virus. This response, as we have heard, has included careful case investigations of suspected transfusion-transmitted infections. This has been done in collaboration with blood centers and hospitals, most of which are AABB member institutions.

It also includes prompt communication of new information and discussion of policy issues in a timely and open manner with the medical community, the media, government, the general public, and leading to this very important workshop.

Now, into the substance.

It appears very highly probable that West Nile transmission by transfusion has, in fact, been documented. We know that despite the short period of West Nile viremia, the large number of infected persons in certain geographic locations during the 2002 epidemic combined with the asymptomatic course of the infection in the majority of infected persons has resulted in the concern that the potential spread of this agent may exceed that of other

transfusion-transmissible agents for which no screening is currently performed.

So, this concern has led to evaluating the need to screen all blood donations with a West Nile assay in order to protect transfusion recipients during either the 2003 or subsequent transmission seasons.

In addition to this workshop's goal of identifying the practical issues involved with implementing a screening assay, the AABB would like to encourage a discussion of some of the public policy issues associated with West Nile donor screening, and I think that is going to come in the panel subsequently.

The first major issue is to establish the threshold that needs to be met for the decision to implement screening to be made. Several questions arise in this regard.

Firstly, based on the documented transfusion transmission cases in 2002, have we already reached the threshold to screen?

If not, what additional data will have a bearing on whether to implement a screening assay? Will data from

the donor/recipient research studies described earlier today be important in this decision?

Will the extent of mosquito-borne transmission in 2003 be considered in this decision, or for precautionary reasons, do implementation decisions need to be made prior to that time frame?

Now, in comparison to other infectious agents for which donor screening is performed, it appears that West Nile is unique in two respects.

Firstly, like other arboviruses in the U.S. that have been associated with meningoencephalitis, it is likely that the current large-scale epidemic will not persist and that the infection will become endemic with long periods of low-level enzootic and human activity, punctuated by local outbreaks.

It is of interest, and we have heard it described at this meeting, that a similarly large epidemic of another arbovirus, St. Louis encephalitis, occurred in regions of the U.S. in mid-70s, but has not since recurred.

Anticipating that West Nile may follow a similar course, the AABB encourages discussions of how decisions will be approached regarding the possible discontinuation

of widespread West Nile donor screening if, in fact, it is implemented.

Secondly, arbovirus infections are seasonal and coincide with the breeding of mosquitoes in warmer weather. This prompts the question of whether it is reasonable to use resources to screen donated blood for West Nile in the northern U.S. during the winter, for example, and whether consideration has been given to formulating a strategy that allows for seasonal screening linked to a regional assessment of West Nile risk.

Obviously, there is no precedent in U.S. blood banking for such an approach, however, we believe this should not in itself preclude thinking about its merits. Obviously, we have heard some opinions expressed, pros and cons, on this, and I am sure that it will be discussed further this afternoon.

Now, if the decision is made that implementation of West Nile screening proceeds, then, the AABB would like some clarification as to what regulatory format will be adopted, and I think we got some of that this morning.

Will the national IND structure that was formerly utilized for HCV and HIV NAT be the mechanism used for West

Nile Virus testing? Finally, in answer to a question, I think we heard this morning that it will be. If so, what level of enzootic or human transmission will be the criteria for donor screening in various geographic areas and will individual blood collection organizations make their own decisions about whether and when to test?

Speakers from other organizations will address in more detail the considerable logistical issues involved in adding a West Nile NAT screening assay to current HIV and HCV NAT.

AABB would like to reiterate or actually since I am going first, I guess, would like to make the one essential point, which is that the automated testing platforms to support individual donation NAT of the entire U.S. blood supply do not currently exist, and I am sure you are going to hear this discussed by several other speakers.

So, AABB would like to focus on several other issues that will arise in the context of a West Nile screening assay. The first is the cost of blood components for hospital transfusion services. Undoubtedly, the cost of West Nile screening will be passed on from blood centers

to hospitals, and there still remains no mechanism for hospitals to recover this cost in a timely fashion.

To address this problem, the AABB urges that the FDA maintain a liaison with the Centers for Medicare and Medicaid Services to be sure there are appropriate payment adjustments to blood intensive DRGs to cover these costs.

The second item relates to the possible procedure or procedures that will be used to confirm a positive West Nile screening test result. Possible modes of confirmation include alternate NAT assays or follow-up donor testing by NAT or serology.

The issue of confirmation of West Nile viremia is crucially important in formulating policies related to West Nile screening. Related to confirmatory testing are issues of donor management, including donor deferral and counseling, consignee notification, and lookback investigations after a West Nile screening test is implemented.

Will distinctions be made in these policies based on whether the screening result is confirmed or not confirmed? If testing proceeds under multiple manufacturers' INDs, which seems likely, the AABB asks the

FDA to standardize approaches to these matters across the INDs and to communicate the approach to these issues to the transfusion medicine community in the near future.

Based on our current state of knowledge, the AABB supports a donor deferral period of four weeks after a positive West Nile screening assay using direct viral detection.

When a West Nile viremic donor returns to donate whole blood after more than four weeks, no special reentry testing should be necessary, the donor should be allowed to donate if he or she is asymptomatic and their unit should be used if the West Nile screening assay which would be applied to the new unit is negative.

Similarly, the AABB supports consignee notification and possible recipient notification for components made from donations collected up to four weeks prior to the identification of a viremic donation.

These short periods of time for donor deferral and consignee notification will only actually affect apheresis donors. The AABB encourages compilation of research data from the proposed studies, so that these

current draft policies can either be supported by such data or changed appropriately.

Policies with respect to consignee notification, product retrieval, and recipient notification when a donor reports post-donation symptoms, such as fever and flu-like symptoms, in a geographic area where mosquito-borne transmission of West Nile to humans has been documented have been set forth in the recent FDA guidance document, and we heard that described this morning.

We would just like to note that a review of such policies will need to be undertaken if West Nile screening is implemented. For example, it is possible that the index of suspicion for product retrieval could be changed with post-donation information based on a negative West Nile screening assay.

This may be highly dependent, however, on the sensitivity of such an assay. Basically, we just want to alert all participants that reexamination of these policies should be ongoing as new data become available.

The AABB has and will continue to encourage its member institutions to participate in CDC directed case-based investigations of possible transfusion-transmitted

West Nile. The AABB has encouraged blood collection facilities to save retention samples from each donation during the epidemic season for at least 90 days and has also supported the reporting of patients with West Nile Virus infection who received a transfusion within the past four weeks, so that case investigations can be conducted.

Again, if a West Nile screening assay is implemented, the AABB would like to continue to work with CDC to examine whether the criteria for ongoing case investigations should remain the same or be altered.

One vital component of the donor/recipient research study that you heard about this morning involves lookback investigations of recipients who received components from West Nile viremic donors and their enrollment into the study protocol.

As an organization, the AABB will encourage its members to participate in this study so as to compile the largest possible database relevant to answering questions related to West Nile transmission.

In summary, the AABB supports the priority that the FDA and PHS have given to consideration of screening the blood supply for West Nile. Obviously, there are a lot

of open questions that we will hear about this afternoon. The AABB will continue to work closely with PHS agencies on all issues related to West Nile transmission and blood safety.

Thank you.

[Applause.]

DR. WILLIAMS: Thank you, Steve.

The second speaker is Dr. Susan Stramer representing the American Red Cross.

ARC

DR. STRAMER: Thanks, Alan. Good afternoon.

I just would like to start by saying three things in preface to my presentation. Hopefully, they won't take my eight minutes.

We have developed at the Red Cross the presentation as a team approach involving multiple departments at Red Cross. I am going through a specific level of detail just so you can appreciate the complexities of implementation. The last thing I would like to mention is implementation of West Nile Virus testing. We should benefit from the lessons we have learned from HIV/HCV NAT.

I am just going to read through the slides as quickly and painlessly as possible

[Slide.]

For our implementation, firstly, we will need an organizational approach and related policies in order to begin our implementation activity, some thoughts or some considerations that there is no FDA licensed tests that we are painfully aware. Testing is not required or mandated as of yet, and it can't be prior to licensed tests.

FDA will require a multiple year cycle for review and licensure. IND kits are anticipated to be available for blood donor screening by July of next year, and the FDA endorses, at least I believe they endorse a national IND approach.

West Nile Virus testing will likely become the standard of care as the industry implements this test under IND. An open question is obviously will the hospital want to pay an extra cost for the new test that we have to pass on to them, that it is not required or mandated. We have these issues with HIV/HCV NAT

[Slide.]

Implementation has an impact on our testing labs related to procedures and systems, our computer systems, which I am going to go through in painful detail for you.

In our BIS group, which is Biomedical Information Systems, controls all our computer related activities. Multiple systems will need revision and this will impact on currently scheduled releases.

The activities that we have related to compliance and et cetera are scheduled in computer changes long before those changes have to be implemented, so we already have a whole wave of scheduled releases that are ongoing, and West Nile implementation will obviously impact those.

It will affect our regions because we are going to have to replace a critical computer system that our computers use to receive lab results, since our DMS system can no longer accept any new results, so DMS will have to be replaced.

Our hospitals, we don't know pricing for NAT reagents yet, but we can guess that they are going to be somewhere in the 2.00 to $5.00 per donation range, and that is for reagents alone.

Red Cross has an extensive compliance improvement program. West Nile implementation will obviously cause these activities to be reprioritized or will cause us to require more resources.

The impact on above depends on the timeline for implementation. As we have talked about July of next year may be the earliest date for the initiation of an IND, will we initiate nationally or will we first implement in those areas with the highest mosquito or human activity.

[Slide.]

Now, I am going to go through testing. Our testing assumptions are very simple and they have been covered. Screening will be by a NAT assay in our five existing Red Cross NAT labs using the current assay platform. If this changes, that means the timeline just extends considerably.

IgM would be used most likely for donor follow-up and donor counseling. We would implement a serologic test and a single NTL, so that all of our follow-up samples would go to that single location.

Another assumption is that we would use our current pools of 16. Obviously, if West Nile Virus can't

be tested in a pool of 16 or if the viremia is too low and we have to look at a lower pool size, those will be looked at, but currently, to implement smoothly, we need our pools of 16 to be maintained.

We have discussed individual unit testing as not being feasible. Steve Kleinman just highlighted that in the AABB statement. The September BPAC that we just had clearly identified that we are not ready to do individual unit screening yet.

Lastly, a testing assumption is that our sample handling requirements will be identical to those of current NAT.

[Slide.]

We have taken a poll of all the testing labs as to what they need. This is an estimate over what they have given me and that I have collated. We need an additional 22 FTEs which range from 3 to 6 per lab.

We need additional equipment because our testing volume will double. It will quadruple if we have to implement pools of 8, for example. We need dedicated assay readers, which in the system we use are luminometers. We

need additional storage freezers for reagents, and we will need other miscellaneous equipment.

As far as an impact on transmission of test results, we don't believe that there will be an impact, however, there will be a one- to two-hour delay in generation of test results. Cost, excluding the reagent costs, just for this estimate that I have given you, we approximate $1.5 to 2 million.

[Slide.]

Of course, everything has to be validated, but validations can only occur after we have developed, for example, SOPs, so we need to firstly develop SOPs. All of our NAT SOPs will require modification except those in sample management and pooling.

Once the SOPs are developed and validated, we will have to train all of our staff on each of these new procedures. Any additional equipment we have, we will have to go through an extensive validation procedure, as well as the assay itself, all software and systems in general including what we do with the end of our implementation, which is a process qualification to ensure that everything from the beginning that is sample receipt through the

generation of test results leaving the NAT lab are in control with the change, and the timeline for this is unknown.

[Slide.]

Our biggest impact, though, is with our biomedical information systems. Our parent computer system that sits in our 36 regions is called NBCS, our National Biomedical Computer System. We have to transmit test results from the NAT labs to NBCS, so that they may manage all products and donors.

The systems that will be impacted by NBCS changes and all the other changes that have to occur include our Procleix assay software. This is the actual software that will read the results in the NAT labs.

Those results are transferred to something called the NGTL automation system, NAS. NAS will then translate pool results to individual whole blood numbers and then those results must get collated with all the other test results and sent to regions.

Currently, we do this one of two ways. We use DMS, but since there is no connection between NAS and DMS,

we have had to implement another computer system called Surround, so these two can communicate.

We also have a replacement system for DMS in the works called LIS, but that hasn't been implemented in all regions as I will review. So, DMS and LIS interface with the regions, that is, with NBCS, and I have listed there the extent to which we have these systems in the Red Cross. Again, NBCS is where everything finally resides.

[Slide.]

Where are we with NBCS? We are now on what we call Release 1.5.2, and even though we use the Chiron Procleix assay that was licensed on February 27th, we have not yet fully implemented all IVD aspects of testing.

Currently, what we are waiting for is the system I told you that converts pools to whole blood numbers. We are still pending 510(k) approval. There are some package insert changes that we require before we can fully implement NAT under IVD, and then there are other issues that we will remain under our IND policy for donor and product management until there is final guidance from FDA.

Now, as I mentioned, we have subsequent NBCS releases that deal with many other issues at Red Cross.

These are Releases 1.6 and 1.7. They have long ago been defined, and again, they deal with multiple compliance issues that we have made commitments to, to FDA

[Slide.]

So West Nile Virus NAT will require a new NBCS software release. What we call it and where it will fit in, I don't know. That is why I have 1.5.X or 1.6, where we will squeeze this in. We cannot do donor and product management manually. It will be fraught with errors and one thing we have learned from the implementation of NAT is we cannot do that.

We are also talking about implementing other tests, West Nile Virus is not the only test on the horizon. This year we have made a commitment, an organizational commitment to implement bacterial detection. We have to implement some type of parvovirus B19 testing.

We have had discussions with FDA about Chagas, and this was reviewed at the last BPAC Committee, so Chagas isn't going away. We are investigating a number of transfusion-transmitted Babesia cases, so new agents keep coming, West Nile is not the end of the road.

The impact on timeline for other releases that have already been defined, and this is excluding any other testing, are unknown, but we should expect delays and some level of prioritization will be required. Again, we don't know the timeline or costs for West Nile Virus special release.

[Slide.]

DMS, this is again the way we used to and the way in most cases we transfer all test results from the labs to the regions. DMS is an antiquated system and as I mentioned, we cannot add any more tests to the system. It is in place in the majority of the Red Cross system.

We have implemented Ortho Lab LIS, whatever we want to call it this week, as our replacement system for DMS, but that hasn't been completely successful. We have identified some major issues, so that system will not be implemented further.

What we will do is take Surround, which is an IDM system, and that will replace all of our DMS use and LIS, but in order for us first to talk about adding any tests, the DMS in place in 31 regions will absolutely have to be replaced.

We will then need an interface with NBCS and again, the timeline and costs for all of this is unknown

[Slide.]

Lastly, for BIS, the two issues that relate to the lab are a NAT assay software, which is the Procleix software, that will be developed by the manufacturer, but we will have to validate the system, the software. We will have to have dedicated equipment most likely to read the results, so that we are not interpreting test results on our IVD system. Our assumption is we would need a separate dedicated IND system to crunch IND results.

Again, I mentioned we have software that translates pool results into whole blood numbers. We have submitted a 510(k) for a current HIV/HCV NAT. We are awaiting 510(k) approval, but if we change that, we will have to submit another 510(k) for approval.

Now, I don't think that will be required for an IND, but it will certainly be required for a licensed test. Again, the timeline and costs are unknown.

[Slide.]

What happens in the regions? I mentioned that we have to replace DMS, so the equipment and software

validation, procedures, training, all of that is going to have to happen. Donor deferral, will the policies change relative to what FDA has mentioned? We are kind of using a 28-day period as two times the viremic period prior to symptoms.

Will donor reinstatement just occur by allowing the donor to come back and test West Nile Virus NAT negative, or will we need a follow-up sample, which we will be doing anyway under IND, and then the donor tests NAT negative.

We will have a IgM positive result that we will be able to tell the donor you were infected in the past and now you have cleared your infection, or if you are negative, will that indicate that the West Nile Virus test was a false positive.

[Slide.]

We have talked already about in-dated products and previous and subsequent products. My point here is will any of these procedures change with a licensed NAT assay and also recipient notification.

[Slide.]

One thing that we haven't talked about is our favorite, retrotesting. At the time that we have a test under IND, will we need to replace all of our frozen, that is uncooled fractionater, products from the epidemic year and from the epidemic areas.

Our FFP turnover is estimated to be about three months, but how much from 2002 would be untransfused? Could we relabel those FFP units from this year as recovered plasma for fractionation? Then, what about our liquid inventory, would we have to test and replace that?

All of the regional processes which are governed by BSDs would need to be modified, that is, about 8 to 10 would require modification with half of those requiring FDA review and approval prior to our implementation.

Lastly, what do we need to do in testing HIV/HCV NAT, so an IND needs to be written, it needs to include IRB review and approval of all of our human subject involvement, our donor consent materials, donor notification and counseling. These processes and procedures will need to be developed that require validation and training.

Lastly, as has been highlighted all during this meeting, the IND or the implementation of testing needs to be a collaborative effort between the test kit manufacturers, the blood collection facilities, and the FDA, and again, the timelines and costs of this are unknown.

Thank you for your attention.

[Applause.]

DR. WILLIAMS: Thank you, Susan.

The third speaker is Dr. Celso Bianco representing America's Blood Centers.

ABC

DR. BIANCO: This has been an excellent, excellent meeting and what I think makes it excellent in part is because all the questions that we all are raising now have been raised during the meetings. We didn't get the answers, but we raised the questions

[Slide.]

America's Blood Centers has 75 member centers, being 74 in the U.S. and 1 in Canada. It distributes about half of the U.S. blood supply, that is, 7.2 million red blood cells in 2001. It provides transfusion services and

stem cells, cord blood, tissues in many cities, over 50 cities, and recruits 40 percent of the marrow donors. Several of our centers carry major research programs.

[Slide.]

NAT is not new. We implemented NAT in March 1999. Our centers cover both technologies. Those centers here in green are centers that are using the current GenProbe technology, and the centers that are using the Roche technology for a total of 15 laboratories

[Slide.]

When we asked the question, the many questions that we have here, they are the ones that I am going to try to address - why, where, what do we know, can we do it, how could we overcome the challenges, what do we need, and when.

[Slide.]

Why test blood donors? Robin, this is your answer. As of November 1st, there were 3,475 cases of West Nile Virus and 201 deaths. If the ratio of 1 to 150 for serious illness is correct, over 500,000 people have been infected in 2002, and West Nile Virus is primarily transmitted by mosquitoes, but has been transmitted by

transfusion in at least 6 cases, more are being investigated.

Eighty percent of the individuals that are infected are asymptomatic, and the actual risk to transfusion recipients is unknown. Still here I want to remind you that I wish we had the same amount of information about variant CJD when we decided to defer 5 to 7 percent of our donors.

[Slide.]

The scientific challenges were all raised here. It is assumed that the viral titers are low, but what are the actual viral titers in infected humans, what are the viral titers in implicated blood donors and recipients with positive PCR results?

How can we translate measurements used in animal studies into numbers that make sense for a licensed test, or what has tortured all of us during this meeting, what is the correlation between PFU, TCID50, genome equivalence, copies, or I am sure that somebody is going to come with a concept of a unit to resolve all those issues.

[Slide.]

Implementation challenges, I think that Sue Stramer raised very well all the issues that all blood centers are confronted with, the need to reduce the minipool size for West Nile Virus NAT, and do we have to increase the amount of specimen that we collect for testing, will we exceed current guidelines.

There are very strict guidelines about the volume. If we collect an extra tube or a higher volume of sample, we may exceed these and we may have to defer donors or not accept donors at the lower weight limits.

How do we plan validation for a test that we do not know, where will we find external controls to comply with CLIA, and will false positive results be an issue? I hope the syphilis issue with antibody tests doesn't show up anywhere else.

[Slide.]

How should we confirm the NAT test results, how much more staff will we need, how much training, how can we develop and validate the required software for assay and product management in a timely manner, and how will we deal with frozen components, particularly frozen plasmas.

[Slide.]

The issue of pool size was extensively discussed at the BPAC, and from our point of view, the current technologies, and the point of view of the majority of our members where HCV and HIV using minipools are not sufficiently automated to allow for individual donor testing for HCV, HIV, or a potential test for West Nile Virus.

The implementation of single donor testing or reduction of pool size will probably interfere with the quality of HIV and HCV NAT.

[Slide.]

If we think about pool size also, and I am actually more optimistic about these calculations than I was before we started this meeting, but if we just consider pool sizes, and we consider the specimen tests--a specimen has about 1,000 copies per ml--we will have, in a pool of 20, that is the average pool size that we use. One manufacturer uses 16, the other uses 24, a pool size of 20 specimens also will have 250 copies ultimately.

[Slide.]

Jim Gallarda from Roche actually helped me do the Poisson calculation in this. I expect from the numbers

that I saw here, very few and limited, that, yes, that we will be able to detect many specimens. If we have 500 copies, we could expect over 90 percent detection in our pools of 20. If we have 2,500 copies, we will be close to 100 percent.

[Slide.]

The viral load in a symptomatic infected individual has been estimated to be 1 through 5, and that is limited data. Based on these theoretical numbers, a 1,000 copy per ml sample could be diluted approximately 20 fold and still be detected more than 95 percent of the time.

Thus, current pool sizes may be able to detect a relevant number of samples with West Nile Virus sequences and prevent release of the corresponding blood components for transfusion.

[Slide.]

There are potential things that manufacturers may do to help us in terms of sensitivity and in terms of implementation. We could add NAT if we have the current platform and averaging the same obviously, we will implement assays in a more efficient manner.

Sensitivity may be less than optimal if lower viremia levels are confirmed. We could add NAT for West Nile Virus using the yet unlicensed automated platforms. These could resolve the issue of viremia if we have to, and obviously, we had some discussion about viral concentration procedures, but we want to remind manufacturers that at one point, it is desirable, but we do not want it to be an obstacle too fast, development of the test, the availability of detection for other viruses particularly Dengue and togavirus, because we ignored those issues--or not ignored--but accepted those as facts of life in the past, and it seems that we changed, and they are not acceptable anymore.

[Slide.]

Obviously, to remind all about the applicability to tissues, cadaverian organs and issues.

[Slide.]

In the absence of ideal assays, in order to implement an assay for the summer of 2003, we may have to accept screening of minipools with assays of limited sensitivity. It is better to screen and be able to

eliminate half of the potentially infected samples than not to do anything.

The second thing that we could try to do in a limited fashion is to screen a limited number of donations for recipients at high risk, the CMV model, but not exactly because even for the CMV, we screen a lot of units today using automated tests that allow us to provide sufficient numbers of CMV seronegative units for all patients with some risk. Here, we will focus on transplant recipients and highly immunosuppressed recipients.

[Slide.]

if we assume that we have a satisfactory assay by July next year, one question was raised, test only hot zones, I think that there are some facts there that we cannot ignore. The epidemic spread very fast in 2002. What would be the trigger? Birds falling off the trees.

How many days we have after there is evidence of epidemic zoonosis in a region between seeing that, detecting that epidemic, and implementing assays for humans. We want to be preventive.

It is unlikely that we can just have all these systems set up and we turn the switch on or off. So, my

sense is that for many of those reasons, and for the reasons that we cannot clearly define the borders of the hot zone, that we may have to test in the entire country.

However, I should note what Sue Stramer said here, as we implement those assays, we certainly should focus on those hot zones as priorities.

Again, in this area, and I think that Steve Kleinman dealt very well with all issues of donors and modification, but here, just to remind ourselves that we are not just telling a donor that you have a lifetime disease that is HCV or HIV, we counsel them, deal with the issues, refer them.

Here, we are going to reach these donors with a positive NAT result for West Nile Virus. After this donor is okay, the viremia is gone, there is nothing else. We have to recreate a lot of the materials, a lot of the methods, a lot of the things that we do in terms of donor notification, counseling.

[Slide.]

What do we need? There is a point where we have to come back, we need support from assay manufacturers. We need support from the CDC for information, prevalence

studies, and all that they have done for us in this sense, we need support from FDA overcoming these regulatory issues in a very timely fashion. This is going to be very important.

We need support from CMS and the health care system to deal with the added costs, and obviously, we may have to knock on the doors of Congress. They had several hearings, and we can tell more of our stories and the issues that we are confronting.

[Slide.]

Not to forget that we have competing priorities, Sue Stramer was very detailed about that, but we are looking at how to deal with contamination. That became most important infectious disease risk in terms of transmission by transfusion. We are dealing with parvovirus B-19, and we are dealing with NAT for HBV as priorities. They are next to us.

[Slide.]

In terms of when, ABC member centers will be ready to implement NAT for West Nile Virus when recommended by the public health system and assays become available,

and if anyone would like a copy of what I presented, my e-mail is at the bottom of the slide.

Thank you.

[Applause.]

DR. WILLIAMS: Thanks, Celso.

The next speaker is Michael Kanaley from the Plasma Protein Therapeutics Association.

PPTA

MR. KANALEY: Thanks very much. Good afternoon, everyone. My name is Mike Kanaley of the Plasma Protein Therapeutics Association.

I will not be using any slides this afternoon, but I would like to read into the record a statement on behalf of the Association.

PPTA is the trade association and standards-setting organization for the world's major collectors of source plasma and manufacturers of plasma-derived and recombinant analog therapies.

Our members provide 60 percent of the world's needs for source plasma and plasma protein therapies. These include clotting therapies for individuals with bleeding disorders, immunoglobulins to treat complex

diseases and immune deficiencies, albumin for burn and trauma victims, and alpha-1 anti-trypsin for alpha-1 anti-trypsin deficiency.

PPTA members are committed to assuring the safety and availability of these medically needed life-sustaining therapies. With regards to West Nile Virus, PPTA member companies have already taken actions to address the potential risk posed by the virus to plasma protein therapies.

As demonstrated earlier today by industry experts, the emergence of West Nile Virus has not changed the risk-benefit ratio for plasma products for fractionation. The viral inactivation and removal processes as used by the plasma protein industry provide significant safety margins against the transmission of West Nile Virus via these therapies. However, the benefits of fractionation and the subsequent inactivation and removal technology are limited to manufactured plasma protein therapies.

It is PPTA's position that based upon the scientific evidence presented at this meeting, the institution of source plasma donor testing or additional

in-process control measures would afford no significant increment in the safety of plasma protein therapies with respect to West Nile Virus.

The industry does recognize the importance of donor selection prior to testing as a step toward product safety. As such, current industry practices for donor selection complement the enhanced screening measures in the FDA's recent guidance.

To date, there has been no known transmission of West Nile Virus by finished products manufactured for plasma for fractionation. Data from industry's viral safety experts confirm that West Nile Virus is inactivated or removed during the manufacture of plasma protein therapies.

This is borne out by studies with model viruses such as bovine viral diarrhea virus, BVDV, a virus closely related to West Nile Virus, as you heard much about in the last two days.

In addition, as demonstrated today, PPTA member companies have begun studies to investigate primary inactivation steps using the West Nile Virus and have reported their up-to-the-minute data. Again, we remain

confident in the safety and quality of the plasma protein therapies with respect to the West Nile Virus.

Notwithstanding these robust viral inactivation and removal procedures in place, PPTA members have already implemented the recent FDA guidance that donors report any post-donation illness and the temporary deferral of donors suspected to be infected with West Nile Virus. PPTA believes that these are appropriate and necessary precautionary measures.

In addition, PPTA members are uniquely situated to identify and interdict donations from donors who are at risk for West Nile Virus infection. Current industry QSEAL standards requires plasma protein manufacturers to hold all donations for a minimum of 60 days prior to manufacture.

This inventory hold period provides an opportunity to obtain post-donation information, including information about suspect West Nile Virus infection. If such information becomes available, the donation in question will be traced and discarded rather than entering the manufacturing pool.

PPTA's Qualified Donor standard applies to individuals who want to donate source plasma. All new

donors must first successfully pass two full sets of medical examination and viral marker screening before either donation can be accepted and used for manufacturing.

This introduces yet another layer of safety to plasma protein therapies. The combination of the inventory hold and Qualified Donor standards provide PPTA source plasma collection centers with a greater opportunity to obtain important health information from donors both pre- and post-donation and allow the source plasma industry to better manage potential health risks such as those posed by West Nile Virus.

In conclusion, PPTA believes that with respect to source plasma, neither donor testing nor institution of additional in-process control steps are necessary for West Nile Virus. The additional donor selection measures for post-donation reporting and temporary deferrals in FDA's guidance adequately address the potential risk posed by West Nile Virus to the plasma protein supply.

PPTA's voluntary standards and the robust viral inactivation and removal processes maintain the safety of these life-saving therapies for the consumers who rely on them.

Thank you for the opportunity to speak on behalf of the plasma protein therapeutics industry.

If you would like a copy of our statement, there should be a copy on the desk out front, and it will be on our web site, as well.

Thank you.

[Applause.]

DR. GREENWALD: Next, we have Dr. Martin Mozes from the Association of Organ Procurement Organizations.

AOPO

DR. MOZES: Good afternoon. Thank you for inviting us to present before this group.

[Slide.]

The AOPO Organ Procurement Organizations are not laboratories as such and probably most of them contract out their laboratory work, and we are not banks, we are not organ banks as such, so I feel that I am kind of free to present all the list of dilemmas and requirements that plague those two environments.

What I would like to do is to tell you about AOPO, which may not be familiar to most of you and about the organ procurement environment and our specific logistic

environment as it relates to screening and transmission avoidance.

AOPO, the Association of Organ Procurement Organizations, and this is from its web site, www.aopo.com, incorporated in 1984. This association is a private, nonprofit organization, recognized as a national representative of the organ procurement organizations, the OPOs.

AOPO is a professional organization dedicated to the special concerns of all 59 member OPOs.

[Slide.]

The mission statement for this organization is that it represents and serves organ procurement organizations through advocacy, support, and development of activities which maximize the availability of organs and tissues and enhance the quality, effectiveness, and integrity of the donation process.

[Slide.]

The working arm of this activity is the Organ Procurement Organizations, and I am just giving you one sample of a mission statement of an OPO, which happens to be the one that I am personally associated with, and that

is to save and enhance the lives of as many people as possible through organ and tissue donation.

Now, to fulfill this mission, the OPO strives to maximize donation, and this is through activities, such as public education, professional education, and developing appropriate systems within hospitals to respond to the opportunity of organ donation using the Department rules, et cetera.

Part of the mission then is accomplished by optimizing the quality and safety of the transplanted organs, and this relates to obviously the physiologic or functional quality of organs that we provide to recipients, as well as the safety of these transplanted organs both in terms of minimizing the risk of disease transmission, as well as minimizing the risk of, for example, transmission of malignancy.

So, it is both the quality, which is basically anatomic quality, physiologic functional quality, and the safety which are paramount in this activity.

We also strive to optimize the organ utilization for transplantation through effective systems and

implementation of efficient allocation and distribution of this scarce resource.

[Slide.]

Having said scarce resource, you saw some of the illustrations that Dr. Freeman showed yesterday in yesterday's session. The number of patients on the waiting list for organ transplants is growing exponentially at approximately 10 percent per year and currently stands at over 80,000 patients on the various organ wait lists, and this is after subtracting organs transplanted throughout the year, as well as patients who have died while waiting on the list, and that number is approximately 9,000 per year.

Now, the number of deceased organ donors or cadaveric organ donors we used to call it has increased in the past decade by about 35 percent, so when you saw yesterday the curve of the waiting list and this almost flat, flat line of the number of donors, that is not as flat as all that. Actually, there has been an increase in the past decade, but it is hardly enough to keep up with the growing demand.

So, as a more or less undesirable byproduct of this organ shortage, the number of live donors, by necessity, during this period has increased by 154 percent, so just to give you an idea of the numbers that we are talking about in terms of the need for screening, et cetera, we are at about 6,000 deceased donors per year in the U.S. and approximately 6,500 live donors.

This is actually the first year, 2001 was the first year where the number of live donors actually exceeded the number of cadaveric donors because of the shortage.

Now, the results of this increasing gap are the critical allocation issues as to equity and utility of this scarce resource, the longer waiting times and the increased morbidity and mortality on the wait lists.

[Slide.]

Now, I said before that the safety of the organs is of paramount concern. It has to do with the clinical responsibility to patients to provide effective treatment, the clinical responsibility to avoid exposing patients to risks of harm from transplanted organs, such as transmission of infectious diseases or malignancy, and not

least, the ethical responsibility to maintain the public trust in the safety and integrity of the system since this donation is primarily an altruistic voluntary activity.

[Slide.]

Let's get to how this actually happens. When a potential donor is referred, we have a very detailed, across the system, across all OPOs, very detailed methodology for taking a medical and social history, and this would include active or recent cancer, active sepsis especially fungemia, and questions relative to active viremia.

Now, when you take this history, it is not the same as taking a history from a prospective blood donor. This is, as was explained yesterday, these patients are not able to give their own personal history in that situation. The family is also under quite a bit of stress, and it is at best unreliable secondhand history what you are talking about.

When you are talking about vague symptoms, such as might be associated with West Nile Virus, and on top of that, when 80 percent of these patients may be asymptomatic totally, obviously, this is not a good screening tool.

We go on with a physical examination and other tests, which include x-rays, CT, at times others to rule out active cancer, significant infection. Then, we get into laboratory testing, which I will talk about in a minute.

These three tests actually constitute absolute ruleouts for organ donation. Of course, afterwards we have to get into organ-specific evaluation for the appropriateness of donation of specific organs.

[Slide.]

Now, I think this is probably the most important slide in this presentation, because this tells you how time sensitive this process is and what our requirements are.

Starting here, the donor case usually starts with determination, after determination of brain death, confirmation of brain death, consent from next of kin, and, when applicable, consent also from the medical examiner or coroner.

At the beginning of the case, lymph nodes are obtained for tissue typing, and blood is obtained for the serologic and other testing. At times, blood can be used also for tissue typing.

As far as the timeline for this, for the product of this activity, you can see that we reconfirm the ABO type of the donor, we obtain the HLA type, and that is available within a couple or three hours.

The cross-matching that is done with the trays of recipient sera that are in the histocompatibility laboratory take longer, and that relates to subsequent allocation and distribution primarily of the kidneys and pancreas, which require cross-matching routinely.

The serology testing, the results are usually back by five or six hours, and that is pretty much the requirement within this scenario, because during this period, the donor is managed in terms of clinical management. These are donors who are on life support in the ICU, sometimes hemodynamically unstable as a result of their brain death condition.

Evaluation of organ function takes place here, and then these organs have to be placed at different transplant centers for different recipients based on allocation lists which are printed out from a central location. This requires multiple phone calls.

Subsequently, when all of this is done or sort of superimposed on this, we need to do the coordination of the organ recovery itself, which occurs after all of this has been done, after the serology has been cleared, and then the organ recovery takes place somewhere here. Nowadays, it is frequently a multi-team, multi-organ recovery, so it takes quite a bit of coordination.

The thing is that in order to clear this donor, in order to ascertain that the donor is free from risk of transmission of infection here, we need to know this as soon as possible, because there is a lot of logistics committed along the way here, so it is not enough to get a result at this point here, because this is kind of late in the game, and subsequently, there is not a lot of time to wait for results.

The heart has to be transplanted within four to six hours of removal, as Dr. Freeman mentioned. The liver has a few more hours, but there is a lot committed at this point including the recipients, which have to be coordinated and lined up at this time.

[Slide.]

This is the testing that is currently performed for organ donation. All tests are performed on pre- and post-transfusion samples. Now, there is a limitation to this in that the donor may receive further transfusion in their management, and there is no further testing after that.

As I mentioned, these three tests currently constitute absolute exclusion donation.

[Slide.]

Finally, the requirements for screening tests in this environment are that the tests must be readily available locally at any time in every OPO service area, the results must be reported prior to organ procurement, preferably within six hours from start of the case.

The tests must be reliable with high sensitivity and specificity, just an example of the HIV test that we have currently, and obviously, false positives result in wastage of this scarce resource, and that would be very undesirable.

The tests should also assess the temporal relationship of active infection and transmission potential we talked about, serology versus RNA testing.

[Slide.]

In summary, this is a critical, complex, time-sensitive process. The transplant community has the obligation to maximize the number of organs available for transplantation.

The risk of transmission must be minimized although, as was stated yesterday, clinical judgment of risk-benefit ratio is frequently applied where there is an imminent risk of a potential recipient's life, such as status one, hearts and livers.

Of course, we must have a rapid and reliable test to screen for this virus as soon as possible.

Thank you.

[Applause.]

DR. GREENWALD: Next, is Ms. Jackie Malling speaking on behalf of the Eye Bank Association of America.

EBAA

MS. MALLING: Hi. My name is Jackie Malling. I am with the Minnesota Lions Eye Bank in Minnesota. I am the director. I am happy to represent the Eye Bank Association of America, and we appreciate the invitation to speak today.

[Slide.]

Just real quick for those of you who might not be real familiar with the Eye Bank Association of America history, it is the oldest transplant organization in the United States. It originally was established in 1961 when corneal transplants really became a lot more common and more routine medical procedure.

There are approximately 94 eye banks that are members of the EBAA. It is recognized as a national accreditation organization, and approximately 99 percent of the domestic corneal tissue supply is supplied by member eye banks of EBAA.

Just a point of reference in terms of as you are looking at the other donation organizations, there are approximately 90,000 donors, eye donors that are screened every year, so it is a pretty high volume.

[Slide.]

The EBAA has extensive medical standards that really are developed specifically for cornea transplant. They are developed by the Medical Advisory Board, which consists of a lot of physicians and some eye bankers like myself - nurses or eyebankers.

It is a very dynamic group that addresses issues, a wide variety of issues that come up again specific to eye donation. All the EBAA medical standards that are passed originally by the EBAA are run by the American Academy of Ophthalmology for approval.

[Slide.]

It is interesting to note as we sit here and talk about West Nile Virus and I just say wow to the challenges that you all have ahead of you here, we are fortunate to have I think a very good safety record for cornea transplantation.

In the United States, the first cornea transplant was in 1905. Again, they became a more common procedure in the 1950s, I believe it was, and nearly one million cornea transplants have been performed since 1961.

There have been no known systemic disease transmissions via cornea transplant since 1986, so I think this speaks to at least in some way the evidence that our medical standards are being effective in providing safety for cornea tissue.

[Slide.]

The EBAA has an ongoing rule in the safety, of course, of cornea tissue. We take safety to the heart. We monitor information that is going on with cornea transplants, we issue alerts. The EBAA will issue medical advisory alerts or other sorts of alerts to the membership very quickly.

They require eye banks to seek and report adverse reactions. This third point here, the adverse reaction reports, I think we also were the first transplant community to have an adverse reaction reporting system, where not only were we required to maintain adverse reactions, but we are required to report them to the EBAA, who will then, of course, forward on systemic disease transmission reports to the FDA.

We also monitor the safety of other transplant communities, hence, I am here today.

[Slide.]

Of course, we know that the FDA has made recommendations, you have all been talking about them today and yesterday. We are aware of the recent information that the FDA issued to blood and organ organizations, and we also note at this time that there are not additional

recommended changes in the screening of organ and blood donors.

[Slide.]

For West Nile Virus and eye donation, what does this mean? Well, currently, there is no evidence to suggest that West Nile Virus is transmissible via cornea transplant, and we are going to have to watch this as this goes, but regardless of that are the current EBAA medical standards to rule out donors.

They do evaluate for sepsis, which at least for 20 percent of the population that has a West Nile Virus Fever or West Nile Virus infection, we might catch some of those although it has been highlighted here today and yesterday that donor medical and social history interview, and also reviewing the medical records for these symptoms, we are not going to catch the majority of the cases.

[Slide.]

Regardless, just to point out, and I won't read all of these, but some of the EBAA medical standards that would rule out an eye donor, that might have West Nile Virus Fever, would be, on this slide, death with neurologic disease of unestablished diagnosis would relate back to

maybe more the severe cases of West Nile Virus in terms of if there is actually severe neurological symptoms going on.

These two are more relevant, I think, in terms of catching the more mild cases or at least the second one, active septicemia ruleout including bacteremia, fungemia, and viremia. So, if we know about that, those donors are not going to be suitable for transplant.

[Slide.]

Again, active bacterial or fungal endocarditis and intrinsic eye disease, and this covers a lot. It basically covers inflammation within the eye, so if we noted in the medical record or in the social history that the donor had eye pain, that would be something that we would look into and possibly consult further.

[Slide.]

So, our current role in monitoring with West Nile Virus and what is going on related to current cornea tissue, again, the EBAA would report any known or suspected cases of systemic disease transmission via cornea transplant. The EBAA will consider new information seriously and take decisive action and work with other agencies.

[Slide.]

In terms of eye donors and cornea transplant, it is going to be important to consider the cornea transplantation apart from other organ tissue, organ transplant, or blood transfusions as we get more information and as we are trying to discern how to proceed.

A few points about cornea transplant that are different. Currently, of course, the excellent safety record that we have, but also I know AOPA just spoke, and Dr. Mozes pointed out that donors with a history of cancer would be ruled out, but for eye donation, a lot of cancers are suitable, and there is no known transmission of certain, like whole organ cancers.

Also, I think a lot of you probably know, and I know that Dr. Solomon does, that the cornea is avascular. We say this all the time, but it does make a big difference as we are considering these new emerging infections that are coming up.

We do have required tests for eye donation. It would be HIV-1 and 2, hepatitis B, and hepatitis C. That would be hepatitis B surface antibody for hepatitis C and the antigen for hepatitis C.

Also, in terms of the recovery time for eye donation, we like to recover and transplant, well, surgeons like to recover and transplant within five days typically. The tissue is suitable, according to the manufacturer's recommendations, a little bit longer, up to 14 days, but most surgeons in the United States will really want to transplant that tissue within 3 to 5 days, so we, of course, have time constraints as well.

[Slide.]

In summary, we really feel that the eye donor screening measures are sufficient at this time. The EBAA will, of course, evaluate new information quickly as it comes along.

We would like to collaborate with other agencies as needed, if there is something the testing labs need from us in terms of determining what an adequate, suitable blood sample would be, we would be happy to work with them and comment, and then we would evaluate any new donor screening or testing methods that come along.

This is to remind me that I am going back to Minnesota where it is freezing and snowing and the mosquitoes are dead.

Thank you very much.

[Applause.]

DR. GREENWALD: Next, we have Dr. Mark Damario for the American Society for Reproductive Medicine.

ASRM

DR. DAMARIO: I would like to read a statement. On behalf of the American Society for Reproductive Medicine, I would like to thank the FDA and co-organizers for the opportunity to speak about the concerns of West Nile Virus and reproductive medicine.

I am here representing both the Society of Assisted Reproductive Technologies, as well as the American Society for Reproductive Medicine.

The Society for Assisted Reproductive Technologies, otherwise known as SART, is a sub-society of the American Society for Reproductive Medicine. This society represents approximately 370 assisted reproductive clinics in the United States.

The American Society for Reproductive Medicine, otherwise termed ASRM, represents approximately 9,000 health care professionals in the area of reproductive

medicine and related disciplines in the Unites States and abroad.

Following the report of the first cases of possible transmission of West Nile Virus by organ transplantation, published in the MMWR on September 6th, SART and ASRM members have been carefully considering whether there is sufficient data to warrant changes to current reproductive donor and screening and testing practices. Those primarily entailed the screening procedures for sperm, oocyte, and embryo donation.

We are aware of the reports of confirmed West Nile Virus, meningoencephalitis or meningitis diagnosed in patients receiving recent blood products, as well as the FDA's final guidance to the blood industry published on October 25th.

We are also aware of the recent reported case of West Nile Virus presence in breast milk and its possible transmission through breastfeeding. At present, however, there appears to be very little information on the potential of West Nile Virus transmission through reproductive material.

Although certainly we do not have a reason to discount its potential, in the absence of national testing or screening standards regarding West Nile Virus in organ or tissue donation, there are no current guidelines for reproductive clinics to follow.

Looking into the future, outside of cases of confirmed West Nile Virus where perhaps the individual should not donate any blood, tissue, or any other cellular material for at least some time period from the onset of illness, how do we effectively prevent the transmission of West Nile Virus from unconfirmed or asymptomatic cases, which is a major theme of our workshop.

Although a secondary consideration, we nevertheless are concerned about the potential economic impact of West Nile Virus testing and reproductive medicine at a time when the risks are not fully known.

I appreciate the opportunity to attend this informative workshop. SART and ASRM are both seriously considering these issues and remain open to suggestions.

Thank you.

[Applause.]

DR. GREENWALD: Next, we have Dr. Judith Woll of the American Association of Tissue Banks.

AATB

DR. WOLL: Good afternoon. My name is Dr. Judith Woll and I am the Chief Executive Officer and Medical Director of Community Blood Center, Community Tissue Services in Dayton, Ohio. I am also a member of the Board of Governors of the American Association of Tissue Banks and I am pleased to present this statement on behalf of AATB to observe the development of tests for the screening of tissue donors for West Nile Virus.

Similar to the other organizations, AATB is a voluntary professional, nonprofit, scientific and educational organization. Our mission is to promote the availability of safe and high quality human tissues for transplantation.

To further this mission, the AATB publishes its Standards for Tissue Banking, a recognized authoritative source for the industry. For more than 15 years, the AATB has also operated its own voluntary accreditation program to ensure that tissue banking activities are being performed in a professional manner and in compliance with

these standards, in addition, AATB educational, scientific, and certification programs for tissue bank personnel.

AATB's membership currently exceed 1,100 individual professionals and more than 70 accredited tissue banks engaged in the recovery, processing, storage, and distribution of human tissue.

Most of the major tissue banks have obtained AATB accreditation and AATB-accredited banks provide most of the human transplantable tissue in the United States.

[Slide.]

To give you a concept of the size that we are talking about, we distribute about 850,000 grafts each year from about 20,000 donors, so that means that each donor gives between 30 and 60 grafts, so we are somewhere different from the blood industry and maybe a little closer to plasma in that sense.

Of course, also closer to plasma is that most tissue grafts are processed in ways that we reduce the viral load.

The AATB's mission has consistently supported FDA regulation aimed at assuring the safe and clinically beneficial use of all human tissue provided for

transplantation in the United States. In 1984, we published first edition of standards, we have set rigorous performance requirements for the prevention of transmission of communicable disease.

Over the years, the standards have been revised and incorporate increasingly stringent donor screening protocols, have been revised to require the use of additional FDA licensed laboratory testing procedures for markers of potentially transmissible diseases as they become available.

The safety of human tissues for transplantation is therefore a primary goal for AATB. While there are no reported cases of West Nile transmission by tissue transplants, the confirmation of West Nile transmission by organ transplantation, and as discussed yesterday, by blood transfusion, raises major concerns for AATB.

[Slide.]

Our screening is similar to that described by the eye banks and the organ banks. We have a medical/social history obtained from the next of kin usually, a physical examination, a review of the medical records and any autopsy records that are available, the infectious disease

screening, and then a medical director must review the entire chart to evaluate the release of the tissue.

[Slide.]

Our standards, similar to the other standards reported, say that we prohibit the release of any cell or tissue for transplantation from donors who exhibit evidence as detected by history, physical exam, laboratory testing, or autopsy, of significant active infection at the time of donation.

So, like everybody else, for those who are symptomatic, we hope we will have excluded them, but the issue is still the asymptomatic patients as has been discussed for the last two days.

[Slide.]

One of the issues is screening laboratory tests for blood, organs, and tissues, and we really have very different issues, and I think that is one of the reasons why we have been mentioning this over and over again, the blood people are concerned about the volume, the logistics of handling 13 million donations a year, the turnaround time for the short shelf life of platelets, and, of course, the cost, because the cost is distributed over only two

products, if you will, or three products from each single donation.

There are major concerns about turnaround time. For tissue, I think our major concern is the quality of the sample and the ability for the tests to be valid from cadaveric samples, and as Dr. Biswas said, we test basically blood from cadaveric donors.

[Slide.]

Just to tell you, as compared to the organ world, well less than 10 percent of our donors are heart beating. Our donors are what are called dead dead, and they may be dead anywhere up to 24 hours if refrigerated before the samples are drawn, because we sometimes get our samples by doing heart sticks at the time of procurement. Therefore, there are many sample issues, such as hemolysis.

Hemodilution was talked about earlier and we do calculations based on an FDA algorithm to assure that there is not excessive hemodilution or we do have to get a pre-transfusion sample. Of course, there could be enzymatic degradation by the RNases in the blood.

So, we, as an organization, respectfully request that the FDA do all in its power to encourage test

manufacturers to develop licensable tests, not just for blood and plasma donors, but also for the use of the cadaveric blood samples of tissue donors.

[Slide.]

As we have in the past, the AATB is willing to work with the FDA and test manufacturers in this important effort. Again, in the past, licensure has focused on blood donors because they have got great numbers, they have a market large enough for the manufacturers to be willing to fund the very expensive IND and BLA studies, and whereas tissue and organ, the number of donors is small and the definitions for qualifying test performance are critical due to the limitations of the specimens.

[Slide.]

Right now what will AATB do? Well, obviously, we are reviewing our standards to determine whether revisions are necessary, whether the standards need to be more explicit. We will add a test when one is available and approved for use with cadaveric blood, and we will certainly assist the FDA and test kit manufacturers in validating cadaveric samples.

On behalf of the AATB and the thousands of our donors and the donor families, our individual members and accredited member banks, and the hundreds of thousands of patients we serve, I thank you for your attention and your consideration.

[Applause.]

DR. GREENWALD: Thanks to everybody.

We are going to see before we drag everyone up here for the next 15 minutes if we actually have any questions for any of our previous speakers.

Discussion

DR. FREIBERG: Space, final frontier. With all that Sue presented and the difficulty in getting all the other things, and I went to computers and staffing and money, I didn't notice whether or not you mentioned that if you could get all of that together, you have space.

If you don't have space, can we do the rest?

DR. STRAMER: What I presented as far as the testing assumptions or what we can do within our current five NAT labs, so the amount of equipment we can add, the amount of people, the amount of storage of test reagents, et cetera, we can add--well, I did a survey of our five NAT

labs to ask them what it would take to go to pools of eight, well, let me back up, what it would take if our current pools of 16, to add one test, what it would take to add two tests, and then if we went down to pools of eight, what it would take to add one test and what it would take to add two tests.

Currently, within our space requirements within those five NAT labs, we can add either up to two tests, samples of 16, or we can do one test if we reduce full size. We know West Nile is coming, and as I said, another critical test that we are going to have to look at this year in the NAT labs is also parvo, so that probably leaves us at pools of 16.

DR. FREIBERG: The reason I mentioned it, and I thank you for your great answer, is that there is other things coming, too. We have peptides B testing we have been talking about.

DR. STRAMER: Yes, I am familiar with that.

DR. FREIBERG: Well, one of the strategies is to start at a discriminatory hepatitis B by itself before the triplex assay and system is set up, and if we look at that algorithm, we are talking about setting up a separate semi-

automated system for discriminatory HBV, and then when the triplex assay is ready, you have to have that as a separate system from the licensed tests and the discriminatory approach for that, all three of them.

So, is it even feasible in 2003 to be thinking about parvo, West Nile, discriminatory HBV, and a triplex test? If not, we have to start talking about what some priorities are and figure out what we are going to do.

One of the things we are here together is to I think talk about what those priorities should be.

DR. STRAMER: Well, I can only answer for the Red Cross and a lot of this still is developing, so it is colored by my own opinions, but I do have some influence, so HBV discriminatory NAT, we likely will not implement as a stand-alone test.

We are very hopeful that one day soon, PRISM will be licensed, and NAT with increased HBsAg detection, will carry us over until Ultrio is licensed.

Now, that brings up other questions as far as the sensitivity of HBV NAT, and the reality of doing it in pools of 16 and NAT automation realistically, the way we would like to introduce Ultrio is on an automated system in

which we either reduce pool size or go to individual unit testing to really maximize our yield for HBV.

That again is a whole another discussion, so our priorities right now, certainly NAT automation is always ranking really up there as number one, but short term what we can do with our existing facilities is only implement the two tests that are probably at our highest priority level, which is West Nile and parvo.

That is the confines of the NAT lab, and then if we look outside of the NAT labs, we are going to have to implement bacteria and bacterial detection, and as soon as PRISM is licensed, we are going to all pedal to the metal, we are going to replace our current technology with PRISM.

DR. STRONG: I think that is an excellent question because we are all struggling with that and many of our laboratories in ABC are about maxed out on space.

Our hope had been that we have PRISM by now, which would free up about three times the space that we currently have to use with the systems that we have. We badly need automation.

I don't think we can do more than one more test with the current system we have on NAT because it is a

semi-automated system at best, and without automation, we are not going to be able to go much further, so if there are any other tests to be done, there is no space in our facility, we will have to find a new space. I think if I were speaking for the New York Blood Center, they have talked about renting out Yankee Stadium, I believe.

DR. BIANCO: I think that was a very important question, but there is another very close second there to space, and I thought you were going to ask about it. There is staffing. We have a tremendous difficulty these days of finding qualified laboratory personnel even in states where state requirements are not as complex and complicated, where they have the local FDAs employed in California.

That is another issue that would be certainly alleviated by more automation, less dependency on individual operators.

DR. GREENWALD: If there is not going to be any more discussion, maybe we can all come back a little bit early, so we can leave early, start about five minutes after 3:00.

[Recess.]

Panel Discussion

DR. NAKHASI: I would like to call the names of the people who need to be up here. Dr. Goodman is here, myself, Liana Harvath, Mahmood Farshid, Darin Weber, Bill Hobson, Glen Freiberg, Robert Lanciotti, Lou Katz, Mike Busch, Steve Wagner, and Thomas Kreil.

We are all here. The purpose of this discussion is basically to discuss what we learned during the last two days and the issues we discussed, and basically, this is focusing the issues which we have discussed the last two days.

This is the time for the audience to clarify the issues and maybe we can sort of answer those. At the same time, I would like to ask Dr. Goodman to sort of give us a little bit of perspective, and then we can open final discussion.

DR. GOODMAN: I didn't necessarily expect to say anything, but I have been writing down some things that might be worth saying, so I guess I am okay.

Maybe I will just make a few comments for people to react to and just again summarize some of FDA's feelings on this issue and some things especially in light of what we have learned both from the factual presentations here,

but I think also from the valuable opinions and controversy that swirl around this issue and will continue to do so because in the absence of knowledge, there will always be controversy. Right now there is a fair amount of absence of knowledge.

The first thing I was going to say is that the reality is, as some have said, that this is a moving target and there is insufficient and evolving knowledge. That is not bad, you know, that is reality.

The fact that we are all trying to do our best under those circumstances means that we will probably do some things we could have done better because we can't predict the future and we don't even know enough right now to learn from all the past.

I think a big issue that is unresolved, that has been brought up from several directions, is the needed sensitivity to be used in an assay, and really the deeper question is what is the infectious dose of this virus and what would we need to detect.

I think one thing we need to be wary of is that there is case investigations and there is again incredibly praiseworthy work that Rob Lanciotti's lab was remarkably

ready to do. I think they really deserve a lot of credit for this.

We still need to realize that right now most of our case definition is based upon results of those studies, and we don't know, for instance, whether, in a whole unit transfusion, you know, could it be that in certain patients, one infectious virion is sufficient to transmit the disease or could it be that there is a dose threshold, and I think some of what we need to look for in the screening test is predicated on that, again, the importance of the kind of studies that Mike and others are talking about, trying to identify as best we can infected donors and look at what the outcomes of transfusion from those donors were. That is a big missing piece.

Connected to the assay, again I want to bring up, I know it complicates it further and I know it complicates it further with respect to the FDA, is this issue of if one develops a screening test, despite some of the technical barriers we heard, the potential long-term benefit of if it is possible to have nucleic acid detection that includes other potential relevant flaviviruses, whether that is

something to mull over a little despite some of the negative early experiences, such as in Rob's lab.

Similar to that and even more important, I think is this issue. I mean we are only even here able to talk about it because people have successfully developed some really good NAT tests and also some really good antibody tests, so we can actually talk about the reality of rapidly applying those in a clinical situation. That is building on successes.

But I think we are so much better off than we were before HIV or hepatitis and some of the recent experiences, but we are also kind of sensing that there is sort of a bottleneck in these technologies and in our ability to bring them along rapidly.

I think both the companies and FDA, to think as much as we can about how we add or potentially in the future subtract pathogens from technologies, how we use platform technologies, which of the new technologies coming along, whether they are gene chips or others, would really lend themselves in the longest term to a more nimble blood testing system that meets public health goals or whether we

are there by adapting current technologies, I think those are issues.

Then, again, are there ways from the FDA's perspective that we can stay within the existing laws and make it easier to use these platforms and move that along, and these are things that we have had some discussion of internally, but we do hear you and appreciate those concerns and want to keep trying to push what we are able to do there.

I think people know, and we said this in the guidance, that our current thinking is unless the current investigations turn out to be erroneous or nothing else pans out or the disease completely disappears from the face of the earth, that this is something we are facing here and that we likely will need to recommend screening for this disease.

However, I think that it is important as people have raised, that there are a lot of issues around that screening, and we want input about it and we will bring it to advisory committees, et cetera, these issues of are there seasonal and geographic targeting for screening.

We have heard about some of the impracticalities of that, which I think are very real, and we need to keep those in mind. I think if we have limited availability of testing at first, because of the incredible timetable we are on and the difficulties of meeting that at all, quite frankly, then, as I think Sue Stramer and others said, it would make sense to target what is available to places where it might have the highest public health yield at least initially, because that is reality.

I think we need discussion of what if no or very limited testing is available, I mean we are placing a lot of hope on the success of the testing, but we don't know yet that it will be available, we don't know yet how good it will be.

So, I think also in the Public Health Service and the blood community, we need to have our backup plans in mind, think about the roles of other technologies or at least studying them, maybe including pathogen inactivation, maybe other strategies for protecting the donor base.

Then, there are the issues of organ donors, and we don't know. I think, as we have said earlier in this

meeting, that the best thing to protect both organ donors and recipients is going to be to protect the blood supply.

I kind of feel good about that because I think we are making a lot of progress towards protecting the blood supply, but there are some other organ donor specific issues.

We heard about the availability, the issues of testing cadaveric blood, the issue about the availability of lymph nodes, which I think is something to keep in mind because I am not completely convinced, well, I think it is fairly unlikely that everybody with tissue infection is going to also have viremia at the same time, so that is something else to keep in mind.

Those are the major at least notes that I wrote down. Again, I know we are all very impressed by the many thoughtful presentations, the practical difficulties of developing a test when we don't yet know the pathogenesis of the disease, the likelihood that just nucleic acid testing will be sufficient, what limits IgM testing can be pushed to because there are some real advantages if that can be pushed.

Those are all things that have been raised and we will have to grapple with as more data become available. We may learn a tremendous amount from some of the planned studies that Mike and others have talked about that CDC is doing, that NHLBI is supporting.

We may learn from some of the data that the diagnostics manufacturers and the blood industry generate in developing these tests. We heard some tantalizing bits of that from Andy Conrad, and I think there is the potential to learn a lot more when tests get available widely out there under IND.

The fact is that the disease, the cases are not so common that we can get good numerator and denominator data without some pretty large populations being studied.

I just thank everybody that is here and look for input from the audience on what the Public Health Service in general, and FDA in particular, can do to be helpful here.

DR. NAKHASI: Thank you, Jesse.

Before we open up the discussion, I just want to basically highlight the two major issues. One is the challenges we have and the facilitating factors we have,

basically taking from Jay, I think challenge is the technology transfer, test the development as Jesse pointed out, establishment of regulatory standards, adequate sensitivity to detect low viremia, what is the level of the virus which is infectious, donor deferral criteria.

But we have also facilitating factors because we have prior experience with the NAT, we have opportunities to use existing platforms, but at the same time we have problems, what other problems will be evolving, as we heard, space, the technical people available, possible use under IND, resource sharing, and ongoing close cooperation. We all need cooperation between FDA, industry, you know, everybody in PHS.

So, I think those are the issues. On top of that, as I just would like to reiterate, the implementation is a major issue also, because how would we, because of the fact, the nature of the beast here, it is transient viremia, it is how long the viremia is, is it chronic infection versus acute infection, which ones, which geographical regions, and do we have studies to address those issues, the seasonal variability, and issues like that.

I think by doing those studies which people have planned, I think we may get some answers to that, and I think we will be having a continuous dialogue about these issues. We are planning to have another mini-workshop on the implementation issues sometime late in December, at BPAC in December, so we will keep on having the dialogue until we get to the point that we will have kind of a test available for the next season.

DR. FREIBERG: Hi, everyone. I am Glen Freiberg. I am representing AdvaMed membership today although I work for GenProbe, and I, like Dr. Goodman, made a little list of things that I thought would be useful to point out and try to move ahead together.

I have a quick question first. Is anybody from CDRH here? No. That is kind of a shame, and the reason I am pointing it out is that many of the things we have been talking about over the last two days, in the end, are not going to be regulated by CBER.

Currently, FDA has two centers that regulate IVDs, and I am sure most of you are aware of that, but some of the IgM products we talked about for measuring prevalence or measuring long-term response to the disease

will likely be regulated as diagnostics through CDRH rather than as blood screening products.

So, we are going to have to figure out how to do that to be able to get the answers we were looking for by the most rapid path, and we would have to negotiate with CDRH to find out whether or not, for this summer, an ASR platform could come out quickly, whether or not it could be a broad IUO test, whether or not the prolonging the investigation rules could be sort of voided under CDRH with the same flexibility as we have under CBER.

So, possibly since they weren't here today, when you have your implementation meeting at BPAC, it might be a good idea to invite some CDRH people and let's start that cross-center discussion, because we really have different types and different kinds of clinical trials that go on in order to get the products cleared or approved through CDRH.

A second point I have, and I only have a few, so I will try to be brief, was a follow-up to hearing that CBER is following some of the CDRH guidances.

CBER has always been very flexible in being able to pick and choose and take from FDA wherever something good was, whether it is the IND rules for an IVD rather

than IDE, or in the case of some of our many meetings, we have been following the guidance from PDUFA whereas, even though our IVDs weren't covered by PDUFA, we followed the type ABC type meetings.

My point on that subject is that there is also a meeting guidance under CDRH, and it is a little different than the one that CBER has been using in the past, and it would be great to get a commitment from CBER to use the CDRH guidance, the main difference being is that there are a couple kinds of meetings. One is a determination meeting, and one is an agreement meeting, in which case you come out of the meeting with a binding agreement with FDA.

That would be a little new and unusual for CBER, but it could be helpful, so I seek that commitment.

Other things on the process that we could discuss changing are some of the traditional things that aren't really West Nile specific, but in general, the CBER process.

Most of us saw, during the HIV-1, HCV clinical trials that in order to respond to what was necessary in the field, we had a really long investigation with a long IND and many, many lots of products, but when it came to

the pivotal clinical trial in the end of the process, we still had the traditional conformance lots. We were only doing three lots for an IND, probably should do conformance lots.

But I think CBER needs to think about some more flexibility especially maybe in the West Nile case where if we have lots of products that come out, product lots, independent product lots that aren't yet conformance lots meaning the final guard bands, the final specifications, but if the product is really working great, then, maybe it is time to say we don't necessarily need three conformance lots to get the license, maybe we can get the other two conformance lots for a post-license submission.

In that way, industry could get the product on the market, licensed much sooner and likely much cheaper for us if we could start talking about some flexibility.

Historically, I think we all understand that FDA or CBER has regulated the process as the product, and I think it is time to maybe reexamine some tradition, some of the emotional things we have always said, and look at the science again of NAT and the robustness of the products or IgM products, and give some consideration to changing that

and regulating the product as the product and also covering the process under regular GMPs.

My last request is a simple one, and that is, for complete review, the first time we send things around, to have it be a complete review. That means it would include the package insert, the labeling.

We may have some more back and forths after that, but the first time around, we can figure out what is the intended use going to say, what are the warnings should we have. We should be able to get a labeling review as part of that first complete review. That would help the West Nile process move along, as well.

So, on behalf of industry, those are some of my thoughts.

DR. NAKHASI: Do you want to comment on that?

DR. WAGNER: I just have a couple comments. I wanted to reemphasize some of the comments by Mike Busch about the virus's fairly quick ramp-up.

When you are talking about sensitivity, it is not only the absolute number of viruses, but it is how steep that curve is, because that really defines what the window

period is, which is going to determine what the percent of people that you missed might be.

So, it may not be as bad as we think it might be in terms of pooling or other things, so I suggest that we wait for the available evidence to come in. We are going to be getting this data over the winter, I presume, and make a determination based on the science.

DR. GOODMAN: I think that is a really good suggestion and a good point. We have heard fairly low levels of virus from the few data points that we have, but there is some suggestion that it may reach other levels at other times, and you are sort of looking at part of the tail of the elephant, or something like that here.

The other thing is in response to Glen's comments, there is a number of technical and regulatory issues, but I appreciate your input and we will discuss the points you made and try to give you a response on those.

DR. NAKHASI: Also, I may add I appreciate Glen's comments also, because I think Glen knows that we have started working closely in contact with the CDRH and streamlining many of the issues which he has pointed out, especially the commitment.

The reason why I say that, because we are now going to be having a presubmission plan type thing, which we are telling everybody before you come up with a plan for IND, please come to us, talk to us as a presubmission plan, the way the CDRH's pre-IDE plan, they call it, and discuss the issues and sort of discuss what number of samples you need, the clinical trial design, and things like that, so that there are no problems down the road.

So, I appreciate your comment, but I think we are striving towards that end, and also, as Jesse said, we will discuss some of these issues internally, and the bottom line is to get the tests on the market as safe and effectively as possible.

DR. GOODMAN: One comment I forgot to make is that there is communication ongoing with CDRH and meetings that have occurred and are occurring. We have been trying to share the science area and the public health area of West Nile with CDRH.

DR. KATZ: First, I wanted personally to thank Dr. Goodman for his moderate rhetoric during the past couple of months. For those of us who are actually talking to physicians in hospitals and transfusion recipients, it

has made our job of what reassurance we can provide much easier, and I think I speak for my organization ABC when I personally thank you.

Everybody has already said everything, and I just have a couple, two or three take-homes from the meeting. The technology issues and the virology and the sensitivity that has driven off what we learn about the virology over the coming months has been nicely covered.

I have a feeling, looking at the epidemiology of arboviruses elsewhere, including West Nile in other parts of the world, St. Louis encephalitis in this country, it may well be that we are going to implement a superb test next year in the absence of an epizootic or epidemic.

That is fine because I think we are being precautionary and careful for the patients that we all care about. So, perhaps the best message at the end of all of this is going to be what we learn as this is a model for rapid response in the future.

I am fairly skeptical about how much West Nile there is going to be next year, but at least we need to be paying very close attention as we go through this process

what aspects of what we are doing worked, because the next thing that comes along will be important.

The only other thing that I wanted to emphasize is that we need to be thinking about the impact of this activity on a large number of other safety priorities occurring in blood centers as we speak.

Sue gave a reasonable list of the other organisms, and I think most particularly bacterial contamination where a very substantial segment of our community has made the commitment to attack what we think is the most important infectious complication of blood transfusion that remains in the post-NAT era.

I can anticipate in my own center and many other community blood centers around the country that the rapid implementation of West Nile Virus may slow down our implementation of bacterial detection for platelets, and how at the end we measure the net benefit of what we are doing needs to include that.

At my own center, this is going to require enormous resources one way or another, and, for example, our hemovigilance program is likely to suffer in its implementation, so that as we try and get into our

hospitals and help them identify patients better, and identify adverse outcomes not related to infection from transfusion, that we are taking some resources that I would apply to my hemovigilance program, and we are going to apply them in this sort of effort.

I think we all need to be thinking about that because at the end, the patient outcomes are what we are interested in, whether it is West Nile or bacterial contamination or TRALI or ABO mismatch, or anything else.

Another question nobody really addressed and I only bring it up in case somebody wants to talk to me about it afterwards, is where we are with hyperimmune globulin, which is used in the Middle East, I know, and whether or not our friends from PPTA have begun to plan now that we have somewhere around half a million people that might have antibody, the production and evaluation of a hyperimmune globulin.

DR. GOODMAN: We don't have a test to find them.

DR. NAKHASI: Celso.

DR. BIANCO: We did not discuss in great detail, but it is a bottleneck in terms of clinical trials and all

that, is the issue of IRBs. Each one of the clinical trials need to have an approved IRB locally.

Maybe the public affairs, considering the urgency of the issues that we are dealing with, could find some method by which we could influence the local IRBs to be more consistent and less finicky about certain of the word changes here and there.

They are really an impediment for the use of common protocols, of common notifications or common things, and this has been a serious obstacle in many of the clinical trials that we are going into.

DR. BUSCH: I will just follow up on that. The truth is the IRBs are being placed in an untenable position. We are being asked to implement mandatory screening under IND. This is not human research, and they are being asked to approve a protocol, you know, giving people informed consent when there is no option.

I really think the issue here is a decision from above saying that this is really not a research issue, this is a mandated implementation of a test.

DR. GOODMAN: I appreciate these difficulties and they don't just occur in this kind of study. They occur in

lots of multicenter studies and public health situations, and I think they are getting more attention by the IRB communities.

I think using an unlicensed test where you don't quite know the implications of positive test result in real-time, et cetera, you know, there are some issues that are relevant to IRBs. I mean I just wouldn't want to say this isn't something that an IRB should pass on, but it is not the same as giving somebody an investigational drug for West Nile Virus.

Unfortunately, what you sense is the regulations are generally designed to be in the most protective sense, so I don't have an answer, but there are circumstances, for instance, where central IRBs can be utilized.

This probably wouldn't raise to that because you would have a hard time arguing that in a trial lasting months, you know, you didn't have the time or opportunity or reason to involve your local IRB, but I think there needs to be more uniformity across IRBs and more willingness to accept the judgment of other IRBs.

DR. BURDICK: Jim Burdick from Johns Hopkins.

I have had the pleasure of putting a lot of things or attempting to put in some cases things through the IRBs, and we have had sort of a revamping of it recently, locally.

The western IRB process, I think it is called--there is probably somebody in the room that knows better about this--but our institution has utilized that central processing, and I think it is a good way to get around the IRB problem.

This is not offering an opinion on what the FDA says we have to do needs IRB approval or not. That is another interesting issue. But local IRBs are very territorial, so I think that has to be taken into account.

DR. FREIBERG: Let me add a quick comment on that because we have recently had a sponsor investigation by FDA for one of our clinical trials where we used the western IRB in a multi-site situation like that.

It was a good idea, it worked fine, but what FDA is also looking for is why you didn't use the local IRB and whether or not you are IRB shopping. So, for the purpose of your sponsor's safety, if you choose to go that route, you really should have something on file to explain it was

multi-site, there was only reason to go to one IRB, something in the files would have saved us three or four hours of discussion just as a tip.

DR. STRAMER: A couple of comments regarding IRB. I have taken a number of issues to the Red Cross IRB, obviously being from the Red Cross and we have our own IRB, and for those of you who have worked with the Red Cross IRB, they are notorious as being the most, well, anal-retentive IRB on the planet.

We have had, with West Nile, I have already put two proposals before them regarding the studies heard this morning, and they have been extremely rapid in responding and very positive. Peter Page has also had very positive experience as far as the CDC case investigations in which we had to put our follow-up materials through the IRB.

So, knowing the critical public health issue of investigating West Nile, and learning more about this virus, IRB has been extremely positive. I also wanted to comment that we have had discussions with actually Mike and with Sally Caglioti about doing some unified IRB approaches.

For example, and I only use the Red Cross as one example, a large IRB that has a lot of experience in blood donor screening, that whether we use it in combination with western IRB or we just use unified approaches like this in which national INDs are used, we bring it to one IRB for the entire national experience, so it is one method perhaps that we could consolidate all the IRB issues together.

I wouldn't say that using the IRB from Red Cross would be IRB shopping because perhaps our IRB would be one that you would want to shop away from.

I would also want to comment to Glen's earlier comments about the length of the IND period. Even though the length of the IND period at least for the GenProbe test, and currently I am going for the Roche test, has been very long and I think we used 10 clinical lots during the GenProbe IND, if my counting is correct, I think actually it was a very good process.

We learned a lot about the test. By the time the test was licensed, we really had very few open issues, and considering the number of open questions we have for West Nile, I wouldn't want to hurry and license a test because

we really don't quite understand as of yet what we are doing.

Until we understand more about test kit performance, viremia in donors, transmissibility in recipients, whether we want to even keep the test beyond an IND period, you know, I would just suggest we make sure we have all of our questions answered before we license and require a test for donor screening.

DR. FREIBERG: I am in agreement, Sue, we have to get all those answers. My point was with those 10 lots, the last 3 didn't add anything to what we needed to know, to know that the test was reproducible, is working fine, and could be licensed. We would have just had the license sooner if we didn't have to continue more and more lots into the conformance lot period.

So, if we don't have all the questions answered, we keep going, but I think that we did.

DR. NELSON: Ken Nelson from Hopkins.

I just wanted to add a postscript to what Celso mentioned about the hyperimmune globulin. Jim Rayhall in New York has a trial approved, IRB approved, for the use of

interferon for treatment of severely ill patients with West Nile.

He presented some data, a handful of cases. It was not placebo-controlled, it was a case study, but it looked like interferon had some effect, although not spectacular, but these were really sick patients.

Since he has got the protocol and everything licensed, if people had patients with encephalitis that were seriously ill, it might be worth considering contacting him and putting the patient on a trial if there was nothing else, and there isn't any known treatment.

He also studied ribavirin and that didn't seem to work either in vitro or in vivo.

DR. TABOR: Glen Freiberg's suggestions may be good suggestions for improving the overall efficiency of FDA review, but in the case under discussion, these complaints about the number of release lots for decades, biologics that are being put up for licensure, would have had to submit three release lots for good reason, or IRBs, which are required by Congress under regulations dealing with IRBs.

I used to be chairman of the NCI IRB for a few years, and there is a place of IRBs even for this type of study, there is a reason for them. But all of these things, number of release lots, a couple of hours talking to the IRB, they are facts of life in today's world of clinical research.

Most of the people sitting up there who are already involved in the studies have learned how to deal with them, and I think we just have to deal with them. These are tangents today. We have enormous technical problems to overcome before we can test for West Nile Virus, and we have to focus on those technical problems and I guarantee you if you come in with a good test before next June, FDA is going to bend whatever rules it can to accommodate rapid delivery of the test.

DR. WAGNER: I would like to make a comment in the complaint vein, and I am not sure whether the FDA is the right forum for this, but--

DR. GOODMAN: Of course.

DR. WAGNER: --but I would like to see some consistency for determination of what viruses are considered BL3 versus BL2. I think that having some

viruses that are similar to be BL2 and others to be BL3 just doesn't make sense, and in addition to that, it limits the number of research laboratories that can work on a problem. That is going to slow down any test development or research, because there is only so many BL3 facilities.

I know I am beating a horse that is not even here potentially, and it is not necessarily from a government agency. It may be an organization that is populated by esteemed virologists who recommend BL3, but still the issue I believe needs to be dealt with.

DR. FREIBERG: I need to go back to the three lots one more time.

DR. GOODMAN: All right, but this is the third time, so there is no more after this. He gets the last comment. That is my ruling.

DR. FREIBERG: In the CDRH PMA process, we also do three lots, and I don't really have a problem with three lots for CBER. The real difference is that in CDRH, we will do a development lot, we will do a pilot lot, and we will do a full-scale lot, and we have to be able to prove they are consecutive and they are all made the same way, they all perform the same way, whereas, in CBER, it is full

size conformance lots using the final process under IUO, and what often happens is that, at full size, we have a ton of material that is going to expire before it gets used.

So, I am okay with the three lots, but let's look to CDRH and learn a little bit here. If you can show your process is the same process, and the same product is the same product, development, pilot, and final, that would make life a lot easier for everybody and still meet the need of a reproducible process.

DR. GOODMAN: Thank you. We will discuss that.

DR. NAKHASI: Having heard the complaints, kidding aside, I think we need to focus what is the goal from this workshop. We had set it ourselves to have I think a couple of things.

We need to get some agreements both from industry and the blood banks, and the various agreements are, are we going to be having the validated tests as of July 2003, will we be having IND testing up and running, and I think those are the issues.

Also, the issues, we should not forget, the blood donor as well as the tissue, because there is another part of which the tissue donor testing, which we had spent quite

a bit of time, also are we going to be validating these tests as far as those samples, too.

I would like to hear from all the concerned parties how are we going to be doing these things.

DR. BUSCH: I will address the issue of should we test. Personally, I think it is very unlikely that we are going to have a serious problem next year given the epidemic nature, and I think the science will bear that out. Unfortunately, the science can't be done before the decision has to be made.

The studies that we are able to do, I don't think even if they were completely negative, would turn the tide away from the current momentum towards deciding to test, so in my opinion, we should leave this room with the consensus that testing should be implemented if possible, and I think in the construct of minipool NAT next year.

We should give the companies a very clear message that that is what we all are committed to trying to do, if feasible, next year.

DR. KATZ: I want to second what Mike just said, not because I think testing is medically smart, but because if they don't have that clear message now, we won't be

there, and if Mike and I are wrong about the epidemic, then, we are going to feel pretty bad.

So, I think we should come away from this with the assumption that tests available, we will get going on or about July 1st is not unreasonable.

I just want to reemphasize that given operational realities in blood centers, there are very few places in the country that can do single donation, so that the best must not be the enemy of the good here, that the other message should be that it is in the minipool formats that we are already using and we are going to sort out the virology as this year and next year goes by.

DR. KLEINMAN: I have two comments or suggestions I guess, and they kind of contradict each other as possible approaches.

The first is whether this test should be brought forward and positioned in a way that was similar to HIV p24 antigen, and that is, it is an interim test, it's here because we think we have a problem.

Depending on what we find, we may decide in the future to forego testing. Suppose pathogen inactivation comes along, maybe this would be a test--and the epidemic

goes away--maybe this would be a test where we want to have some flexibility, and not be locked into it as a licensed test enshrined in FDA guidance or regulation.

That is one possibility. I recognize that introduces some problems. Certainly, it isn't what a commercial manufacturer would like to hear in developing a test, that the test isn't going to be here in the marketplace in future years, but I still think that that should at least be considered at the level of the FDA.

The other somewhat contradictory suggestion is that if we implement now with a West Nile test, because I don't think it is practical from what I have heard to have a broad-based flavivirus detection system at the kinds of sensitivity that we want, in the time frame that we want.

So, if we implement a West Nile test, maybe that step along the way to a first-generation assay, and that that assay could then be revised, not that we append flavivirus tests on top of a West Nile test, but we have a second-generation assay that both looks for West Nile and other potential pathogens that might come into the blood supply.

I realize those two are different ways to go, but I guess the purpose here is to look at possibilities and introduce them. So, I think on top of the commitment that says we should be here next July with a screening assay, which I think should be a clear message, we will at least to consider these permutations on the theme.

DR. GOODMAN: I would just like to comment on that for a second. I think actually those are both very valid and thoughtful suggestions that bear all our consideration. I think another little point that occurred to me as you were saying that is that I think as we go out potentially with a test or tests next year hopefully, we also have to be careful about our communication.

It is still going to be an issue. We are going to be largely functioning again, unless we get a lot more information before then than we have at this moment, we may not know are those tests going to eliminate 100 percent of transmission, 50 percent.

So, I think FDA and the companies involved, you know, this will be if we can come up with anything useful at all, this will be a real success story of partnership and of ingenuity on the part of industry and herculean

effort on the part of the blood community, but we need to be careful not to oversell it.

So, I think we just should communicate as we go in a very forthright way about the achievements, but also the limitations of what we achieve.

The second part of your thing about going from West Nile to a flavivirus, that is an area where I think we would all be very interested in hearing any sponsor's plans or proposals, and we are again trying to be flexible about how one could do that, you know, could maybe be a place to get some mileage in this process.

DR. BULT: I am looking forward for clarification. I hear constantly talking in terms of the blood community and the problems related to the whole blood sector. Mike, I am asking for clarification. You said "we," who is we?

DR. BUSCH: Clearly, the plasma industry should not be testing for this virus. They have inactivation on the back end that I think, from what I have seen, has ample capacity to deal with the viremia that may exist especially the rare frequency viremia, the dilution, and then the inactivation. I don't think you guys should have to test.

DR. BULT: I agree with you.

DR. GOODMAN: And I think we need data, but I think we are hearing that, and again we appreciate those comments.

DR. BUSCH: On the issue of, you know, we are asking these manufacturers to invest a lot of money, a lot of resources in a system that I think many of us feel may be a short-term need. I know they are approaching it with the consideration that this is an opportunity to transition to more automated platforms to move forward in more flexible approaches, and I think that is really the benefit of this whole process.

One of the things, though, that does sort of trouble me is this concept of moving from more specific virus to more generic, multiplex type of assays sounds good, but the regulatory--and I don't think you can fix this--the regulatory issue of once you have a licensed test, you know, to then replace that licensed test with a clearly improved, enhanced, you know, diversity assay is a parallel track that may take years, and a mechanism to upfront anticipate these more automated platforms having

built-in expansion capacity and much more streamlined methods to get the enhanced performance on line.

DR. BIANCO: I think idea number two from Steve Kleinman is brilliant. Idea number one is the kiss of death. I would like just to support that idea, and I am sure that you can find a regulatory measure by which you assume with limited tests like that, just documentation of the sensitivity that is equivalent to the prior version, as we have with many new generation tests, version 1, version 2, version 3.

We have a history, but we always looked at the specific. Here, we have a broader question.

MS. ZYLBERBERG: Claudia Zylberberg from NABI Biopharmaceuticals. These questions are for Dr. Farshid.

I just want to clarify that for viral inactivation today, if we need to submit a new application, we need to look, for example, BVDV and West Nile Virus? Just to make sure.

DR. FARSHID: As I indicated this morning, this issue, there will be further discussion within Office of Blood, but based on the principle that we have always operated on, if you are dealing with a relevant pathogen in

a product validation, there needs to be some way to indicate the capacity of the manufacturing process in clearing the virus.

That has been the approach that we always took in evaluating such studies, but based on what we saw this morning on this data, on a number of the slides, we did not see the actual data, so further denigration is needed to come up with the final decision that needs to be made in the office level and probably center level.

DR. KREIL: Thomas Kreil on behalf of PPTA. I would actually like to have a comment on that. As I would hope we have conveyed with our presentation this morning, the industry is certainly prepared to do further work only we would hope that the center will wait until more information is available before issuing further guidance on this issue.

DR. FARSHID: I think we agreed there.

DR. BURDICK: Could I make another comment about the things to test. From transplantation surgery, fresh frozen plasma is the thing that scares me, because, number one, it has a longer half-life, number two, it gets

transported around. It tends to smear out the otherwise seasonal distribution of things compared to red cells.

If we were in the scenario down the road in which, for instance, we were doing seasonal testing, we were doing perhaps regional testing rather than national, if we had a way of being sure for liver transplants and for the special preparative regimens in kidney transplants that we are talking about, that we could have our fresh frozen plasma clean, I think we have a special need in that area.

It is a small area, but it is much different than the more general use of red cells. So, maybe this is obvious to everybody, but I just wanted to sort of emphasize that point.

DR. BUSCH: I think one idea that came up when we were in the midst of this epidemic was whether we might be able to source blood for particular patients from low-risk regions.

Even now, unfortunately, there is a lot of FFP on the shelves from high-risk regions right now that theoretically could be either diverted, trashed, or we could source it, replace it with safer stuff, so I think those issues haven't been really been thought through.

DR. GOODMAN: Just to be sure that we are clear here, though, the people from the source plasma industry who fractionate plasma are arguing for non-testing of that, that if testing is performed of blood donors, that would encompass fresh frozen plasma, in the future obviously. Right now it is a real concern and we share that with you, and we appreciate any input on that one, as well.

No new original ideas. This reminds me of discussions that Lyle Petersen and Mary Chamberland and I and others had in the middle of the night - well, can we think of anything else we could do.

[Laughter.]

DR. GOODMAN: We just can't think of it.

DR. BUSCH: Actually, just to follow up on that, I really also think that Lyle and Mary deserve enormous gratitude from the industry. The work you guys have done along with Jesse and the lab is incredible, and I think the responsiveness of the Public Health Service in this crisis has been just great.

DR. BISWAS: If we are going away with some sort of a consensus about minipool testing, then, please keep in

mind the sensitivity in the individual donation when you are designing these testing algorithms.

DR. GOODMAN: I would just add to that. I found some of the discussion--and I am somebody who used to do PCR work when you did it by hand and stuff like that--I found the discussions of sensitivity somewhat confusing, perhaps because I didn't sleep that well the last couple of nights, but I think we need to look at this issue very carefully as more data becomes available.

There is obviously no reason in the world not to make the most sensitive PCR assay you can make here.

DR. FREIBERG: Stop calling it PCR.

[Laughter.]

DR. GOODMAN: I am sorry, you are right, okay, nucleic acid amplification. Some of them aren't even that, they are probe amplification, but the most sensitive nucleic acid test. Sorry about that.

I think we just need to make it as sensitive as possible and keep an eye on the data that is going to be accumulating in terms of how we are going to implement testing. Obviously, you can't do something that is impossible, but we also don't want to do something that is

hopelessly stupid, so let's not set this in stone at this point.

DR. FREIBERG: In my understanding of Dr. Giachetti and the way she sets up and presents analytical sensitivity, it is always on an individual donor sample, it is not a group sample.

DR. KLEINMAN: I think this has been said in the morning before, but since this is the summing up part, I think it is worth stating again.

One critical need is for these performance panels and standardization of how we are going to express quantity, so that we can actually compare assays.

It is an absolutely critical need, I think everybody recognizes it, whether it is a CBER release panel or some other panels that are put together during the assay development stage, and a reasonably--I don't know if we can standardize the way that we quantify it, but we at least have to have some methods that everybody accepts whether they will be RNA transcripts or cultured virus or, you know, source virus from various animal species or whatever, but I think we need to come to some conclusions about this very early on, because it affects all the way the data is

going to be displayed, and it affects the ability to go forward with any research studies or screening.

DR. FREIBERG: On my theme of finishing the project, I also need to point out that we need more than one of those. We heard BBI, I think present, and they have often worked with FDA.

If BBI creates a great panel, and we all start to use it, we end up with a little problem at the end of the process, is that FDA doesn't have an independent lot release panel, so we need to have at least two sources of those types of things.

DR. RIOS: Maria Rios.

I just would like to follow up what Steve said about having a uniform way of measuring and determining what we are measuring and quantifying. I think it is a great idea, as we already urged, for you to share resources, and I want to remind you of the vitro repository where we can go in the changing formation and come to a common unit of measurement. We come a long way putting together.

Regarding how we are going to do, we, as the community, the cell activation, it needs a lot of studies

because unlike the HCV, from what I gather from Dr. Brinton, the West Nile Virus carry only one copy of single-stranded RNA, which I don't know how this conclusion was reached, CV carry two positive, it's double copy in each virion, and we need to look at that very carefully and really see how we got to the measurements, PFUs, and et cetera, not only comparing how many particles are there, but if they carry the virion, it's full particle or not.

So, that would be very good to work out together and try to come to a common sense.

DR. BUSCH: I do agree this is so important. I recommend that maybe NIH and FDA convene a small, focused meeting. Actually, we have John Saldanha now in North America, who really ran the NIBS standardization program. I have talked to him, and he would be eager to participate.

So, I think we really need, in the next few weeks to convene the right people to move this forward.

DR. GOODMAN: Or they can just stay here tonight.

DR. NAKHASI: I think, answering Glen's question, I think we are, as you heard from Maria, that there will be a lot release panel developed in-house, so I think that we

won't depend on one panel, and obviously, the standardization of panels is very important.

DR. PHELPS: With regard to the transcripts and the use of the transcripts, I would just like to make a comment. The Industrial Liaison Committee, which I am a member of, right now has a test going on, a study going on to evaluate HCV transcripts as a potential to be used for standardization of all diagnostics and blood screening assays.

We hope to have some data ready to review actually. At GenProbe, there is going to be a meeting next week on Tuesday with the first round to pass on this particular evaluation. If that is successful, I think that might be a model system to be used in this case for West Nile Virus.

DR. NAKHASI: I think if we don't have any more questions, I would like to ask Dr. Edward Tabor to summarize the meeting. The whole thing has fallen on his shoulders, so in the next 15 minutes, what did we learn and what we got out of the discussions.

Concluding Summary

DR. TABOR: One of the most striking things about this two-day workshop on West Nile Virus has been the fact that throughout all the talks, the audience has really seemed riveted, no one has got up to leave, and everyone has returned promptly after breaks. It is really something.

The fact is West Nile Virus is an important issue for people working in the blood and plasma systems in this country and elsewhere, and we are all hungry for any information we can find out about it.

The workshop has been about more than just the West Nile Virus epidemic of this year or the one of next year or the one of the year after. When Jesse Goodman opened the meeting yesterday, he said that the West Nile Virus crisis is a test of flexibility and agility for the blood industry and the diagnostics industry and the FDA.

Many people here see this crisis as a model for how we can approach any emerging infectious disease that threatens the blood supply. Dr. Goodman suggested that we should seek to develop robust technology, platforms that can be applied to each new infectious disease that threatens the blood supply.

The speakers and the audience have followed this suggestion or perhaps they were already thinking along the same lines, because we have heard extensive discussion about how we might be able to create assays to detect West Nile Virus, that could also detect cross-reacting St. Louis encephalitis virus and Dengue fever virus.

In addition, we have heard representatives of NGI and GenProbe discuss the use of their licensed NAT platforms for detecting HCV and HIV RNase in the development of candidate assays for detecting West Nile Virus.

What kind of virus is West Nile Virus and what are its characteristics that are of interest to the blood transfusion and plasma fractionation communities? West Nile Virus, we have heard is a 15-nanometer positive sens-RNA virus, one of the Flaviviridae like HCV, yellow fever virus and Dengue fever virus.

It is most commonly transmitted by mosquitoes, but its incubation period and viremic period allow it to be transmitted by blood transfusion. The viremia generally begins one to five days before the onset of symptoms, we have heard, and it lasts an average of six days or perhaps

it may begin earlier and last much longer. A lot of the facts about this virus remain somewhat uncertain.

During the 1999 West Nile Virus epidemic in New York, Dr. Lyle Petersen has estimated there was a risk of 1.8 to 2.7 infections per 10,000 donations according to his mathematical model.

It is likely that the more extensive the epidemic, the more likely that there would be infected donations, particularly during the peak of an epidemic. Dr. Petersen stated that the height of the risk appears to be highly time-limited. Two weeks after the peak of the epidemic, the risk may drop to 50 percent.

In the 2002 epidemic, there were 6 cases that were confirmed to have been acquired by blood transfusion. Other cases that may have been transfusion-transmitted are still under investigation.

We heard descriptions of several research use assays to detect West Nile Virus infections including assays to detect IgM antibodies to the virus and PCR-based or other NAT assays to detect viral RNA. Some of these have been applied to the 2002 epidemic although the data

are incomplete because follow-up samples are still being collected and analyzed.

Dr. Wong called our attention to the fact that the IgM assays are strongly false positive in half of the patients with past syphilis infections, an observation that had not been made previously by many investigators, if any.

Fortunately, syphilis infections are relatively rare in the United States today notwithstanding the increase from 2.1 to 2.2 per 100,000 reported by the CDC in the latest issue of MMWR, but this cause of false positive tests for West Nile Virus will have to be addressed as the tests are developed.

Most of the speakers felt that blood and plasma donations or at least blood donations could be tested under IND by the time of the next mosquito season, roughly June 2003. Descriptions of plans for test development were provided by eight test manufacturers. Most of the eight described plans rather than progress. Only a couple described the specifications of their assays and preliminary clinical test results from the field were only presented by Dr. Andrew Conrad of NGI.

Using the NAT platform from their licensed HCV and HIV NAT, NGI reported detecting West Nile Virus at an average level of 100 copies per ml.

Although they detected an unspecified number of positive minipools in samples from endemic regions, most had not been resolved to the individual donation yet, however, the one positive result that had been resolved to the individual donation was detected in a pool of 64 samples and had such a high copy number, 196,000 per ml, that it could have been detected in a pool of 512.

The detection of such a high copy number was surprising since most investigators expect to have difficulty detecting low viral load samples using the minipool formats for blood.

Further studies will be needed to evaluate just how prevalent infections at this higher level occur.

Several problems with the NAT assays including TaqMan assays were highlighted by Dr. Lanciotti. The levels of viremia in humans infected with West Nile Virus are expected to be low, particularly early in the infection, with an average level of 18 plaque-forming units per ml.

NAT testing of pools of 16 donations may not be adequate. In fact, it was suggested that only 50 percent of positive samples would be detected by testing minipools of 16 for West Nile Virus RNA.

Another problem is that West Nile Virus RNA and West Nile Virus IgM antibodies are rarely found in the same sample. TaqMan appears to be no longer positive by the first day after presentation in 95 percent of patients according to Dr. Lanciotti, at least using tests with current levels of sensitivity.

IgM also has the drawback that it remains positive for greater than one year, perhaps much longer, long after infectivity has ended.

The differences between these assays contributed to a discussion between blood safety experts and transplant surgeons in which it seemed clear that there may be a separate agenda for testing tissue and organ donations for West Nile Virus compared to blood donations.

Blood safety concerns make it necessary to eliminate donations during the early phases of infection when viremia is present. Tissue and organ donations, however, require screening tests that can be used on

cadaveric serum or perhaps tissue, and tissue and organ donations probably require a longer period of donor deferral since the surgeons are concerned that organs may harbor the virus longer, and the surgeons say that they often function under the assumption that "ever infected" means that a donor should be excluded at least for some infectious agents.

Furthermore, there is evidence from animal studies described by Dr. Kramer that the virus is present longer in kidneys than in the serum of experimental and affected animals.

The point was then made that eliminating West Nile Virus from blood donations may eliminate the vast majority of tissue and organ donation transmitted cases. Nevertheless, there seems to be ample reason for the research community to focus on developing assays for both settings at present.

We still do not know much about this virus and the concerns about its prolonged presence in tissues and organs appear reasonable in the context of how little they know.

Today, we heard interesting presentations about the potential impact of pathogen inactivation methods on the infectivity of West Nile Virus infected donations. Several members of the audience pointed out that we would still want to screen to exclude West Nile Virus infected donations even if blood components could be subject to pathogen inactivation.

This is analogous to the situation of continued serologic and NAT screening of source plasma for HCV and HIV despite the introduction of virus inactivation procedures since screening and deferral add an extra layer of safety.

There are, however, safety considerations related to pathogen inactivation of blood components that have not yet been fully addressed and hopefully, these will be addressed as clinical studies progress.

We are all anxiously awaiting the development of a safe and effective inactivation process for whole blood and its components.

Drs. Busch, Stramer, and Kleinman described an ambitious and extremely important project to generate

answers to many of the issues related to excluding donors infected with West Nile Virus.

Using several large repositories of donor-recipient paired samples, they plan to evaluate several NAT methods to detect West Nile Virus and to establish the specifics about its viremic period viral load, transmission rates in the community and in the blood setting. Studies of this type are essential.

In summary, vast resources are being focused on the problem of blood transmission of West Nile Virus. Nevertheless, it is important to recognize how little we know today. We really don't understand the natural history of the infection in humans at least from the point of view of blood donation.

We need to determine the characteristics and duration of the early symptoms and whether suitable questions about symptoms can be used to exclude infectious donors.

We need to know more about the viremic period and viral load. We need to know if antigen antibody complexes are present in the acute phase sera that are negative by one assay or another. We need to know which blood

components can transmit West Nile Virus and how long the virus can survive in blood components under blood bank storage conditions.

We need to develop reference reagents, test panels, and seroconversion panels to evaluate the tests as they are developed, and efforts are being made to develop these now.

We need to learn more about cross-reactivity with other flaviviruses. We need to develop supplemental assays or supplemental test algorithms using other approved assays, and for the long range safety of blood, we need to continue to develop test platforms that can be easily modified to meet each new infectious challenge to the safety of blood and plasma.

Thank you.

[Applause.]

DR. NAKHASI: Thank you, Ed. Very good summary. I think time is up now. I promised you guys 4:30, it is 4:23, so I would like to thank you all especially CDC, NIH, and all the PHS agencies, the AdvaMed, other organizations, blood organizations, and also obviously HRSA, and all the organizations who helped us to make this possible.

Thanks again. See you soon with a test.

DR. GOODMAN: Thanks to Hira, too.

[Applause.]

[Wherupon, at 4:23 p.m., the workshop adjourned.]

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