Food and Drug Administration

Center for Biologics Evaluation and Research

114th Meeting of the

Blood Products Advisory Committee

November 18, 2016

Great Room, Building 31
FDA White Oak Campus
10903 New Hampshire Avenue
Silver Spring, Maryland

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PROCEEDINGS (8:30 a.m.)

Agenda Item: Call to Order and Opening Remarks, Introduction of Committee, Susan Leitman, Acting Chair, BPAC

DR. LEITMAN: There are no new members of the committee who were not here yesterday but we would like to go around and introduce those who are here again today.

I am Susan Leitman of the NIH Clinical Center. On my right --

DR. ORTEL: Tom Ortel, Chief of Hematology at Duke.

DR. LERNER: Norma Lerner, pediatric hematologist, NHLBI.

DR. DEVAN: Michael DeVan from Walter Reed National Military Medical Center.

DR. SIMON: Toby Simon, Senior Medical Director, CSL Behring, and acting industry representative.

DR. REES: Robert Reese. I am the Manager of the Regulatory Compliance Program for the State of New Jersey.

DR. SANDBERG: Sonja Sandberg. I am a professor of mathematics at Framingham State University.

DR. DEMARIA: Al Demaria from the Massachusetts Department of Public Health.

DR. TEMPLIN: Christopher Templin, consumer representative, user of blood-blood products of blood derivatives.

DR. STAPLETON: Jack Stapleton, University of Iowa, Departments of Internal Medicine and Microbiology.

DR. ESCOBAR: Miguel Escobar, hematologist from University of Texas in Houston.

DR. LEITMAN: Thank you very much. Lieutenant Commander Emery will now read the conflict of interest statement.

Agenda Item: Conflict of Interest Statement, Bryan Emery, LCDR, Designated Federal Officer BPAC

LCDR EMERY: Good morning. My name is Bryan Emery and I am the designated federal official for today's meeting of the Blood Products Advisory Committee. Mrs. Joanne Lipkind, Mrs. Denise Royster and Mrs. Rosanna Harvey are the committee management specialists and they can assist you with any needs at the tables located out in the hall.

I would like to welcome all of you to the 114th meeting of the Advisory Committee. Dr. Susan Leitman is our acting Chair. The CBER press media contact is Mrs. Tara Goodin who is in attendance. Mr. John Bowers is our transcriber.

I would like to request that everyone please check your cell phones and pagers to make sure they are turned off or in silent mode. Please also remember to speak directly into the microphone at all times, and please identify yourself. It is helpful to the public, people attending by Webcast and to the transcriber.

For the members around the table and the audience, coffee, drinks and snacks are down the hall in the kiosk where you entered the building. There are also rest rooms out the doors to the right as you go past the kiosk.

All committee topic and update discussion needs to be done in the public forum and not during the breaks. The FDA and public need your advice and expertise. The public and industry must stay behind the stanchions and in the audience area. Please do not enter into the FDA or BPAC Committee table

area. Please wait until the open public hearing designated time to make any remarks using the center aisle microphone.

Now I would like to read into the public record the conflict of interest statement for this meeting.

Welcome to the second day of the 114th BPAC meeting. Dr. Susan Leitman will continue to serve as our acting Chair for today's BPAC meeting. Mr. Christopher Templin will serve as a temporary voting consumer representative, representing all consumer interests. Dr. Toby Simon will serve as the acting industry representative. Dr. Simon is employed by CSL Behring of King of Prussia, Pennsylvania. Industry representatives act on behalf of all related industry. Industry representatives are not special government employees and do not vote.

Today's agenda will include the following. For Topic III presentations and discussions the committee will hear an informational session on Zika virus and blood safety in the United States. This is deemed to be a non-particular matter. In addition, the committee will hear updates on a transfusion-transmissible infections monitoring system and an FDA workshop on preclinical evaluation of red blood cells for transfusion. These updates are also determined to be non-particular matters.

Based on agenda topics and the analysis of the financial interests reported, FDA has determined that all members and consultants of this advisory committee are in compliance with federal ethics and conflict-of-interest laws.

Under 18 U.S. Code 208, Congress has authorized FDA to grant waivers to special government employees and regular government employees who have financial conflicts of interest when it is determined that the agency's

need for a particular individual's service outweighs his or her potential financial conflict of interest. Based on the agenda topics and all the financial interests reported by members and consultants, no conflict-of-interest waivers were issued to any voting or non-voting member of this committee under 18 U.S. Code 208.

There may be regulated industry speakers and other outside organizational speakers making presentations. These speakers may have financial interests associated with their employers and with other regulated firms. These individuals were not screened by the FDA for conflicts of interest; however, the FDA asks, in the interest of fairness, that they address any current or previous financial involvement with any firm whose product they may wish to comment upon.

We would like to remind the members, consultants and participants that if the discussions involve any other products or firms not already on the agenda in which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement and their exclusion will be noted for the record. FDA encourages all other participants to advise the committee of any financial relationships that you may have with any firms, products if known, or its direct competitors.

This announcement is in addition to the conflict-of-interest statement read at the beginning of yesterday's meeting, November 17, 2016, and will be part of the public record for the Blood Products Advisory Committee Meeting on November 18, today. This conflict-of-interest statement will be available for review at the registration table.

This concludes the reading of the COI statement for the record. Let me hand the meeting back to Dr. Leitman.

DR. LEITMAN: Thank you, Commander Emery. I would like to inform the BPAC members that FDA is not seeking advice or recommendations from the committee on this topic. The committee may ask questions of the FDA and speakers, but if the discussion appears to be veering towards advice or recommendations I will need to halt the discussion and remind the committee that FDA is not seeking advice on this topic.

Again, this is an update only. The FDA is not seeking advice or recommendations at this time.

Topic III: Information Session: Zika Virus and Blood Safety in the United States

With that, let's proceed to Topic III as stated by Commander Emery, Information Session on Zika Virus and Blood Safety in the United States. We will start with the first speaker, Dr. Ingrid Rabe of the CDC, who will tell us about the current status of the Zika epidemic.

Agenda Item: Current Status of the Epidemic, Ingrid Rabe, CDC

MS. RABE: Thank you for the opportunity. In 2007, I started working with the Division of Vector-Borne Diseases in Colorado and I distinctly remember being picked up at the airport by a colleague who informed me that he was working on an outbreak investigation on Zika virus and he asked me, have you ever heard of this virus. And I said no. Little did we know what we would be facing a decade later.

Zika virus is a single-stranded RNA virus. It's in the genus Flavivirus, family Flaviviridae, and it's very closely related to dengue, yellow fever, Japanese encephalitis and West Nile viruses, and it's transmitted to

humans primarily by Aedes stegomyia species mosquitoes.

These pictures show the vectors involved. Aedes aegypti is the more efficient vector for humans, although Aedes albopictus is also a competent vector. These mosquitoes also transmit dengue and chikungunya viruses. They oviposit in domestic water-holding containers and they live in and around households. They are typically aggressive daytime biters, but they may also bite at night time.

This map shows the approximate geographic range of these mosquitoes in terms of where, based on projections around where mosquitoes have been identified, and those obviously are the ones of interest and capable of spreading disease. And this slide shows the typical vector-borne transmission cycles of Zika virus. Similar to yellow fever virus, there is a sylvatic or jungle cycle where mosquitoes transmit the virus between non-human primates but they may spill over to epidemic urban cycles in which mosquitoes feed on infected humans and then become infected and transmit to subsequent humans that are bitten.

In terms of non-mosquito borne modes of transmission, this has really been a very novel and somewhat unprecedented situation in terms of transmission of arboviruses than what we are usually used to working with. We know that there has been much attention around intrauterine transmission resulting in congenital infections. There may also be transmission of Zika virus during childbirth from a viremic mother to a newborn. We have well recognized sexual transmissions now, as well as even previous to this outbreak there were documented laboratory exposure transmissions, and also through blood transfusion on which some probable case reports have been published. There is also the possibility of transmission through other routes such as organo-tissue transplantation or through breast milk.

The Zika virus was originally isolated from a sentinel rhesus macaque monkey in Uganda in 1947, but before 2007 there were really only sporadic human cases of disease of febrile illness. In 2007, there was the first large-scale outbreak that was reported on Yap Island in the Federated States of Micronesia. In 2013 to 2015, there were more than 30,000 suspected cases reported from French Polynesia and other Pacific islands.

In May 2015, the first locally acquired cases in the Americas were reported in Brazil, and currently, outbreaks are occurring in most countries or territories in the Americas including U.S. states and territories.

This map shows the updated Zika virus disease cases reported to PAHO, as of yesterday. The countries are listed on the right with the map on the left. Brazil, Columbia and Venezuela account for around 70 percent of the cases, and then with kind of the next highest numbers listed in the countries and territories listed below including Puerto Rico with 5 percent of those cases. Just one other point on that is that 25 percent of the cases reported to PAHO are laboratory-confirmed.

This slide shows a pie chart that shows what the relative contribution to the total numbers of those cases is from different regions, South America obviously contributing the bulk of those reports followed by the Caribbean and Central America.

This next series of charts is going to show the epidemiologic curves for different countries. This is showing Brazil, and that was going from January to October 3rd, 2016. The next is from Columbia, obviously later in terms of the epidemic progression, and then in Mexico. These data are all derived from the PAHO website.

This is showing the affected municipalities in Puerto Rico, and those are cumulative cases from 2015 to 2016. As you can see, there is widespread affectation across the various municipalities with differing levels of numbers of cases reported.

This is showing the line chart of the cases reported from Puerto Rico and it lists Zika, dengue and chikungunya virus disease cases, although you can barely see the lower line at the bottom with the other two viruses. But that is basically showing an indication of the trends that we've seen as far as the reporting goes.

This is data from our ArboNET surveillance system through which states and territories report cases to CDC. Those travel-associated cases are listed at 4,232 now, and that includes cases that are, for example, sexual transmission cases associated with travelers, and the sexual transmission cases are now at 235 reported to ArboNET and, also, congenital infections included as well.

As far as the locally acquired cases are concerned, those are largely in terms of that 32,000 number that is predominantly cases from Puerto Rico at around 31,000, comprising 98 percent of those cases. And then, within the continental U.S., the 139 cases reported by Florida to ArboNET, and those were data presented on the CDC website as of November 16th.

This shows the state of residence of returning travelers in whom cases have been reported. Actually, it's returning travelers and the local cases in Florida as well. The three states comprising half of those cases are New York, Florida and California.

As far as the mosquito-borne transmission in Florida is concerned, this has obviously generated a lot of media attention and public health concern.

They have had sporadic presumably locally-acquired mosquito-borne cases identified in multiple counties in south Florida, but multi-person transmission was identified in three areas of Miami Dade County, and this was published in MMWR by Dr. Likos and colleagues recently. Because of those multi-person transmission areas identified, this extended recommendations for pregnant women to avoid travel to those areas.

In one of those areas there was no evidence of ongoing active local transmission after they applied aerial spraying and other mosquito control efforts, which was promising

As of November 17th, there were 139 locally acquired cases reported by Florida to ArboNET. The Florida Department of Health does continue to report active investigations in several counties, and the travel advisories for pregnant women and their sex partners who are concerned about potential exposure -- they were advised to consider postponing non-essential travel to all parts of Miami Dade County.

This slide shows the month of onset for reported Zika virus disease cases, and that is as of October 2016. You can see the peak in the month of illness onset in the summer months, so June, July, and August. Obviously, much of that is not just related to the pattern of the outbreak but also to the pattern of travel in terms of when people are traveling to areas that are affected.

This shows, among the returning U.S. travel-associated cases, where they actually acquired the infection. The majority of those were actually from the Caribbean followed by Central America and then South America.

I'm going to describe a little about the clinical features as well as laboratory considerations just so that people get a sense of how cases might be

detected by their physicians, which, obviously for symptomatic cases, is going to be the first step for getting to a diagnosis that is reportable.

Most infections are in fact asymptomatic, and clinical illness is usually mild although the classic constellation of symptoms that has been seen has included rash, fever, arthralgia and/or conjunctivitis. The symptoms typically last several days up to a week, and severe disease requiring hospitalization has been uncommon. Fatalities are certainly rare.

In terms of the analysis of data of U.S. travel-associated Zika virus cases in 2015 and 2016 -- and this is data that was analyzed up to July 13th -- the most common presenting symptom was rash occurring in three-quarters of cases followed by fever and arthralgia and then, in about one-third of cases, conjunctivitis.

The differential diagnosis of Zika virus includes multiple infectious diseases. However, among those, dengue and chikungunya are particularly similar in terms of presentation but also have some other additional factors that make those of particular importance, so distinguishing Zika from dengue and chikungunya is important. They are all transmitted by the same mosquitoes with similar ecology, so if someone has traveled to an area with transmission or lives in an area with transmission there's a good chance they may have been exposed to any of these viruses that were circulating there at the time. And the diseases have similar clinical features. It is particularly important to rule out dengue because proper management of dengue clinically can actually improve outcome.

In terms of this outbreak, newly identified clinical manifestations of Zika include adverse outcomes of pregnancy, that everyone is well aware of now. There were reports of fetal losses and certainly of microcephaly and other

congenital anomalies, but there were also increasing reports of Guillain-Barre syndrome in French Polynesia and now in the Americas as well. Also, some other neurologic syndromes are coming to the fore now as well. Also, there are reports of severe thrombocytopenia.

In terms of pregnancy specifically, existing data show that there is no evidence of increased susceptibility to infection among pregnant women. Infection can certainly occur in any trimester. The incidence of Zika virus in this population is not known, and there is no evidence of women who are pregnant and become infected having more severe disease.

In terms of the microcephaly in Brazil, there were, as everyone may recall, reports of a substantial increase in the number of babies born with microcephaly in Brazil in 2015, although there were not good baseline data to know what the rate was prior to that. However, Zika virus infection has been identified in several infants born with microcephaly, including deaths, as well as in early fetal losses. The incidence of microcephaly among fetuses with congenital Zika virus infection is not known at this time.

However, there have been attempts to quantify the estimated risk, and those papers describing those estimates are listed on this slide. In one of the papers there was a 1 percent to 13 percent estimated risk of microcephaly if infected in the first trimester, and that was based on modeling of the outbreak in Bahir, Brazil, and noted negligible risk in the second and third trimesters. In another paper, there was a 1 percent estimated risk in the French Polynesia data, and 29 percent abnormalities were detected, including two intrauterine deaths, in lab-confirmed Zika virus infections in women with prenatal ultrasounds in Brazil.

So, really, a lot remains to be clarified in terms of this. A lot is going

to depend on the methods of identifying and modeling these.

The main questions that remain for us at this point in time are, what is the level of risk of Zika virus infection during pregnancy, when in pregnancy the infection poses the highest risk to the fetus, what is the full range of potential health problems that Zika virus infection may cause, and what is the risk for later health problems in an infant who does not have overt abnormalities at birth. And, also, whether there are other factors that might affect the risk of birth defects such as co-infections with other pathogens.

For the diagnostic testing we have various methods available. We have PCR molecular methods to detect viral RNA and serum in urine, and there has been work in other samples as well. We are evaluating RT-PCR in amniotic fluid and semen. There is also serology using anti-Zika virus IGM assays and utilizing antibody testing in serum and cerebrospinal fluid. And then for tissue sampling and testing, they can do immunohistochemical staining and RT-PCR. Recommendations vary on different vectors including time after onset or exposure and what appropriate testing should be done.

In terms of who should be tested, it would be patients with a compatible clinical presentation with an onset within two weeks of travel or during the time of travel to an area with ongoing transmission, or if there's an epidemiologic link to a laboratory-confirmed case through vertical transmission or sexual contact. And pregnant women should be offered testing if they have had travel to a residence in an area with ongoing transmission during the pregnancy or sexual contact without protection with a partner who traveled to an area or had exposure.

The biggest difficulty in terms of the evaluation diagnostically is

that there is a lot of serologic cross-reactivity between other relative flaviviruses and Zika. The Zika virus IGM can be positive because somebody has antibodies to dengue, for example. There may even be non-specific reactivity in an assay that causes a false positive.

Neutralizing antibody testing is useful to discriminate between viruses but really only in the setting of primary flavivirus infections. It seems to be less reliable in secondary flavivirus infections because there may be higher rises in neutralizing antibodies to previously infecting viruses. It is also difficult to distinguish the infecting virus if somebody has been vaccinated against another flavivirus.

There are, it feels like, exponentially more places to have Zika testing done now than at the beginning of the year. There are a number of commercially available molecular and serologic tests under emergency use authorization. Testing is also performed at CDC and most state health departments, although neutralizing antibody testing has a fairly limited scope of laboratories able to perform that testing, including CDC and some state health departments. We do encourage healthcare providers to contact their state or local health departments to facilitate diagnostic testing and interpretation of results.

There is no specific treatment for Zika virus infection; it is really supportive. But, as I mentioned before, it is encouraged that clinicians manage as if for dengue because the clinical management is critical to good outcome. We also advise, to that end, not to use aspirin or nonsteroidals until dengue can be ruled out in order to reduce the risk of hemorrhagic phenomena.

In terms of Zika virus surveillance, I mentioned the clinical criteria that we would be looking for and encouraging people to suspect Zika. And there

is also evaluation of women, particularly pregnant women, who have traveled to areas with Zika virus transmission, but certainly women even planning to become pregnant should be aware of where areas of potential acquisition might be a risk. Also, evaluation during pregnancy if there are risk factors identified or infection identified. Also, there is, obviously, ongoing awareness of possible local transmission in areas where the vectors are present.

Reporting of Zika virus disease cases occurs through physicians reporting to health departments and health departments in turn reporting to CDC. That is done through the ArboNET reporting mechanism. CDC has approved a recently revised case definition in June 2016, and healthcare providers, as I mentioned, are encouraged to report the cases to their state or local health department because such timely reporting should enable health departments to react then and especially if there's a potential for local spread to try to mitigate that.

There is unfortunately no vaccine or medication to prevent infection or disease, so the primary prevention remains avoidance of mosquito bites and, if people are in areas where they might be exposed, to use EPA-registered repellants as well as wearing long-sleeved shirts and long pants over exposed skin, using permethrin to treat clothing, and to make use of screens and air conditioning systems to prevent mosquito bites.

Pregnant women should consider postponing travel to areas with ongoing Zika virus outbreaks because of the risk of infections and potential adverse outcomes. If people are infected with Zika virus, we advise avoiding mosquito bites during the first week of illness to prevent further subsequent transmission from an infected mosquito to the surrounding area.

This slide shows some selected resources, although, as I'm sure many of you have seen, there are very many helpful links on various sites with additional information about Zika. On CDC's website we have a number of different sections on statistics and general information both for healthcare providers and health departments and laboratories and so forth.

That concludes the talk.

DR. LEITMAN: Thank you very much, Dr. Rabe.

Our next speaker is from the Office of Blood at FDA, Dr. Hira Nakhasi, who will guide us through FDA guidance and efforts with respect to blood safety and the Zika virus.

Agenda Item: FDA Guidance and Efforts with Respect to Blood Safety, Hira Nakhasi, PhD, OBRR, FDA

DR. NAKHASI: Thank you. I will discuss the FDA's efforts to ensure blood safety from Zika virus. The outline of my talk is basically focused on the basis for concern for the U.S. blood supply.

Based on that, we issued early guidance in February 2016. Then the evolution, and we monitored the outbreak while the Zika epidemic was taking place. Based on that, we revised the guidance in August 2016. And I will also talk about the FDA's efforts to assist device manufacturers to get the testing under IND, and what are the public health benefits of testing. At the end, I will discuss some unresolved issues.

Just to give you a chronology of FDA's response to the Zika virus outbreak, as you heard from Ingrid, the first locally transmitted case was reported in Puerto Rico in 2015, and then in January 2016, the first case in the U.S. Virgin Islands, and then we saw the first documented sexual transmission

case of Zika in the U.S. in Texas. FDA then issued the first guidance for industry on how to tackle this epidemic in February 2016. In March, FDA approved the first IND for the use of Zika NAT assay for blood screening. In April, Zika NAT was implemented in Puerto Rico because Puerto Rico already had a lot of cases at that point.

Continuing with the chronology, in May 2016, FDA approved a second IND, and Zika NAT testing was implemented in the United States where the locally transmitted cases were occurring.

In July, the first reported local transmission occurred in Florida. In August, as I mentioned earlier, we issued a revised guidance recommending universal donation testing, but this was in a phased approach. The three phases were immediate implementation of the ID-NAT in Florida and Puerto Rico where locally-transmitted cases were already happening. Then, within four weeks of the issuance of the guidance, we recommended that the 11 states, based on their risk-based determination which I will discuss later in more detail what was the basis for those 11 states, and then nationwide within 12 weeks. I think by now, today, most people should be implementing the ID-NAT.

The concern for public safety was based on this rapid expansion of the virus epidemic in the Americas since 2015 and the large number of travelers traveling to these outbreak areas. As you heard from Ingrid, the mosquito population can transmit these in the United States, and there was significant morbidity of mosquito-borne Zika infection including congenital microcephaly and Guillain-Barré Syndrome.

There was also known transfusion transmission cases of other flaviviruses already known, and we had also known that in the Polynesian

outbreak in 2013 and 2014, when they tested blood donors who were asymptomatic for Zika, 2.8 percent were positive for Zika, suggesting that there is an asymptomatic phase for this. And, as you heard from Ingrid, 80 percent of the people are asymptomatic with infection. Reports were published in the media in Brazil about transfusion transmission and, also, the presence in semen, potential exposure to sexual transmission.

I will give a little detail on the potential transmission. Transmission, as we heard, there are probable transfusion-transmitted cases in Brazil; 80 percent of the cases are asymptomatic, and 2.8 percent of asymptomatic blood donors in Polynesia were RNA-positive. Viremia can occur prior to onset of the symptoms and up to 18 days after resolution.

I will describe in a little detail three probable transfusion transmitted cases reported in Brazil. The first donor was a 54-year male pre-symptomatic donor of platelets, and three days after donating the platelets the donor reported febrile illness from post-donation information. When the index sample was tested, it was Zika-positive by RT-PCR.

These platelets had gone into a recipient, a 55-year old male who had a liver transplantation, and the serum collected four days post-transfusion was positive for Zika RNA by RT-PCR and cell culture, suggesting there is probable transmission based on temporal coincidence of infection. It was also a sequence virus from the recipient, as well as the donor had a similar sequence.

The second report was the donor, again a pre-symptomatic donor, who had donated leukoreduced apheresis platelets and, five days after donating, reported symptoms, and index plasma and urine samples were positive 14 days post-donation by RT-PCR and subsequently confirmed by IFA and PRNT.

There were two recipients in this case. One was a 54-year old female and the second was a 14-year old female. Both these recipients were negative by PCR for chikunganya, dengue and Zika pre-transfusion; however, following transmission, the first recipient was positive by PCR on day six, and the second one was PCR positive from day 23 and continued to be PCR positive for up to 51 days. Both were seroconverted later, suggesting again that these are probable transmission cases.

Based on that, the FDA, as I mentioned earlier, issued a guidance in 2016 and now you know by heart all that guidance so I don't have to go into detail. However, just to remind you that what was recommended at that time was that in areas affected by local mosquito transmission, we recommended we should stop collecting blood except testing done by NAT or pathogen reduction of components by an FDA-approved test.

In areas which were not affected by local mosquito transmission, FDA recommended a four-week donor deferral for persons with known or suspected Zika infection traveling to endemic areas or sexual contact by a person who had suspected Zika infection. In both affected and unaffected areas, FDA also recommended donor education material, donor history questionnaire, risk-based deferral and retrieval of potentially contaminated collections post-donation.

At that point, because the test was still not approved under IND and in Puerto Rico quite a bit of Zika cases were reported, through federal government support, blood for Puerto Rico was outsourced from the continental United States for one month beginning March 17 until the first investigational testing started on April 2nd.

This slide shows you the weekly Zika detection rate in Puerto Rico donors using one of the tests under IND, and you can see the number of Zika-positive donors kept increasing up to almost 1.8 percent approximately by June-July and hovered around that rate up to August, and in the later part of August and September the rate started going down. As of yesterday, the rate is up .17 percent. That is in Puerto Rico.

The Zika cases in the United States, as you heard from Ingrid, are both locally acquired and travel-associated cases in both the United States and U.S. territories. As you saw, the territories have a large number of cases and very little travel-associated which makes sense; whereas, in the case of the U.S., most of them are travel-associated, and locally acquired cases are mostly in Florida.

In the last couple of days I was at the ASDHM meeting in Atlanta. Obviously, there was Zika discussion going on. I attended one of the sessions and a Florida Department of Health person stood up and said -- because somebody also presented the same 139 number, and the reason I'm saying that is because the person from Florida Department of Health corrected that person saying they have now around 250 but didn't provide any evidence of that.

In addition to the number of cases having locally-acquired, the interesting part of Zika is that it can be detected in several body fluids and blood. In serum, RNA can be detected. Whether it's an infectious virus we do not know, but the RNA can be detected up to one to two weeks in serum and, in pregnancy, up to 46 days.

In whole blood, it can extend up to 81 days, and in the whole blood, it mostly is associated -- I will show you a slide in a minute courtesy of Mike Busch's REDS-III studies -- it can extend for up to 81 days. And most research is

related to the red blood cells. It can be detected in the urine up to 91 days, in semen up to 62 days and, in some cases, up to 188 days, and in saliva up to 91 days.

This is the slide courtesy of Mike Busch's REDS-III studies where he is using his follow-up of these donors, and basically, it is that Zika RNA persists in whole blood longer than in plasma and is primarily associated with red blood cells. Initially the peak was at one week, then three weeks, six weeks and three months. I think at six weeks you can see it sometimes in the plasma, but very little actually by three weeks and six weeks, and by three months it is completely gone in plasma but you still see it in the red blood cells.

Whether that is clinically relevant; *i.e.*, is it infectious, this RNA associated with the RBCs, remains to be seen and the studies are undergoing. Also, can it be infectious in the presence of antibodies.

Besides the persistence of this virus in other body fluids, the sexual transmission is another issue with Zika virus. As you heard, sexual transmission has been reported predominantly from infected males to their partners, male to male, male to female, and female to male. It has not yet been established in female to female. In addition, the number of sexually transmitted cases of Zika has been increasing in the United States and the latest count was around 34 cases as of the beginning of November.

So, sexual transmission of Zika raises a potential concern about epidemic spread of Zika outside the recognized areas of mosquito-borne transmission. Even though the mosquitoes may be gone, people may be infected and asymptomatic and it can be transmitted through sexual contact.

Based on this evidence, FDA revised the guidance in August

because of these evolving experiences and basically mandated universal donation testing for ID-NAT in a phased approach immediately in all U.S. states and territories which are affected by local mosquito-borne transmission, which is mostly Florida and Puerto Rico, and then phased in within four weeks in the highest-risk states, which are 11 states, and nationally within 12 weeks unless the blood components -- basically plasma and apheresis platelets -- are pathogen reduced because there is no approved pathogen reduction test yet for red blood cells.

In addition, there are differences from the February 2016 guidance. We extended the donor deferral period to 120 days and the look-back product retrieval to 120 days after the positive NAT in the donor.

In addition, blood establishments may discontinue screening for Zika risk factors, which in the February guidance there was a donor history questionnaire and they were asking questions about sexual contact and other things. Here, if it is universal screening, there is no need to ask that question.

The rationale for the policy change -- By now you must have gotten my point; however, I want to repeat this point. The evidence of expanded Zika epidemic in the continental United States, that was the reason why we revised the guidance. Also, the delay between occurrence, recognition and confirmation of local mosquito-borne transmission in an area. Logistics complexity and limited effectiveness of donor screening for risk factors in the face of evolving areas of local transmission. Increased concern about sexual transmission as a mode of spread of the epidemic independent of mosquitos, and potential impact of travel-based deferrals, because previously if you deferred based on the trial without testing it would have an impact on the blood supply.

In the phased approach, the reason for four-week implementation in those blue states is basically the proximity to these areas, the local transmission states like Florida and the Texas area and New Mexico, Arizona, and because locally-acquired cases have been found in the northern part of Mexico.

Presence of mosquito species capable of transmitting -- You saw Ingrid's slide that showed the distribution of the mosquito aegypti as well as the albopictus, mostly aegypti, and other epidemiological linkage (travel associated density). That is basically in New York. There are a number of trial cases associated in New York, the highest number around 900 cases.

That was the guidance. We were not just focusing on only the guidance document; we were also busy in getting the implementation of the NAT testing under IND. In addition to that, FDA was also involved in developing the reference reagents which can be used for validating the NAT assays.

This work is from Dr. Merieux's Lab in the Laboratory of Emerging Pathogens under Dr. Sanjay Kumar's able guidance. Two human Zika isolates were used for the viral stocks in supernatants of infected cell culture. One is from a Puerto Rican strain and another Cambodian -- Cambodian is an Asian -- because, as you know, in the Americas, most of the Zika is from Asian strain.

Reference reagents were formulated and these were analyzed by several laboratories using probit analysis of NAT detection in end-point dilutions, and the reference reagents were then unit agents. These reagents were also used in the WHO's reference international standard preparation.

So CBER's reference reagents are now available on request -- will be available; not yet -- to validate NAT assays and may be used for lot release of

licensed test kits.

In summary, the impact of testing using ID-NAT is, one, ID-NAT testing under IND has identified and interdicted more than 300 likely positive blood donations in Puerto Rico, so the blood is safe, and in a significant number, but small, of positive donations in the continental United States. And you will hear more detail about those cases by the two speakers from the industry about their respective number of positive cases. And implementation of donation testing for Zika RNA has prevented potential cases of transfusion-transmitted Zika virus, assuring a safe blood supply.

However, there are several unresolved issues which are relevant to the risk of transfusion-transmitted Zika. Further studies are needed to determine (1) what is the minimum infectious dose of Zika in blood components; (2) adequacy of NAT to detect virus at or below the minimum dose of infection; (3) need or no need to test the whole blood instead of plasma because, as I said, there is significant parasite burden when parasite remains attached to the red blood cells, and whether that is infectious or not remains to be seen; (4) the viability of Zika in stored blood components; (5) whether Zika-contaminated blood from a seroconverted donor is infectious; and (6) possibility of recurrent viremia from tissue reservoirs because these can stay longer in different tissues and can be detected at least in the different tissues, and whether those small amounts of virus staying there can then be reactivated when the person becomes immunocompromised.

In conclusion, I want to acknowledge the people who really helped us in getting this testing and getting the guidances out and also the reference reagents. Starting from CBER, which is the OBRR and other FDA components,

the Division of Emergency Transfusion-Transmitted Diseases, which I am part of, Human and Health Services Department, Office of the Assistant Secretary of Health, BARDA, CDC and, obviously, last but not least, the test kit manufacturers and the blood establishments who reacted immediately to this epidemic. And this goes to say, again, we have gone through this path several times in the past when the West Nile epidemic came, and the blood establishment and the test kit manufacturers reacted and helped us to get these tests done, and our colleagues from different parts of the PHS. Thank you very much.

DR. LEITMAN: Thank you very much, Dr. Nakhasi.

The next two speakers are from the companies that make the tests that are used to screen blood donors -- Dr. Lisa Pate from Roche, and Rainer Ziermann from Hologic. We will hear an update on IND testing in the U.S. and territories.

Agenda Item: Update on IND Testing in the United States and Territories, Lisa Pate, MD, JD, Roche Molecular Systems, Inc., and Rainer Ziermann, PhD, Hologic

DR. PATE: Good morning, and thank you for the opportunity to address you today. My name is Lisa Pate. I'm going to talk to you a little bit about what Roche has done to contribute to the protection of the blood supply from Zika.

By way of disclosure, I am an employee and shareholder of Roche Molecular Systems. Also, another important point is that cobas Zika screening of Puerto Rico donations is and has been supported by BARDA, which requires the inclusion of the following acknowledgement, basically saying that we are funded in whole or in part by federal funds for this important purpose.

The cobas Zika test has been authorized by FDA for use only under a specific protocol by U.S. blood-screening laboratories and collection organizations enrolled under the IND. A little less than a month ago, the FDA approved the cobas 6800/8800 systems on which the cobas Zika test is run, as well as the cobas omni reagents for us for blood screening in the U.S.

My objectives for my talk will be to describe one Zika virus blood screening strategy introduced in the U.S., to describe a bit about the development of the cobas Zika assay, the screening we have done in the U.S. and its territories, most importantly Puerto Rico, and a little bit about what's next and, really more to the point, what's happening today by virtue of the FDA's August guidance.

Others have already described how we got here. I'll give you my spin on it. Zika became epidemic in Brazil in 2015 and spread very rapidly through the Americas. By February, Zika was active in more than 30 countries and the Caribbean and South and Central America. The first cases were reported in Puerto Rico in December, and travel-related cases began to appear in the U.S. in very early 2016.

Around the same time, Zika's possible link to microcephaly, which has now been confirmed, sparked international alarm. In mid-February, as Dr. Nakhasi described, the FDA issued guidance prohibiting the use of blood collected in Zika-active areas, which included Puerto Rico, unless the donations were screened with a Zika NAT test or pathogen reduced, which was only available and remains only available for plasma and platelets, not for red blood cells. So the impact was that local whole blood collection in Puerto Rico was halted on March 7th and blood was imported from the mainland U.S. to Puerto Rico for nearly a month.

In early 2016, basically, we came back from our Christmas break having seen many, many new stories already about Zika and its impact in Brazil in particular, and began thinking about what we might do to help combat Zika. We used a proprietary *in silico* design tool to identify very rapidly candidate primers and probes that would be good choices for a Zika assay and, through various levels of stratification depicted with this funnel, chose primer and probe sets that were maximized or their performance could be optimized with the thermocycling parameters and chemistries of our omni reagents.

Within a very short period of time we identified three primers and probe test sets for wet lab testing and chose one as the test and one as the reference method for the assay we hoped to develop. Once we chose these, we did some additional testing and, using a third-party quantitated material, determined that the 95 percent limit of detection of the test was about eight copies per millimeter at the time, and until very recently there was no international standard for Zika. One I think has been accepted or approved by WHO in the last few days, so our copy number versus the copy number that the next speaker will tell you about can't exactly be compared because they were based on different standards.

We were able to develop our test in an astonishing 10 weeks and sought a way to make the test available for blood screening application initially in Puerto Rico and then in the 50 United States. We developed a study protocol designed to evaluate the specificity of the Zika test which was approved under IND the end of March, and individual donation testing was required at the time and has been used throughout our testing of blood donations both in Puerto Rico and in the U.S.

As I said, the assay was designed for use on our new cobas 6800/8800 systems. Initially, the test was used to screen donations from Puerto Rico at Creative Testing Solutions in St. Petersburg, Florida. Since the early days of the testing, in April two other blood centers, Qualtex and Gulf Coast Regional, have also begun testing some donations from Puerto Rico.

As Dr. Nakhasi said earlier and as I said as well, collections were halted in Puerto Rico at the beginning of March, and because a test became available under the IND, whole blood collections resumed in Puerto Rico less than a month later, on April 2nd, and we tested our first donations, or CTS tested our first donations, with the cobas Zika test on April 3rd. Interestingly and probably not a complete surprise, we detected Zika on the very first day of testing on those blood donations.

As I mentioned, the test was made available under a study protocol, and part of the goal of assessing the specificity was to use other methods to confirm that our test was, in fact, detecting Zika. The way we do that is do an initial reactive -- or plan to do it at least; this was based on CDC guidelines available in March when the protocol was written. The initial reactive is tested twice at the testing laboratory with cobas Zika and then also in a simulated pool of six. As you probably are well aware, many pathogens are tested using a pool strategy and then a reactive pool is tested further to identify the individual contributors of the reactivity. I'll show you some data about the simulated pool testing in a few minutes.

We then had the samples of serum in plasma from the reactive donations sent to Blood Systems Research Institute in San Francisco where it was then tested with an alternative NAT, which is the CDC NAT with an increased

input volume. About 1/40 is sensitive or maybe even less sensitive than our assay, and viral load is estimated and serology IGM and IGG are also performed.

Donors who have reactive donations are invited to enroll in a follow-up study, optimally with two follow-up visits where the first occurs within the first two weeks following the index donation and the second from two to eight weeks following the index donation. Those donations are tested once with cobas Zika at the testing laboratory and then serology is done on the follow-up samples as well.

This is some very current data, the week ending this past Saturday evening. We have tested to date nearly 45,000 donations collected in Puerto Rico with cobas Zika and have identified 335 initial reactives. More than 94 percent of those reactives on subsequent testing have shown either repeat reactivity with the cobas test on index or follow-up donations or have shown evidence of Zika IGM positivity on the index or follow-up donations. Many of the donations, because the testing is still ongoing, have either incomplete or equivocal results.

The viral load for these samples has ranged from undetected -- which is around 1,000 copies per milliliter - - up to 2.5 X 10^{10} copies per milliliter. The overall initial reactive rate in Puerto Rico has ranged from zero in a few weeks to nearly 2 percent -- I'll show you a graph of that which Dr. Nakhasi also showed. The overall initial reactive rate is about .74 percent and that is through November 12th.

This is the graph that shows you the peak of activity at the beginning, so the highest point on the graph with 19 donations at a rate of 1.78 percent was the week of July 7th -- just for a little orientation to weeks. As you can see, the rate of reactivity in donors ramped up fairly quickly by mid-spring, and

hovered at 1 percent or above for most of the summer, and we are now seeing a bit of a decline although it hasn't completely dropped to zero. We have gotten a few reactive donations in each of the last few weeks.

I mentioned we do a simulated pool of six on the reactive donations to simulate what kind of reactivity we would see in a pool of six, and in only about 70 percent of those pools is Zika detected with the cobas assay, which suggests that a significant number of donations do require individual donation testing in order to detect Zika and that many have fairly low viral loads at the time of testing.

These are the five collection centers that first started testing for Zika in the U.S. As I mentioned, CTS in Florida began testing Puerto Rico donations in April and added Florida donations I believe the last week of July. Gulf Coast in Houston, Texas was the first to begin testing U.S. donations and they began that testing of donations collected in and around Houston on May 23rd. Qualtex in Norcross, Georgia began testing in July; Blood Connection in Greenville, South Carolina began testing in August, and the Blood Center in New Orleans began testing in September.

These are the first seven Zika reactive donations collected in one of the 50 U.S. states. Not all but most were collected in Florida. This gives an example of what I mean when I say additional evidence of Zika, so it's either repeat reactivity on a cobas Zika test, reactivity in a pool of six on alternate NAT at BSRI, or one of the IGM markers.

Of the U.S. donations we've screened as of November 12, so last Saturday evening, we've screened 564,571 donations and detected 36 initial Zika reactive donations, 13 of which have been repeat reactive on cobas or alternate

NAT. Another seven are negative on the repeat NAT test but positive on GM serology. And 16 have either no additional evidence of Zika or incomplete or equivocal results. The seven on the no additional evidence of Zika are seven donations collected prior to September 3rd, so that means the eight-week follow-up period has completely lapsed and we can determine that these donors are not going to be able to show us any further evidence of Zika.

The viral load detected in the U.S. donations is a little bit lower than what we have seen in Puerto Rico with a peak about 8 X 10^6 copies per milliliter.

The specificity -- We based this on the donations collected through September 3rd because we know that we are not going to have additional data that would change these numbers. The specificity is quite good at 99.998 percent. I did some preliminary calculations based on the 564,000-plus donations. Even if all 16 that are either equivocal or we haven't been able to confirm fall into the "not able to be confirmed" category, the specificity is still 99.997 percent.

What is next is really kind of what's happening actually, because today marks 12 weeks after the FDA guidance. The second guidance came out on August 26 which requires testing of all donations collected in the U.S. and its territories where Zika is active to begin today, and the donor deferral period has been increased to 120 days.

The purple stars that are in the northern tier states are those centers that were added between September 23rd and November 18th. All of them begin testing, I believe, through today. Three centers -- Blood Work, Mississippi Valley and Community Blood Center of Appleton, Wisconsin began testing in the last few days.

These are just some of the very many people who have contributed

to this work and continue to do so every day. Thank you.

DR. LEITMAN: Thank you very much, Dr. Pate.

The next speaker is Dr. Rainer Ziermann.

DR. ZIERMANN: Good morning. My name is Rainer Ziermann. I'm responsible for clinical affairs at Hologic, and first I would like to thank the committee for giving our company an opportunity to present an update of our data on Zika.

As you know, the assay is in development. The product is currently not FDA cleared or approved, but we provide testing under the IND protocols. There is a conflict of interest because I am an employee of Hologic; therefore, I'm a stockholder and have an interest in this company.

What I want to talk about today is briefly give an overview of the design goals we have for this assay, present some assay performance characteristics, in particular, analytical data, then talk about additional applications of this technology that we use in blood screening, and finally, of course, talk about the current status of the Hologic-Grifols investigational new protocols in the United States.

We don't need to spend much time on this slide; many people have talked about it already. The Zika virus infection is usually asymptomatic in 80 percent of individuals; however, presence of viral RNA even though there is no activity necessarily has been confirmed for up to six months and even longer. Virus titers with high levels of viremia, again, maybe not necessarily all infectious, may be present during the asymptomatic period. Titers can reach very high copy numbers of more than 8 million copies, and transfusion transmission has been reported, and Dr. Nakhasi presented the data of the Brazilian infections.

Currently, as of today, there is no licensed blood test available in the United States; however, through the FDA guidance it became necessary to implement IND testing. The Hologic-Grifols IND protocol actually came into effect on June 20 and we started testing in what we call mini-pools or pools of 16 and also individual donor testing. Initially, we focused on the southern continental United States.

The revised guidance came out in August 2016 which superseded its previous guidance, and the previous speakers talked about it so I don't think I have to. But we did implement -- as of today, we are testing at 12 sites, so we are trying very hard to comply with the recommendation to implement nationwide ID-NAT screening.

The current protocols that we have detect Zika virus RNA in plasma specimens from donors. We use alternate NAT and serology for confirmation of reactive samples. Non-reactive donations are labeled accordingly for use, and we did provide some updates of our protocols to the agency where we added particular options of testing of red blood cells or whole blood, and we proposed some minor changes to confirmatory algorithms.

We also have additional protocols in preparation for organ tissue donors and cellular products from living donors. In fact, we submitted this a little while ago to the FDA and we have another protocol in preparation for cadaveric specimens.

Here is a picture of the Panther system that we use to run our assay. The assay design goals we initially came up with were based on the existing assays. We have assays for HIV, HBV, HCV. The goal is we want to be comparable to the other screening assays with at least 95 percent detection in the range of 10-

30 copies per ml. We want to have specificity of at least 99.9 percent if not higher, and we try to be able to pick up the genetic variants of Zika virus, meaning the African and Asian strains. We use two regions for amplification and detection of the virus, and that clearly should enhance sensitivity and reduce risk of false negative results.

Here is a quick schematic of the two regions that I just talked about. Our assay is based on transcription media amplification, TMA, which is distinct from PCR. We introduced some redundancy here to mitigate risk of false negative results, and you can see roughly how the different target capture oligoes, detection probes and primers and where they are located. We introduced some redundancy just to ensure should there be any mismatch that the 3-prime end or the reverse primers will be able to pick this up, and the two regions enhance the sensitivity, clearly.

Here are some analytical sensitivity data where we used an African strain diluted in buffer, and you can see the copy numbers on the left hand side of the slide that we tried to achieve. We tested between 20 and 72 replicates and the percent reactivity, if you look in the middle column, between 10 and 30 copies, it's 92 to 100 percent with a fairly high signal-to-cutoff ratio and relatively low percent CV. All of this data was actually generated by Jeff Linnen's group at Hologic.

We also tested an Asian strain from a Brazilian donor in a similar type of fashion. Again, between 20 and 72 replicates were tested. Percent reactivity is shown here. It's 100 percent at 10 copies per ml; it goes down to 86 percent at 3 copies per ml. Initially it has very high signal to cutoff ratios that then go down. The lowest concentration is in the percent CV inversely increases

with the lower concentration of the virus.

We also tested virus in urine. This is due to the fact that we have a version of the assay which did obtain emergency use authorization from the FDA. Here, when we tested copy numbers from zero up to 90 copies per ml, we found that this assay in urine has a sensitivity which is similar to what we saw before; namely, with 10 copies per ml we have 100 percent reactivity. With 3 copies per ml it drops to 46.7 percent. So clearly the virus works very well. What we did here is we spiked virus in urine and added this to the urine transport medium prior to the testing, and the urine transport medium increases the stability of the virus.

This slide is a summary of all the preceding slides. When you carry out probit analysis and you look at the right hand side, to 95 percent detection, we have a detection of 5.9 copies per ml for the Brazilian donor specific, 13.4 for the *in vitro* synthesized transcript that we had, and in processed urine, the data from the last slide I just showed you, the detection is 8.5 copies per ml, which seem to me when compared to the previous speaker, Dr. Pate, I think it is fairly similar to what we just heard in the previous talk.

Here is data from an analytical repeatability similar to a reproducibility study. We tested 54 or 108 replicates at different concentrations and we assessed inter-day, inter-operator, inter-instrument, and intra-run variability. As expected, the intra-run variability was the highest. We have a percent CV of 4 percent. If you do the statistics on all this data, the total percent CV for all the different concentrations is 4 percent with, as I mentioned, the intra-run factor being the largest source of variability.

Here are some data from a research use only study that was carried out at Hologic and also at the American Red Cross. A number of plasma and

serum samples were tested as well as 9,000 plasma specimens at the ALC. When you look at the specificity, we have 100 percent specificity for plasma, the Hologic-tested samples; the same for the serum samples tested at Hologic. The American Red Cross had one sample which was initially reactive; it did not confirm so it's a false positive. So that gives you a specificity of 99.99 percent -- very similar as the data we just saw from Dr. Pate -- and that is based on roughly 10,000 data points in total.

Here is data that we obtained from cadaveric specimens. It shows control, meaning living donor, and cadaveric specimens, and we spiked Zika virus into those specimens at a concentration of roughly 18 copies per ml. The reactivity is 100 percent for all of those -- plasma, serum or combined. Similar at the bottom -- specificity meaning non-spiked, the same type of experiment, and none of those samples was reactive, so here we have a specificity of 100 percent. The data is very promising. Right now we do not have a claim for cadaveric but we strive to get that claim.

Probably most interesting is the update on the IND data that we have. As of today, we actually have 12 testing centers up and running. My team went out earlier this week to Rhode Island and to Colorado to set up two more sites. We have one more site coming up in December, so even though the guidance requested was today the deadline, we tried our very best to make this happen.

It actually poses, especially on my team, quite a burden because all of those sites are managed -- It's an IND protocol; it's not a commercial product. Therefore, clinical affairs manages all those sites. So we do whatever we can possibly can do to get everybody onboard, and here is the status of where we are

today. When you look at all those sites in the different states it covers a variety of the country -- Virginia, Texas, Missouri, Oregon, Oklahoma, Tennessee, Rhode Island and Colorado.

The data on this slide is up to November 5 updated. A little later I'll show you more recent data, but based on this, the American Red Cross tested 9,000 pools resulting in about 127,000 test results. Sorry. In 127,000 individual donations for a combined number of 270,000 donors tested. Three initially reactive results were found, and initially reactivity means, as the footnote implies, this could be false positives, meaning those that are non-repeat reactive, seronegative and alternate NAT negative, or they are confirmed positives.

Among those three there is really one that could be counted as a confirmed positive result. I put it in Italics simply because based on our IND protocols that we have in place, even if a sample is repeat reactive -- and this particular donor is -- it has not yet been confirmed with any of the other methods; therefore, per protocol, we cannot count it as confirmed. But all indication is that this is clearly a Zika virus-positive result.

Data from five other centers is indicated here. They tested about 234,000 individual donation samples, which gives now a total of over 500,000 samples that have been tested. Among those five centers, 18 were found to be initially reactive, and of those, three of them are confirmed. So that gives us a total of 21 initially reactive results, a lot less obviously than was found in Puerto Rico and Florida, as expected, I would say, and of those three plus one, really, because we have four confirmed cases as of today.

This is a very busy slide but it lists those four individual presumed positive cases. The first one was an individual from Reno. This sample was picked

up with our test with a relatively low or medium signal-to-cutoff ratio of 18.75. When it was retested twice it was not reactive; however, it was strongly reactive for Zika virus IGM and IGG serology test. It tested negative as an alternate NAT test and RT-PCR-based test; was dengue virus IGM negative but IGG positive, which I think is well established that there is some cross-reactivity.

A viral particle neutralization assay was carried out. I don't know too much about this assay. I think it's a single replication assay which is very specific. It was positive here. And finally, red blood cell positive tested by another RT-PCR test.

This donor actually came back for follow-up. It was non-reactive with our test. Serology continued to be positive. This may be a donor that picked up at the tail end of the infection potentially, but it is clearly confirmed.

The second case is a case from New York. Similarly, it was picked up initially with our test, was reactive with a high signal to cutoff ratio but did not -- when it was retested twice, was both times nonreactive. A very similar picture with serology for Zika virus, positive; IGG for dengue virus positive; Zika virus neutralization, and red blood cell positive. We also have some follow-up data. As you can see, that is certainly a confirmed case.

The third case was picked up in Arizona. This one tested actually reactive initially and on the repeat test, one of the two repeats was, in fact, positive; the other one was nonreactive. Again, Zika virus serology is positive; dengue IGG is positive, and red blood cell was tested and is positive, so we could report this one as confirmed as well. There is no follow-up data available yet. A lot of data is pending. I wish I could show you this result but as of today I don't have the data. It will be coming very soon.

The last case is a case that Susan Stramer presented. This one was positive with our test with a fairly high signal to cutoff ratio of 32.4. It was retested three times and every time it had a very high signal to cutoff ratio of over 30, so this one is the one that I could say was positive even though as of today we have no confirmatory testing for this result, so per IND protocol we cannot count it as confirmed positive.

On the right hand side column you see these were all travel-related cases.

Dr. Stramer provided this slide as recently as yesterday or two days ago, and it shows now that the American Red Cross testing from June to November covered over 3200 zip codes with almost 5,000 donations per zip code, and you can clearly see it is concentrated in California and Georgia, that area, and up in New York.

Similarly, when you do the same kind of data by donor residence you can see that almost 298,000 donations were tested, which include almost 144,000 mini-pool and 153,000 in ID-NAT. These include the three non-repeat reactives that I just talked about and the one repeat reactive. This slide was just updated two days ago, so I am very grateful for this information.

You can see that all across the United States and some of the territories, individual donations pop up even though they are really not the primary areas where the blood is collected.

This slide shows you the AABB Zika virus biovigilance data that is up and running. This data is as of November 16th, and it posts it confirmed three cases. It posts 36 unconfirmed cases primarily down in Florida, and I don't know what the goal is. This is all the data that Dr. Pate talked about. Some of this data

may be uploaded more recently. And eight false positives are reported on this website. I should mention that AABB has a slightly different algorithm to confirm positivity than we have in our IND protocol.

This slide is a little older. It was provided by Dr. Williamson from CTS. There are two testing centers in Phoenix shown in blue. And in Dallas -- I should mention the blue spots indicate the collection sites that are tested in Phoenix; the red spots indicate collections that are tested in Dallas.

Here are some data that, again, Jeff Linnen's team at Hologic carried out. We did additional testing of the donor who was confirmed positive in Reno and we tested the red blood cells from this donor. The top line shows than when 29 replicates of the plasma were tested in-house only two were reactive, which gives you a percent reactivity rate of 7 percent. However, when you test red blood cells even diluted down to 1 to 30, you still get 100 percent reactivity. A little bit lower, it drops to 40 percent and finally down to zero percent.

In summary, we have so far found three plus one, or four, confirmed positive donors that have been detected in our IND studies. These donations are originated from collections that are outside of areas of active transmission; namely, Nevada, New York, Arizona and Texas. All of those have travel histories ranging from 28 to 97 days prior to the donation. Two of those individuals developed symptoms consistent with Zika virus infection shortly after their return from those countries. Overall, it looks like the three out of the four donors that turned out to be positive had very low viremia, and this is based on replicative alternate NAT testing and other PCR testing. However, the American Red Cross apparently has a very high titer.

We know that high levels of Zika virus RNA is associated with red

blood cells from the index donations. How far this is linked to infectivity I think is an open question, as was pointed out earlier today. All these donors show strong IGM and IGG neutralizing activity which is still increasing after a couple of months at least for two of those cases. We saw an absence of dengue virus IGM, but very weak cross-reactivity with IGG ELISA.

As I mentioned, the American Red Cross case from Texas was initially reactive and then three times repeat reactive with consistently high signal to cutoff values. Further testing is pending but it indicates that is a high titer sample.

Overall, in conclusion, the assay was designed as a two-region amplification detection system, which is really expected to increase sensitivity and substantially reduce the chance of false negative results. We showed analytical sensitivity of 6 to 13 copies per ml. The preliminary specificity data shows that specificity is more than 99.9 percent, confirmed by testing under the IND protocol.

We did preliminary testing of cadaveric specimens and showed assay sensitivity and specificity were not affected at all by these somewhat more tricky specimens, which reflects the robustness of the assay.

All of these four that we found that were initially reactive or repeat reactive were linked to travel in areas with local transmission, and three of those four had a very low viral titer. We are really trying our best to support the US FDA guidance for implementing nationwide ID-NAT screening and I think they are getting there.

Finally, I would like to thank a series of individuals, in particular Jeff Linnen who is a very gifted scientist working on all the blood screening

assays. I also want to point out Petra Pavlickova who is heading the regulatory team for blood screening, and her expertise and experience is really invaluable in guiding us through all the questions that arise frequently.

Dr. Stramer from the American Red Cross has been very helpful in providing data and information. Dr. Mike Busch from the Blood Systems Research Institute is heavily involved in many of the testings here. Dr. Williamson from Tempe, Arizona, from CTS, and some more individuals. Also, our colleagues from Grifols. As you know, this product is marketed by Grifols, who work in strong collaboration with Hologic.

At this point I will stop. Thank you very much.

Agenda Item: Questions for Speakers

DR. LEITMAN: Thank you very much, Dr. Ziermann.

I would like to open the next part of this session for questions from the committee to the prior four speakers.

DR. SIMON: This is a question primarily for Dr. Nakhasi but perhaps also for the other two speakers as well. Particularly from the conference that AABB had a few months ago, it seemed like there was exemplary collaboration between the FDA and the IND holders in getting this developed in really record time. I wonder if you could comment on the communication with the blood banks and blood bank community that had to respond and put this into place so rapidly. How did you work with them in terms of execution?

DR. NAKHASI: Basically, we had direct interaction with the test kid manufacturers and it became confirmed that the cases were occurring.

But with the blood establishment, as you know, they instituted the AABB arbovirus liaison meetings which were every other Thursday, every two

weeks, so they would talk about it, and the IND holders would provide the information about the positives to that group and that's how the interaction was.

It was just basically the model was similar to what we did for West Nile. As you remember, we just had a workshop and we asked there. But I think in this situation we had it through the liaison committee. We didn't have enough time to go and have a workshop because the cases were springing up so fast.

Bottom line is, again, collaboration between the blood establishment and the test kid manufacturers. It was fantastic, and both the test kit manufacturers reacted immediately in developing the assays, and whatever help they needed from us, the regulatory point, we provided. At the same time, we had discussions with the blood establishment through the AABB arbovirus liaison meetings to appraise where this epidemic was going.

DR. LEITMAN: I wrote down that I wanted to introduce the question period by stating that I think all the committee members expresses their congratulations and appreciation for the lightning speed with which all this occurred -- less than a year from the first U.S. documented infection, involving both the documentation and education services of the CDC and the response of the FDA and of the manufacturers of these tests. It is good to know that our surveillance and response mechanisms for emerging agents in CDC and FDA work so extraordinarily well and collaborate so well with the blood establishments and with industry. I don't think we have ever seen something happen this quickly.

DR. NAKHASI: Thank you very much for that comment.

DR. STAPLETON: I think this is also primarily for Dr. Nakhasi but possibly for the industry folks as well. You mentioned the low viral loads in the

late samples, and you mentioned also the need to look for infectivity. Do we know the specific infectivity in verocells, or is there another cell culture system that would give us ideal specific infectivity of the RNA so that we would have a better idea of --

DR. NAKHASI: Yes. Those experiments are still going on because I think there is a collaboration between Dr. Busch's lab and our group and they are getting the samples and trying to find out whether it is infectious or not. Mike, do you want to comment on that?

DR. BUSCH: Mike Busch, Blood Systems Research Institute, and I am involved with every company here.

The virus persistence on the red cells -- Based on partitioning studies and lysis and pelleting studies, the RNA is associated with the red cell membranes during the persistent stages, and the current hypothesis is we can actually infect hematopoietic progenitors and erythroid progenitors. So our current thinking is that this persistent signal is a product of red cells surviving which were essentially born during the acute infection phase, the progeny of infected erythroblasts.

Infectivity is being studied in a variety of settings -- cell line inoculation, mouse inoculations, enhanced cell line -- in collaboration with Maria Rios here. We have large numbers of red cell components that are derived from Puerto Rico donations and then longitudinal follow-up samples that we can process literally the next day that are being used in these inoculation studies.

We have now been funded also to expand the originally planned macaque infectivity studies to include studies to directly investigate infectivity by transfusion of leukoreduced packed red cells into macaques to really rule out

what we think is probably a non-infectious persistent signal.

DR. STAPLETON: That partly addresses my next question which I think I know the answer to. Does leukoreduction reduce infectivity or do you know?

DR. BUSH: The acute phase, there is infectivity associated with PBMCs, but on subsequent follow-up the PBMCs are completely negative for virus. Leukoreduced standard blood bank components, the red cells that are leukoreduced, that's where we're seeing the persistent signal. So we don't think the viral infectivity -- Whether the virus is able to infect leukocytes acutely has not been carefully studied.

But whether leukocytes that are naturally derived from acutely infected people are infectious is going to be studied, but those don't persist, so that signal is only detectable during the period where plasma viremia is detectable. And importantly, the blood screening NAT tests are so sensitive that they are able to pick up that plasma viremia for weeks, and occasionally now we're seeing months with that very low level signal after acquisition.

DR. STAPLETON: And the last question is partly related to that. Do you have any evidence that cross-reactive flavivirus, the antibodies, enhances their detection in whole blood --

DR. BUSH: There is published data that dengue -- You know, 90 percent of the donors detected with Zika in Puerto Rico had pre-existing dengue antibodies that are rapidly anamnestically boosted, which is why there is such a challenge with the serologic discrimination. Within days of Zika viremia you see boosting of neutralizing activity against all the other dengues followed by a Zika-specific neutralization. There is monoclonal antibody-based data that suggests

that dengue virus antibodies can enhance infectivity of Zika and dissemination.

There's a lot of research going on in an NIH-funded program in Puerto Rico and the United States specifically looking at enrolling and following 80 dengue antibody-positive donors who go through a Zika infection and 50 dengue antibody-negative donors to specifically look at both disease penetrance and virologic and immunologic issues and acquire the critical samples, the longitudinal samples, during that boosted immune phase to look at enhancement in both directions.

DR. STAPLETON: It may enhance the detection in the whole blood if you have immune aggregates being pulled down --

DR. BUSH: Yes, we have looked at the binding *ex vivo* and with and without antibodies, and there's actually no significant binding of virus to mature red cells, which is why we're focused on the infection, and with or without antibodies, so we've done a bunch of mixing studies. So that doesn't appear to be the mechanism of binding.

Importantly, a small percentage, about 10 percent of infected donors from whom we have excellent index and serial follow-up samples do not develop the red cell-bound virus. We don't know why. Is that some receptor or something? But testing whole body is probably not needed for blood screening. There you have half plasma, half red cells, so that's kind of, from a diagnostic perspective, the way to go.

DR. LEITMAN: Thank you. That was Dr. Michael Bush from Blood Systems Research Institute responding. Don't sit down yet.

You mentioned the extreme rapidity with which the IGM Zika virus antibody can be detected in patients, and that's in the literature -- within three to

four days. Can you comment on that extreme rapidity?

DR. BUSH: Most of these donors that we're picking up in Puerto Rico and even the travelers in the U.S. who traveled to their home countries, they had dengue antibodies prior to Zika infections. If you don't have dengue antibodies, the Zika IGM comes up pretty much as the viremia starts to drop and comes up briskly and stays high.

If you have pre-existing dengue antibodies -- and we have done a lot of careful collaboration with the CDC labs to make sure we're running a really optimized macrolides to monoclonal antibody capture IGM assay, and 95 percent of these Zika viremic donors who have pre-existing dengue antibodies convert their IGM pretty quickly, within days. About one-half of the donations at index already have IGM because they were picked up in the evolving phase. We're picking people up in serial stages of acute viremia, so about one-half of the index donations have IGM.

Those that do not on follow-up develop IGM by the first follow-up visit a week or so later, but then IGM reactivity in people who have prior dengue antibodies is, one, not seen 100 percent of the time, so there's a small proportion of acutely infected donors with Zika who do not convert their IGM, and those who do convert it, it is much more transient and may last only weeks to months compared to people who are dengue-naïve who get Zika, who boost a nice primary response that is more persistent. But they all convert IGG and they all develop neutralizing activity.

DR. LEITMAN: Thank you.

DR. ESCOBAR: I have a couple of questions for Dr. Rabe. In the initial outbreak in Micronesia and Polynesia, were there any reports of anomalies

in the babies during that time? I think we have seen everything from Brazil forward, but in those days were there any reports?

DR. RABE: There was nothing in the Yap outbreak specifically but that was a very small population, so that may explain why there was nothing specifically detected. But there were no subsequent reports either that we are aware of.

With French Polynesia, yes, they did look at retrospective data and did find associated anomalies, but this was also -- Once there was additional data on the Americas outbreak there was a lot of interest in retrospectively looking to detect those, and they did find an increased rate.

DR. ESCOBAR: My other question is, in one of those slides you showed there were about 680,000 cases reported in Latin America but only 24 percent were confirmed by lab tests. We assume the majority of those were clinical diagnoses?

DR. RABE: Correct.

DR. ESCOBAR: And since there are other viruses down there that might have similar symptoms, I guess we could maybe have a false positive diagnosis in a lot of those cases. Also, do you know what tests they are using there to make the diagnosis, since there is cross-reactivity and maybe we're getting a lot of false positives.

DR. RABE: The cross-reactivity is challenging, and really in terms of confirmatory testing it's primarily through molecular detection. The serology in many of the countries where they have had and continue to have dengue circulation particularly, the serology becomes very difficult to interpret and definitively give evidence of a diagnosis.

But on the basis of the molecular testing and detecting high levels of activity by molecular assay, and in the absence of confirmatory testing for dengue in an area, it is assumed that the bulk of those cases where they are getting a lot of molecular signal would be attributable to Zika.

But it is a valid point. We do exercise caution in looking at those numbers for that very reason, because we do expect that some of that would be attributable to other viral infections as well.

And the serologic cross-reactivity is obviously just an issue, where with dengue and Zika, chikungunya would not cross-react at all.

DR. ESCOBAR: Thank you.

DR. ORTEL: I have a question also for Dr. Rabe. Since this virus has been known for almost 70 years, is there any understanding or insight into what has led to this potential change in infectivity, or do you think it just got into a sufficiently large population that allowed it to explode like this?

DR. RABE: I think all of those factors are at play. I think there are a number of different postulates in terms of what may explain that difference, whether the earlier cases really didn't have a sufficiently large susceptible population, or whether exposure to other flaviviruses in an area may have had some additional effect in early childhood of somehow preventing or mitigating later exposure that would result in these effects. But I think there is still a lot of work ongoing in terms of what the reasoning is for that specifically.

DR. ORTEL: And are there similar efforts worldwide as to what is going on here? It's very impressive how quickly we're moving in developing testing and implementing strategies. Is the blood bank population in Europe concerned similarly, or is this a U.S.-centric phenomenon?

DR. RABE: I'll defer to --

DR. NAKHASI: As I mentioned in my talk, they already, under the WHO, had developed an international standard for Zika, so they are concerned and they are getting ready for that. So I think the answer to your question is yes.

DR. ORTEL: Actually, in Europe, the current policy under the recommendation of the ECDC is to test in endemic areas if they have any local outbreak and otherwise to defer travelers or sexual partners who may otherwise be at risk. They don't have a routine universal testing in place to my knowledge.

DR. EPSTEIN: My question for Dr. Rabe -- Could you elaborate a little bit about the issue of public health reporting and also reporting of viremic collections from blood banks? We are aware that Zika virus is nationally notifiable, but what does that mean exactly in terms of who reports what to whom, and how CDC might consolidate reporting under ArboNET?

DR. RABE: There are sort of two arms to that question. In terms of the disease case reporting or reporting of infections detected into ArboNET, that is working through the reporting jurisdictions primarily of states and territories reporting directly into ArboNET. Those cases that they report in are based on the CSTE-approved recent revision to the Zika case definition and includes the capacity to report both symptomatic and asymptomatic infections, which has been a shift from what was previously reported under other arboviral infections where it's typically symptomatic cases that are reported.

During the process of all of this, the states have been -- the health departments have been integrally involved in the counseling on where the patient should be tested and, also, doing a lot of the testing themselves and facilitating testing through CDC. So there is a lot of awareness of cases that are occurring in

states outside of the blood screening setting. That is operating the way, as it usually does, into ArboNET.

But as far as the blood screening is concerned, that is also appropriate for ArboNET reporting and would occur from blood collection agencies that are aware of positive screening results to report those to their health authority, usually to the state health departments who would then report those to CDC as well.

I think many blood collection agencies and health departments have been doing this process for West Nile for many years now and are familiar with those channels of communication. I think the one subtle difference to be aware of is that, given the current outbreak situation and the desired opportunity for mitigation should there be any suggestion of potential local transmission, does make it more time-sensitive to make sure those reports are actually going through to the health departments. So I would just encourage that to be done as well and that would flow through that mechanism again but by the blood collection agencies.

DR. PATE: I would like to add something with respect to blood centers. I and, I believe, Hologic report each week all the new cases both in Puerto Rico and in the U.S., with a weekly report that goes both to FDA and CDC and some other participants so that they are aware.

In addition, for the U.S. cases, from the first one I had made an agreement with Dr. Nakhasi and my contacts at CDC to report them as soon as I heard about them, so, for most of the 36 cases there have been texts or phone calls within 24 and, in many cases, within three hours of my hearing about them, and we provide additional information as it becomes available. So the

collaboration for describing what is happening with Zika as opposed to what happened, what typically happens with a clinical trial, has been a real important part of this, I think.

DR. EPSTEIN: If I might just clarify a point here, the reporting that Dr. Pate is discussing is under the IND, and those data remain confidential within the government agencies.

What I'm trying to get at is making the outcome of screening public information. That can occur through the ArboNET, but what you heard is that the CDC reporting, at least at the present time, is dependent upon the blood center reporting to the state public health authority, which then decides whether they have what they consider a confirmed case and only then will report it to the CDC to post. This has caused some disconnect. What you heard is that information may flow in the direction from the IND holder to the CDC, but that information does not get directly posted.

Also, Dr. Rabe didn't elaborate on this, but the criteria for confirmation aren't the same in all the different players. The states may or may not have now agreed on consensus criteria. You were suggesting that CSTE has now reached an agreement; that's good. But perhaps not. You heard that AABB may be using different criteria. Also, you saw that AABB, on its GIS map, was posting initial reactives. I know that the dataset goes deeper than that, but there is reluctance by the states to post anything that isn't confirmed.

And then you have also heard that the criteria are highly specified under the IND, but you have also heard that additional things are being done beyond what's necessitated through agreement with the FDA and the IND.

So I don't think we have clarity on who reports what to whom and

by what criteria as far as it concerns the public domain, and that's the point I'm trying to press. I think we need to get an agreement on how prompt information gets posted publicly. We are not quite there yet.

DR. STRAMER: I just wanted to elaborate -- I'm Susan Stramer with the American Red Cross -- on the points that Dr. Epstein made and Dr. Nakhasi made. We did have meetings every other week with CDC, CSTE and the AABB Arbovirus Task Force to talk about what is the best way to collect national data. Is it through public health via the CDC, or is it through the IND holders, which is confidential information? That's why we established the AABB Zika Biovigilance site so there would be a public domain for initial reactives, as soon as they are reported or posted, and we are refining the definitions of confirmed positives to be more robust, and as soon as something is confirmed positive it should be posted.

It is also true what Dr. Epstein said that some states; *i.e.*, Florida, have not yet allowed the posting of confirmed positives on the AABB website. So, when Dr. Ziermann showed you the map of Florida and you saw all the red dots -- because Florida is an active Zika virus area -- those samples are not yet posted as confirmed positives even though, as Dr. Pate showed, as many as 13 of the many that are posted in Florida may be confirmed positives, but they are not posted as confirmed positives. And there are only three posted as confirmed positives now which we recognize is a problem on the AABB site -- the one that we have posted from Dallas, the one from Reno that was described, and the one from New York.

So, in order to make national communication worthwhile, this posting has to be done in real time and it has to be that confirmed positives must be added or we will be in the dark as far as what's happening nationally.

DR. LEITMAN: I have a question for the manufacturers. Part of the reason that individual donor NAT is being done under the IND I guess is to capture those very low level donors where you have three, four or five viral copies per ml, but the infectivity or transmissibility of the agent from such donors is not known. Can you comment on whether you think long term this will have to remain an individual donor NAT versus a pooled donor or multiplex NAT, the way the other agents are?

DR. PATE: I think the answer to that is I don't know, with all due respect, and it's probably not the appropriate question for me but, rather, for Dr. Epstein and others sitting here. I think our data shows that there is a question about the detectability in pools, and that question needs to be investigated further to see what is appropriate in the future.

DR. BUSCH: As Lisa showed, a substantial proportion -- over time actually an increasing proportion -- of the ID-NAT yields from Puerto Rico were negative on simulated mini-pools, just 1 to 6. And it makes sense that that dropped over time because, as the epidemic evolves more donors are coming in, in that tail end of viremia that can persist at low levels in the presence of antibody.

So, to the question of infectivity, a small proportion of those ID NAT-only donations are antibody-negative for Zika and are front-end low-viral loads that probably are infectious. A large proportion are tail-ends; they are low-level persistent RNA in the presence of Zika neutralizing antibody. Again, there is this red cell-bound virus as well.

So the studies are funded by NIH to transfuse into macaque serial collections from infected donors through the course of acute pre-antibody

viremia to understand the relationships between the minimal infectious dose and the detectability of ID versus mini-pool NAT assays by the manufactures and, most important, to address the question of the infectivity of these tail-end infections.

But there is no question that, just like West Nile, all of these viruses, there is going to be a low rate of infected donors during high-level epidemics that would be low level on the front end, mini-pool negative, likely infectious, which is why, of course, we trigger ID-NAT with West Nile. If there were ever an opportunity to evolve part of the country at least to mini-pool, it would be similar to West Nile where we would be using mini-pool to detect any incident infections. The problem I guess we have is that almost all the infections in the continental U.S. are not outbreaks where donors got infection locally; they are travel-acquired cases.

So it's different in West Nile where mini-pool surveillance would detect a regional outbreak, and triggering ID-NAT temporally, locally, makes great sense and has worked wonderfully. Here we're dealing with mostly travel-acquired cases and many of them are these tail-end infections because people come back and donate and are detected months later.

DR. LEITMAN: Thank you.

DR. DE VAN: I am not sure who to direct this to, but I'm wondering if there are any data on viability or infectivity of Zika in previously frozen and de-cholesterolized red cell units. The answer may be we don't know but I'm just wondering if anybody knows.

DR. STRAMER: We don't know, but we would assume, just like with any other viral agent, they are going to survive in frozen decholest rat cells.

I just wanted to add a comment to what Mike said about West Nile. Clearly, we do pool testing and we do pool testing as a surveillance tool, and when there is activity, we trigger. We know that greater than 50 percent of the West Nile yield we have each year is not detected in a pool; it's required by ID-NAT. So the triggers that Mike mentioned that we use very effectively have really maintained the safety of the blood supply in the United States.

But, as such, it is not perfect, and since 2003 when we initiated West Nile mini-pool NAT, due to refinements of triggers and other issues, we have had 13 transfusion transmissions. But again, that's a background now of 14 years. So we can assume that even using a West Nile model we may see one breakthrough a year.

DR. LEITMAN: Thank you very much.

The question period is over. We are staying on time today, so I would like to open the open public hearing part of the session. I will read the open public hearing announcement.

Agenda Item: Open Public Hearing

DR. LEITMAN: Both the FDA and the public believe in a transparent process for information-gathering and decision-making. To ensure such transparency at the open public hearing session of this meeting, FDA believes it is important to understand the context of an individual's presentation.

FDA encourages the open public hearing speakers, at the beginning of their written or oral statements, to advise the committee of any financial relationships they may have with a company or group that is likely to be impacted by the topic of the meeting. For example, the financial information may include the company's or group's payment of travel, lodging or expenses in connection

with the speaker's attendance at the meeting.

Likewise, FDA encourages the speakers at the beginning of their statement to advise the committee if they do not have any such financial relationships. If the open public speaker chooses not to address this issue of financial relationships, it will not preclude them from speaking.

With that said, we have only one person who has submitted a request in advance to speak at the open public hearing, and that is Dr. Susan Stramer of American Red Cross who is also the Chair of the AABB Transfusion-Transmitted Diseases Committee.

DR. STRAMER: Thank you. First of all, regarding my conflicts, I paid for my own travel. I live in the D.C. area but I have financial conflicts to disclose. I have received honoraria and my laboratory gets support from Hologic-Grifols, Roche, and Ceres, all of which are impacted by the Zika guidance and the discussions we're having today. But today I am presenting on behalf of the AABB, America's Blood Centers and the Red Cross.

First of all, thank you for the opportunity to present. AABB, America's Blood Centers and the American Red Cross appreciate the opportunity to present this statement focused on the August 2016 guidance and recommendations for donor screening, deferral and product management to reduce the risk of transfusion transmission of Zika virus.

AABB's Transfusion-Transmitted Diseases Committee and its Arbovirus subgroup assisted in drafting this statement. America's Blood Centers and the American Red Cross provide representatives to the TTD committee.

We recognize the nature of the worldwide Zika-related health emergency and are supportive of the objective of HHS to minimize or prevent

infection from blood transfusion, particularly of pregnant women with the consequent risk of harm to the fetus. While we support the delivery of the safest possible blood products and services, we are concerned about the processes used to develop and implement the guidance, the balance of resource commitment to potential benefits, and the potential for future expectations for blood donation testing.

And I think our response addresses the question that Dr. Simon was asking earlier.

The agency issued recommendations related to several regulations and utilized authority outlined in the May 2015 final rule -- requirements for blood and blood components intended for transfusion or for further manufacturing use -- making the content of the guidance a non-negotiable mandate. The guidance appears to be based upon an extreme interpretation of the precautionary principle and rejects the concept of tolerable risk. However, it should be noted that a primary tenet of the principle is that actions should be taken only if they will not cause harm. In the absence of any formal risk assessment, and since the blood community was not consulted during the development of the guidance, we do not believe that this aspect was fully evaluated.

Further, responsible commentary on the precautionary principle advocates against policies based upon zero risk and calls for a response that is proportionate to the risk and commensurate with the measures previously undertaken in similar circumstances.

In this context, we recognize that the current circumstances are extraordinary with little or no precedent but are, nevertheless, concerned that

there has been no public quantitative assessment of the potential risks, benefits or research usage required by the guidance. We consider this wholly inappropriate at a time when both healthcare and public health resources are limited.

As noted, the lack of consultation with the blood community in the development and issuance of the guidance, is of particular concern. No attempt was made to determine whether the guidance could be implemented by the blood community in the required timeframe without adverse effects on the safety and adequacy of the blood supply. Neither was any attention given to the resources required to implement the requirements of the guidance.

Lastly, estimates are that the program will incur direct costs well in excess of $100 million per year. This sum must be measured against the responsible estimate of the potential benefits occurring from implementation of the guidance. Further, the investigational new drug cost-recovery regulations under which centers can bill for this testing -- that's 21 CFR 312, Part 8 -- allow recovery only of the direct cost of testing. Approximately 30 percent of the total cost is indirect and not allowed under cost recovery of this nature. If this were a licensing clinical trial, for example, both direct and indirect costs could be captured. Thus, the costs for this FDA mandate are not yet fully recoverable.

We strongly recommend that FDA establish a continuing formal, public review of the policies recommended in the guidance with the specific objective of modifying the guidance to achieve an appropriate balance of benefits and resource usage.

Despite these concerns, the blood community has risen to the challenge, and we believe that we and our suppliers should be commended.

However, we wish to emphasize that this should be viewed as a unique response. Neither we nor the FDA can determine the concrete benefits and associated adverse effects of implementing this guidance. For example, ongoing safety and quality-related projects were put on hold, laboratories were configured, and we are burdening our hospitals with another IND cost recovery increase without concomitant data demonstrating efficacy.

Every collection site having testing performed under one of the two investigational protocols that you heard about today is also required to have institutional review board approval of the protocol and all documents that interface with human subjects. This task alone has been especially burdensome and challenging to the FDA-required time line. We do not believe that under the current circumstances the blood community could be expected or able to repeat a response to another regulatory expectation of this nature.

Thus, in closing, while we support efforts to minimize or prevent transfusion-transmitted Zika virus infection, our concerns focus on the lack of transparency of this guidance process when there were ample opportunities for fruitful interaction with the blood community. We are also concerned about the balance between the cost and overall value of this initiative. You have seen the initial yield in the continental United States to date.

Finally, we are uncomfortable with the precedent that this process appears to have established.

Thank you for the opportunity to offer these comments.

DR. LEITMAN: Thank you, Dr. Stramer. I would like to take the prerogative of asking the committee if there are any questions for Dr. Stramer or comments.

DR. SIMON: Well, Dr. Nakhasi mentioned those regular calls that he had with the blood community. Did these not assist with the process?

DR. STRAMER: No, they did not, although what we discussed on the calls -- I am the chair of those calls. We invite FDA; we invite those members of TTD who are part of the Arbovirus Task Group, and CDC is on and CSTE. For multiple weeks while the guidance must have been under development there was no discussion on the calls from FDA other than talking about what yield had occurred in testing that was already ongoing.

So it was a complete surprise to us -- Well, August 25th we had a call. FDA was not present on that call. At the end of the call, a few of the Arbovirus subgroup members stayed on the call as we were getting increasingly concerned because we heard rumors that guidance may be coming. From that point forward, I telephoned FDA and the next morning we were notified that guidance was coming out on that.

So, during the Arbovirus subgroup calls hosted by the AABB, the development of a new guidance that would require universal ID-NAT with the timelines that are in the guidance was never discussed.

DR. LEITMAN: I guess at the heart of this is the .006 or .008 percent confirmed positive -- and that's on the low side and you mentioned why that is -- that's being seen from the initial IND data. So the likelihood that those units, if there wasn't testing, would be transfused to a woman who is pregnant or her sexual partner -- which is the real concern; it's not the viral syndromes because they are self-limited and they occur in a very small percentage of patients. So, that's the real concern. The likelihood of that happening is so small I can't quite get at it -- I need some kind of statistical model. But America is paying

$100 million to prevent those extremely rare events. Is this the tolerable risk that you're talking about?

DR. STRAMER: Yes. No one is arguing about the fact that the blood industry wants to do everything we can to keep blood safe. That's really not the issue. It's really the issue of the process and are there other models for which we can achieve comparable safety.

DR. LEITMAN: And there are also other mechanisms to prevent donations from people who might be infected with Zika virus, and those are the travel histories. But if one didn't test and you re-implemented those, there would be a significant donor loss. So they are weighing that as well.

DR. STRAMER: What we have seen in the data that the IND holders have shown, Florida and Puerto Rico, those are active areas; there's no question. Under any model we would be doing ID-NAT. We would consider, as mentioned, the southern tier of the United States doing ID-NAT.

But every other case that has been documented thus far has been in a traveler. And of those, only one, the case that we obtained, is really, I believe, from a front-end likely infectious unit.

DR. LEITMAN: Okay. Any further comments or questions?

MR. TEMPLIN: As I sympathize with the cost that it costs just to keep the blood supply safe, the blood banks, at least AABB as a nonprofit, go out and raise the kettle and get some more money from the public to fund your mission. But I commend the FDA and CDC for doing everything they can do to keep the blood supply safe because of those potential infections that were, say, from the sexual partner or the pregnant woman, that potentially save a family from having to deal with a child with microcephaly. It's great that we can do that

today, so thank you for keeping the blood supply safe.

DR. LEITMAN: Dr. Epstein?

DR. EPSTEIN: I would like to read a statement. We anticipated -- what shall I say? -- concerns that might be expressed by the industry because of the burdens imposed, so we have prepared a statement.

First, of course, we appreciate the comments that we heard from the blood industry organizations regarding the agency's guidance, the revised recommendations to reduce the risk of Zika virus by blood and blood components that we just heard in the open public hearing. We recognize the magnitude of this undertaking, and we understand the concern that the guidance imposes a burden on the industry.

FDA's guidance was issued to address an exceptionally urgent and evolving situation. Zika virus is a transfusion-transmitted disease which can cause potentially severe consequences including microcephaly and Guillain-Barre syndrome. The requirement to test blood donations for Zika virus has already resulted in interdicting contaminated collections, confirming the value of testing.

More generally, wherever feasible, FDA engages stakeholders in developing guidance; however, the situation with Zika virus emerged very rapidly and necessitated swift action and consideration of testing throughout the United States in order to protect public health. While we agree that policymaking should not be driven by a mandate to achieve zero risk, we do believe that issuing these recommendations was warranted given the potential public health impact of the Zika virus.

FDA continues to evaluate the situation in real time and is committed to re-examining its recommendations for donor testing as additional

information on the Zika epidemic and the safety impact of safety donor testing becomes available. Thank you.

DR. LEITMAN: Thank you very much, Dr. Epstein.

I don't see any hands or lights going off for questions. Is there anyone else from the audience who did not submit a formal request to speak at the open public hearing who would like a couple minutes to address the committee?

I don't see anyone, so let's take a break for 15 minutes and please return at five minutes after 11:00.

(Brief recess)

Agenda Item: Committee Updates:

DR. LEITMAN: The last topic for this BPAC meeting is committee updates, and, to repeat, in this session FDA is not seeking advice or recommendations from the committee. The committee may ask questions of the FDA and speakers, but if the discussion appears to be veering towards advice or recommendations, you will need to stop that discussion, and we will remind you that the FDA is not seeking advice or recommendations on the topic. These are updates only.

The first update will come from Dr. Alan Williams of the Office of Blood at FDA, an update on the transfusion infections monitoring system.

Agenda Item: Update on the Transfusion Infections Monitoring System, Alan Williams, PhD, OBRR, FDA

DR. WILLIAMS: Thank you and good morning. Those of you who were members of the committee in December 2014 will remember discussions in the context of potential revisions of policy for men who had sex with men and at

potential HIV risk, and there was a lot of discussion related to a safety monitoring system for the U.S. blood supply as well as a target discussion on HIV tests. This will be an update on some of the developments that have taken place since those discussions and, specifically, an overview of the transfusion-transmissible infections monitoring system.

Remembering Mindy Goldman's talk from yesterday, while we have an acronym, we haven't agreed on how to pronounce it, so sometimes you'll hear TTIMS, and sometimes T-TIMS and sometimes just TIMS.

The program is designed to be a representative and sustainable system to measure epidemiologic variables among U.S. blood donors that may reflect changes in blood safety. The program has already had a publication which serves as an overview to the program. Brian Custer is the first author and was published in Transfusion just earlier this year.

While the development of a safety monitoring system for transfusion in the U.S. is certainly relevant in the context of major policy changes like we experienced over the past year or two, the development of a system has been under discussion for some time.

Listed here are some of the formal recommendations that have taken place related to establishment of a blood safety monitoring system among donors, the first being the HHS Advisory Committee on Blood Safety and Availability before Tissue was added to its mandated, and that goes back to August 2006. Subsequently, there was an HHS gap analysis white paper on biovigilance in 2009 that also recommended a system.

The HHS committee with tissues added to its mandated again recommended it in 2010, 2013 and 2014. Of course, the BPAC discussed it in

December 2014 without a specific vote but a lot of discussion recommending a monitoring system. And then it was referenced again in the context of revised MSM policy by the FDA Commissioner in December 2014. That is just to document that, in fact, thoughts related to a system have been in place for a long time, and I think the progress just reflects what had been in people's minds for some time.

It is important to recognize the very firm foundation that TTIMS is based on, that was established by the National Heart, Lung and Blood Institute-funded RED-II program, and this was an epidemiologic study of much the same thing, to establish epidemiologic data for blood donors and participating blood centers. Some of the foundations that were created by that program were development of protocols, identification of capable blood establishment participants, establishing the feasibility of standardizing and centralizing large volumes of blood collection and operational data. The rather ominous job of providing consensus test result definitions, because when there are different tests for screening in place and different confirmatory tests in place, often it takes a lot of work to reach a consensus definition that can be entered into a central database, and this was done throughout the REDS study.

And there were data collected for the 2011-2012 period reflecting demographics, TTI markers and risk factor data for both seropositive donors and controls. Importantly, these data were collected in the same centers which ended up being participants in the TTIMS and resulted in data that is, in fact, antecedent to the revised FDA donor deferral recommendations that were published in December 2015.

These same foundations are also relevant to investigations of any

future emerging transfusion- transmitted agents that might threaten the blood supply because no matter how many surprises agents might carry there is always the core need for donor-related epidemiology and samples that could be made available for further testing.

The TTIMS program is funded through a five-year funding contract, and the whole intent is to create a sustainable monitoring system. These were competitive contracts awarded in September 2015 for two coordinating centers. The first is a donor database coordinating center, DDCC. The second is a laboratory and risk factor coordinating center, or LRCC.

In terms of governance, there are two standing committees. The first is a steering committee which is the broader group which has representatives from all of the participating blood establishments as well as comprehensive participation from all stakeholder PHS agencies, including CDC, NIH, FDA and other agencies. The Executive Committee is a smaller group comprised of the PIs for the coordinating centers as well as representatives from FDA and the National Heart, Lung and Blood Institute which provide the primary funding.

The program in its first year developed both protocols and manuals of operating procedures covering the coordinating center, the LRCC and the overall study governance, and these are in place. And all IRB board approvals for proposed programs have been submitted for review and approved, and a certificate of confidentiality for certain aspects of the study has been obtained.

A little bit of detail about the Donation Database Coordinating Center. The American Red Cross was awarded a contract for the DDCC on September 30, 2015, and Dr. Susan Stramer is the PI for that coordinating center. The data coordinating center subsequently contracts to obtain data for other

participants in the program. The Red Cross itself, of course, contributes data and also obtains data from Blood Systems, New York Blood Center, One Blood in Florida and from the central testing site, Creative Testing Solutions.

The scope of the DDCC is to maintain a central database reflecting more than 50 percent of the U.S. blood supply so as to monitor Hepatitis B virus, Hepatitis C virus and HIV markers in U.S. blood donors. And the DDCC is responsible for sponsoring and adhering to consensus test result definitions, providing validated data exchange between the data collectors and the central database, and producing quarterly and annual data analyses related to the prevalence in donors, prevalence on a donation basis, estimates of incidence -- which I'll say more about in a few slides, but basically there are various ways to arrive at incidence including NAT-only donation samples called NAT yield, repeat donor seroconversion and HIV antibody recency analyses.

The study will also produce residual risk estimates for safety of the blood supply. This is a function of incidence and window period.

Some of the specific functions of the DDCC you will see illustrated in the next slide, but just to point them out in a tabular basis, DDCC receives and centralizes daily test data from the American Red Cross national test labs and the other centralized testing, Creative Test Systems testing facilities. It receives monthly donations -- that is, denominator data -- from all participating sites. It receives and shares daily lists called PIC lists of potential positive donors from blood centers, Creating Testing Solutions and the LRCC. It shares donation data from positive donors with the laboratory site, the LRCC. It logs results of additional research tests received from the LRCC and establishes linkage of follow-up test results to indexed donation data.

This is shown schematically on a slide received from the DDCC. I won't go again through all these relationships but the schematic will show the directionality of some of the data flow. I think one of the main messages is it's both comprehensive and very detailed in terms of how data are shared across the study. This has all been developed and is currently in place.

Progress made so far -- The DDCC has produced a protocol for its activities as well as a more detailed manual of operating procedures. The data transfers between the participating sites are now operational. Data comes in to a large master file and then, after quality control processes, ends up in the permanent study file available for analysis, and all of these data transfers and data report generation are being developed on schedule as specified by the contract.

Some additional ongoing work which I think gives a little bit of a window into the complexity of the study -- In addition to processing new data which come in there needs to be integration of new data with relevant pre-existing data. For instance, for a donor of interest, the blood center may well have a prior record, and those all need to be pulled and integrated into an analyzable format.

There needs to be linkage of follow-up sample results to original sample results in the database. At some point when analyses occur, there needs to be an adjustment for different dates of policy change -- for instance, an MSM donation policy change -- because they may occur on different dates at different blood establishments. In fact, that is already the case with the MSM policy change.

The DDCC also is responsible for reporting quarterly data

summaries and working with the LRCC and the Executive Committee to compose and conduct relevant targeted analyses and report those.

Moving to the Laboratory and Risk Factor Coordinating Center, this is a contract that was awarded to Blood Systems Research Institute in September of 2015, and then it was expanded to make it more flexible for addition of potential future studies a year later in September 2016. The LRCC makes use of data and samples, again, contributed throughout the study both from Blood Systems itself, from the American Red Cross, the New York Blood Center, from One Blood and from Creative Test Solutions.

The scope of the Laboratory and Risk Factor Coordinating Center is, first, the risk factor interviews which will be used for seropositive donors for HIV -- all HIV-positive donors -- and Hepatitis C virus-infected donors who have evidence of new or incident infection, and Hepatitis B-infected donors who have new or incident infection.

The risk factor data from donors will be correlated with marker data from those donors and, as a group, will be compared with correspondence markers and risk factoring data from other time points that might be available from elsewhere within TTIMS or prior REDS-II marker data.

The LRCC will also be developing a bio-specimen repository for all the samples that are received with the intent of having this available not only for TTIMS investigations but also future sharing with investigators and availability for panel reference and that sort of activity, because these should be highly valuable samples.

The LRCC will also be conducting state-of-the-art laboratory studies which are detailed on the next slide. Both the HIV and hepatitis samples

will be assessed for viral genetic sequence to produce a genotype assessment as well as drug resistance assessment. Donor HIV antibodies will be looked at with assays capable of characterizing a recent infection. This is work that is far advanced for HIV and several assays are available for doing this recency testing. There also is a possibility now of doing antibody-based recency testing for Hepatitis B and Hepatitis C.

TTIMS will provide samples for pilot studies of stored donor materials to assess the performance of recency tests in a blood donation setting, and then, assuming that these results which are anticipated based on population studies are valid in a donation study, it is hoped that these recency analyses could be used to estimate infection incidence with a stronger level of power than one might get from some of the other measures of incidence because the window of measurement is a little bit larger.

One potential application would be to look at incidence among donors before and after a policy change. This was discussed by the Blood Products Advisory Committee in December 2014, and the committee generally supported use of recency tests for this purpose.

To summarize the LLRC functions, the LLRC will receive donation samples from the Red Cross and CTS, distribute these samples to the core laboratories for testing, establish the repository, receive interview data from participating blood centers, maintain databases for interview data and research bio-informatics, and work with the TTIMS data coordinating center and Executive Committee to propose and conduct targeted analyses.

Similarly, here's a schematic for the LRCC which shows these inter-relationships within that part of the program. I won't go through this in detail

because the functions were listed on the prior slide.

The risk factor interview itself is of note because this type of study has been done many times, and the questionnaires used for risk factor assessment have evolved over time. Even with the late development of the REDS-II interview instrument, the LRCC has modified the TTIMS interview questionnaire to capture new potential categories of use.

The first would be the inclusion of transgender categories capturing an employment field, asking the question about monogamy, refinement of sexual risk exposures, questions about pre and post-exposure, prophylaxis and antiretroviral therapy, because in fact, there have been, at points in the country, donors who have been found to be on pre-exposure prophylaxis and discovered subsequently to have acquired -- or had that particular exposure at the time they were interviewed.

The questionnaire will be administered online and, as with the REDS-II study, the languages of administration will be both English and Spanish.

With respect to the questionnaire, this is the only area of the study which still needs federal approval. The OMB 60-day notice was published as of September 30th, and we are hopeful that within a period of months the OMB will have the study approved and it can then move forward.

Within TTIMS, a shareable bio-specimen repository has been established. This will contain both current and historic HIV-concordant positive plasma samples for validation of potential measures of infection recency, and all HIV and HCD NAT yield and HBV NAT yield plasma samples will be part of the repository for molecular surveillance work.

I wanted to say a word about outcome measures within the study.

Being a safety study, one would want to be able to change or determine over time measures of safety reflected by donor marker incidence and prevalence. Prevalence is of somewhat limited usefulness because although it's easy to measure and is fairly stable over time, it reflects infections that have been detected by screening and removed from the blood supply so that prevalent infections are no longer in general a threat to blood safety; whereas, incidence or new infections reflect the potential for a window period that could be missed by a screening test.

Currently, there are several ways to measure incidence. The first is through NAT yield, as mentioned, which is NAT-only samples before the development of antibody, reflecting very recent infection. The second is seroconversion, which is a data-based measure for repeat donors when one would document a negative infection followed by a positive infection in the same donor at a later time. And, finally, recency analysis, which, as mentioned, is based on specific antibody characterization studies which denote a period of time within which an infection likely occurred -- potentially a period of months up to a year.

Another outcome measure which will be derived is a residual risk estimate among donors derived from some of these other measures. Importantly, the risk factor profiles will themselves be a potential outcome measure not only for seropositive donors but for control donors who are interviewed, because, in fact, one can detect some level of risk generally in control donors interviewed in a blood donation setting so that differences in time between the control donors could also be a potential outcome measure.

This illustration is just prevalence over time. This is actually from the REDS-II publication authored by Roger Dodd, who is here, and it shows rates

starting with .248 per 10,000 in 2011 through quarter four of 2012 with .28 per 10,000. You can see it's a relatively stable curve. So that is statistically showing a difference in time in relation to the change in policy. If a change really is there, it is reasonably straightforward from a statistical basis.

Contrast that with NAT yield cases. This is an updated slide provided by Dr. Stramer based on Red Cross NAT yield cases where the NAT yield cases per year between 1999 and 2016 range from a low of one to a high of 11. With this kind of variation over time, you can imagine that finding a statistical difference in time, if it's really there, would certainly take a long period of time to reach significance. That's why the power of something like recency analysis is potentially important to help increase the statistical power of potential analyses.

By way of acknowledgments, there are many people already associated with the study. Many of these folks are investigators who have been in the field for 20 or more years, so the study was actually developed to a very sophisticated level very quickly. The Executive Committee is comprised of Steve Anderson, who is the Chair and with FDA; Susan Stramer from the American Red Cross; Brian Custer, who is the PI for the LRCC who is with Blood Systems Research; Simone Glynn from NHLBI, and myself.

Also, we certainly want to acknowledge -- and it will be a long list of names when we do publish it -- all the TTIMS participant at the U.S. blood organizations who have really worked very hard to help put this together. And we want to acknowledge support by the FDA, by the National Heart, Lung and Blood Institute and by the Office of the Assistant Secretary for Health through Jim Berger.

The final two slides are to draw attention to a Federal Register

notice that was published recently and the docket number is listed. It can be found very quickly simply by doing a search on the docket number. This is a request for comments from the FDA related to blood donor deferral policy for reducing risk of human HIV transmission by blood and blood products.

It establishes a public document which one can submit comments to and is specifically seeking scientific evidence such as data from research regarding potential blood donor deferral policy options and specifically including the use of individual risk assessments as opposed to a time-based deferral for risk. It also requests suggestions as to design of potential studies to evaluate the feasibility and effectiveness of such alternative deferral options.

Once received, FDA will take the comments received into account as we continue to reevaluate and update blood donor deferral policies based on new scientific information. Additionally, through TTIMS and other studies that might be developed, the comments could well serve as a basis for consideration of future scientific studies on the topic.

The docket was opened on July 28th of this year and closes 11/25 of this year, so there is still time to get materials in. Of course, FDA will receive comments at any time but there are major advantages to getting comments submitted through the document because that has an established process.

With that, I will stop. Thank you very much.

DR. LEITMAN: Thank you very much, Dr. Williams.

The next speaker is Dr. Jaro Vostal of the Office of Blood at FDA, and Jaro will address a summary of the FDA Workshop on Preclinical Evaluation of Red Cells for Transfusion.

Agenda Item: Summary of the FDA Workshop on

Preclinical Evaluation of Red Blood Cells for Transfusion, Jaro Vostal, MD, OBRR, FDA

DR. VOSTAL: Hello, and thank you very much for the opportunity to present to you the summary of a workshop we had recently. This workshop took place in October at the NIH campus, and it focused on the preclinical evaluation of red cells for transfusion.

The objective of the workshop was to discuss new methodologies for preclinical evaluation of the safety and efficacy of red blood cell transfusion products. We had sponsorships from NHLBI at NIH, Department of Defense, the Office of the Assistant Secretary of Health and also the FDA, and we appreciate the effort these entities provided for us.

What are the reasons for taking a look at updating the RBC evaluation process for the FDA? It turns out that FDA has been applying the same criteria for approval of red blood cells for transfusion for about 30 years. During this time, our common knowledge about red cell function has really advanced significantly, so it's about time for us to reconsider whether our process should be updated as well.

In addition, there have been some recent animal studies and some clinical trials that indicate that transfusing cells that meet current approval criteria can sometimes cause harm to the recipients. As far as we know, the current testing does not identify changes in red cells that mediate these adverse events, so we're trying to figure out whether additional testing would be able to provide some insight into these connections.

Furthermore, there are new transfusion products that could soon be available and the current testing may not be able to identify any loss of red cell

efficacy or any changes that could introduce unanticipated toxicity. Some of these products that we anticipate are the extended storage of red cells, pathogen-reduced red cells and, also, stem cell-derived red cells.

The workshop started off by going over the background for the use of the red cells for transfusion. We had talks that summarized our current understanding of the role of red cells and delivery of oxygen to tissues. We heard that the delivery was the sum of many parts including the cardiovascular system and the red cells, and the new insight that was presented is that the red cells actually can influence the cardiovascular system.

We had talks that summarized the history of red cell storage and the realization that there's a generation of a storage lesion that encompasses biochemical and morphological and rheological changes. We had a talk that summarized the current red cell use, and this talk highlighted the decline of red cell use in transfusion. As an example, in the years 2011-2013 there was about a 12 percent decline in red cell collection. In order to deal with these changes, blood banks have changed their storage strategy to move it from just in case providing red cells, to just in time.

Let me show you a few highlight slides that were presented in these background talks. This was a talk by Dr. Allan Doctor. He talked about the new realization that the red cells can actually influence perfusion of hypoxic tissues. So it has now been recognized that oxygenating hemoglobin can also bind nitric oxide and that being oxygenated actually drives the uptake of nitric oxide. The red cells can actually produce derivatives of nitric oxide, and when those red cells are then coming through tissue that's hypoxic where the oxygen is unloaded from hemoglobin, this also drives the release of nitric oxide derivatives and these cause

relaxation of the blood vessels and, thus, the red cell can actually control perfusion of hypoxic tissues.

The suggestions is that if you're going to try to evaluate effects on red cells caused by storage or processing, we should be looking beyond the ability to just bind oxygen and there may be other processes that are involved in oxygen delivery.

This is a slide from a talk by Dr. Jim Zimring. He talked about the history of blood collection and red cell storage, and he summarized the changes that have been recognized in red cell storage that include changes in metabolites, protein chemistry, redox biology, the cell surface biochemistry. All these changes are also reflected in the pictorial description of the morphological changes in red cells that they go through as they're stored through the end of their shelf life.

This is a slide from John Hess' talk on the use of red cells in the U.S., and he points out the changes in the strategy of blood banking, at least in his center, the Puget Sound Blood Center. You can see that back in 2003 there were a lot more red cells that were cross-matched than were transfused, and over the last 15 years this has changed, particularly in the last two or three years where they have altered their strategy and now they try to match the number of cells available to the number of cells transfused.

The next part of the workshop focused on what we are doing currently to evaluate red cells. The advances in red cell storage have been made based on optimization of the biochemical energy states, and we have been focusing on ATP and 23DPG during storage and, also, the retention of red cells in circulation. This is evaluated by radio-labeling studies in healthy volunteers.

FDA has accepted this approach, and we used a maintenance

biochemical function in *in vivo* survival as a basis for approval for new red cell products. We have been doing this since 1985 when the 75 percent *in vivo* recovery at 24 hours was initially adopted. It was pointed out during these sessions that there are some gaps in this approach. Particularly, it was pointed out that the current preclinical process does not evaluate the effects on oxygen delivery potential or development of red cell-mediated toxicity.

The session that followed that was on the new methods that are available to test red cells and the quality of red cells. We started off with several talks that covered omics, the science of omics like proteomics, metabolomics, lipidomics and systems biology that can use this data to generate hypotheses. Together, this is a very powerful methodology that can catalog a large number of biochemical and genetic changes in processed and stored red cells.

These changes, in order for them to be of practical use, need to be correlated with clinical outcomes in transfused patients which is going to take clinical trials. The predictive utility of specific markers for clinical outcomes is complicated by the genetic variability of the donors, the collection and processing effects, and variability in patient conditions at the time of transfusion.

This slide highlights the complexity of the data that is being generated currently. This is a slide from Dr. D'Alessandro, and it's a metabolomics readout from red cells that were stored in AS3 for up to 42 days, and you can see that a change from blue to red is an increase in level of metabolites and red to blue is a decrease in level of metabolites. The complexity of this system is that the number of metabolites that can be followed is overwhelming, and if you think of this as a haystack, there's a needle in there somewhere. The problem is we don't exactly know what the needle looks like, so

it makes the whole discovery process even more difficult.

Also in this session we had a talk by Dr. Aker, and he talked about the testing that can be done in the clinical laboratory. What you see here is a number of tests listed. Some of them are relatively new technology. The new technologies are highlighted by the pictures of the different devices on the side. His point was that the tests that are highlighted in blue are what we're actually using currently for evaluation of clinical products, and we are not really looking at some of the other things that are going on in the red cells during storage.

This processing issue was highlighted with this slide that Dr. Aker put up. This is a study that was conducted by Nancy Heddle and reported in Lancet Hematology. What this study shows is that processing of cells can actually affect -- There may be something in the processing of blood cells that could be correlated with adverse outcomes. In this study, they compared fresh red cells stored for less than seven days prepared by whole blood filtration, and these cells are associated with a higher risk of in-hospital mortality than transfusion of middle-aged, stored red cells, ones that have been stored for 8 to 35 days.

We have been concerned about the quality of red cells declining during storage and we were trying to figure out what are the markers of the decline. This study points out that there could be problems with red cells that we're introducing with the processing that's available today and sort of points out the urgency of trying to figure out what's going on with the red cells because this correlation is with mortality of patients.

After the session where we talked about new technologies we moved on to discussion of animal models for evaluation of oxygen delivery. A number of models were presented; I have summarized some of them here. These models

have been developed to identify toxicity issues and sometimes efficacy issues in red cells. The guinea pig has been used with a transfusion exchange of stored red cells and is highlighted for potential for renal damage that's related to free hemoglobin toxicity. There was a talk on a humanized mouse, which is a nude mouse transfused with human cells. The stored red cells are associated with low oxygen saturation and accumulation of the red cells in the lungs of the animals. The interesting aspect of this animal is that you can actually visualize the blood flow and interaction of the blood with different cells through intravital microscopy in live animals.

We went on to discuss a hamster and a hamster microcirculation model. An interesting aspect of this model was that it reported that an increase in hematocrit actually causes an increase in blood viscosity and reduces the blood flow in the animal and reduces oxygen delivery.

Finally, we discussed a method that could be applied to live animals as well as patients. This is electron power magnetic resonance, or EPR oximetry. This method can measure oxygen directly in tissues and it could be applicable to live animals as well as humans. There is already some clinical data available and this may be something that we will see more of in the future.

Then we moved on to a specific animal model to model a specific transfusion situation, and that is resuscitation of shock trauma patients. This is of specific interest for Department of Defense, so they had two presentations. One was on non-human primates, and this is a very useful model because it can receive human transfusion products for evaluations. The results from these studies can be directly extrapolated to humans. However, working with non-human primates raises highly complex logistics issues, particularly regulatory

oversight, and there is a very high cost associated with these animal models.

More frequently used is a swine model. This has been well studied. It is hemodynamically similar to humans and is a lot more cost-effective compared to the non-human primates. However, there are problems because you can only use porcine blood which may develop different storage and processing lesions compared to human blood.

After the animal model section we had a number of talks that focused on red cell-induced toxicity after transfusion. I borrowed a slide from Dr. Paul Buehler and his talk to summarize what has been going on.

What he highlighted was that during storage there is release of free hemoglobin that combine nitric oxide, that can release heme and can release iron. All these compounds are then transfused into the patients and can cause adverse events. The severity of those events depends on the clinical state of those patients. In addition, there is also potential for thrombus formation, micro particles and micro-RNA delivery by the transfused unit, and a strong immune response to the transfusions in patients.

So, all of these things are things we need to consider when we are trying to develop different processing or storage methods for red cells.

In summary, we found that the red cells are one part of a complex system of oxygen delivery to tissues that includes lungs, heart and blood vessels and endothelial cells. In addition to carrying and releasing oxygens, the red cells contain systems that influence the function of the vascular system -- and that's nitric oxide and its derivatives. The processing and storage lesions for red cells can impact the interaction of these cells with the vascular system and, thus, have an effect on oxygen delivery.

Our current methods of red cell evaluations focus on cell integrity and biochemistry *in vitro* and retention in circulation, but they do not focus on oxygen delivery and they do not focus on potential red cell toxicity.

There have been new methods developed that we have been exposed to during the workshop to evaluate the effects of processing and storage of red cells. These can look at oxygen delivery and nitric oxide metabolism for efficacy, and for cell toxicities through iron-free hemoglobin on free radical generation. Now, the physiological consequences of changes in these parameters will need to be identified with animal models and then finally confirmed in clinical trials.

Finally, clinically validated red cell parameters for efficacy and safety could be used to better evaluate red cell quality.

My final slide focuses on what we are going to do with this information. Based on what we heard, the research should continue to focus on changes in red cells that correlate with poor clinical outcomes; look at the role of donors in determining the quality of stored and processed red cells; and, also, look at the clinical state of recipients of transfused red cells and outcomes. We think the manufacturers should consider new validated methods of red cell evaluations including metabolomics, proteomics, lipidomics, ektacytometry and micro-particle generations, to mention a few, to better characterize their products so that clinical consequences can be correlated with the new parameters.

In terms of updating the regulatory approach, we plan to review the information that was presented at the workshop, and we're going to consider updating the process to include the new red cell parameters and models that correlate with clinical outcomes.

Thank you.

Agenda Item: Questions for Speakers

DR. LEITMAN: Thank you very much, Dr. Vostal.

These two presentations on updates for the committee are open to questions from the committee members.

I have a question for Dr. Williams. In showing us the TTIMS data on total HIV-positive donations detected, that was through 2012. But two years ago, this committee voted in majority and the FDA implemented a one-year deferral for MSM, men who have had sex with males, instead of a lifetime deferral. Is there an update on that data? This is not NAT yield data but total prevalence of HIV detection in donors presenting in the United States in the past year or two years -- continuing that trend through 2012.

DR. WILLIAMS: I think FDA probably is not in a position to comment on that because we don't routinely collect those data. That's exactly the type of data TTIMS will be gathering, but I know we have major blood center operators who probably can comment on that.

But recognize that not all blood centers have changed policy at this point.

DR. STRAMER: Susan Stramer, Red Cross. I was just going to say the majority of blood centers may not have implemented the MSM change yet. For example, the Red Cross, in getting all of the software changes, all the changes to the DHQ --

PARTICIPANT: (Off-mic)

DR. STRAMER: Until December 12th. So you will see all of these data at upcoming BPAC meetings when TTIMS provides updates.

DR. LEITMAN: Thank you very much.

DR. ESCOBAR: I have more of a comment than a question to what Dr. Vostal presented. I think, as science really has evolved, it's having an impact on the way we are treating patients right now in our institutions, I think mostly academic institutions.

The data that he showed here regarding toxicity and some of the other things especially on the red cells is really having a big impact on the hospitals and academic centers like in trauma centers where we are really trying to decrease the amount of transfusions that we're giving to our patients, absolutely. We even have dropped our threshold for transfusion. We are now down to seven grams per deciliter in hemoglobins. Those are all the things we're trying to do, again, based on this data that I think is quite important.

It seems like it seems to be working. Certainly, we need more prospective studies looking at the effects of all the products that we are giving our patients.

DR. LEITMAN: Thank you. If there are no further questions or comments from the committee, then I will open this part of the meeting to the open public hearing and make the announcement again.

Agenda Item: Open Public Hearing

DR. LEITMAN: We have no formal requests to do presentations at the open public hearing, but I will open it up to comments from the audience and guests and state that both the FDA and the public believe in a transparent process for information gathering and decision-making. To ensure such transparency of the open public hearing session, the FDA believes it's important to understand the context of an individual's presentation.

FDA encourages you, the open public hearing speaker, at the beginning of your written statement to advise the committee of any financial relationships you may have with a company or group that is likely to be impacted by the topic of the meeting. This financial information can include the company or group's payment of your travel, lodging or other expenses in connection with your attendance at this meeting.

FDA encourages you at the beginning of your statement to advise the committee if you do not have such financial relationships. If you choose not to address this issue at the beginning of your statement, it will not preclude you from speaking.

Do we have any questions or comments for the committee from the audience?

(No response)

Hearing and see none, I will now adjourn this 114th meeting of BPAC and thank the committee members very much for your participation and thank the speakers for their participation. Thank you.

(Whereupon, the meeting was adjourned at 12:00 p.m.)