U.S. FOOD AND DRUG ADMINISTRATION

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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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BLOOD PRODUCTS ADVISORY COMMITTEE

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89th MEETING

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THURSDAY,
APRIL 26, 2007

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The meeting convened at 2:00 p.m. at the Hilton Washington D.C. North/ Gaithersburg, 620 Perry Parkway, Gaithersburg, Maryland, Frederick P. Siegal, M.D., Chairman, presiding.

COMMITTEE MEMBERS PRESENT:

FREDERICK P. SIEGAL, M.D., Chairman
JUDITH R. BAKER, M.H.S.A., Consumer Representative
ADRIAN M. DI BISCEGLIE, M.D., Member
WILLARDA V. EDWARDS, M.D., MBA, Member
MAUREEN A. FINNEGAN, M.D., Member
LOUIS M. KATZ, M.D., Non-Voting Industry Representative
HARVEY G. KLEIN, M.D., Temporary Voting Member
MATTHEW J. KUEHNERT, M.D., Member
CATHERINE S. MANNO, M.D., Member
KENRAD E. NELSON, M.D., Temporary Voting Member

COMMITTEE MEMBERS PRESENT: (CONT.)

GEORGE B. SCHREIBER, Sc.D., Member
SIMONE A. GLYNN, M.D., Msc., M.P.H., Temporary Voting Member
IRMA O.V. SZYMANSKI, M.D., Member
WILLIAM W. TOMFORD, M.D., Temporary Voting Member
DONNA S. WHITTAKER, Ph.D., Member

FDA PARTICIPANTS:

DONALD W. JEHN, M.S., Executive Secretary
JAY EPSTEIN, M.D.
ROBERT DUNCAN, Ph.D., DETTD, OBRR
MELISSA A. GREENWALD, M.D., Commander, USPHS, DHT, OCTGT
HIRA NAKHASI

GUEST SPEAKERS:

MICHAEL P. BUSCH, M.D., Ph.D., Director, Blood Systems Research Institute
BRIAN CUSTER, Ph.D., M.P.H., Assistant Investigator, Blood Systems Research Institute
SUSAN P. MONTGOMERY, D.V.M., M.P.H., Parasitic Diseases Branch, DPD/NCID, CDC
SUSAN L. STRAMER, Ph.D., , Executive Scientific Officer, American Red Cross

PUBLIC SPEAKERS:

CELSO BIANCO, M.D., America's Blood Centers
SCOTT BRUBAKER, , Chief Policy Officer, AATB
LINDA FRASER, , Executive Director, Rochester Eye & Human Parts Bank and Secretary, Eye Bank Association of America
BEN MARCHLEWICZ, Ph.D., Program Manager, PRISM R&D, Abbott Diagnostics
BRIAN McDONNOUGH, Vice President for Donor

Screening, Ortho Clinical Diagnostics

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A G E N D A

P-R-O-C-E-E-D-I-N-G-S

(2:01:00 p.m.)

MR. JEHN: I would like to welcome you to this 89th meeting of the Blood Products Advisory Committee. I am Donald Jehn, the Executive Secretary for this meeting. This meeting will be freely open to the public.

At this time, I'd like to introduce the individuals seated at the table. Would the temporary voting members please raised your hands as your names are called. To the left of me we have Chairperson Dr. Frederick Siegal, Medical Director, Comprehensive HIV Center, Saint Vincent's Catholic Medical Center of New York. To the right of me going around the table is Dr. William Tomford, Professor of Orthopedic Surgery, Harvard Medical School. Next, Dr. Simone Glynn, Branch Chief, Transfusion Medicine and Therapeutics Branch, NHLBI. Dr. Harvey Klein, Chief of the Department of Transfusion Medicine, NIH. Dr. Kenrad Nelson will be here shortly. Dr. George Schreiber, Vice President of Health

Studies, Westat. Dr. Irma Szymanski, Professor of Pathology Emerita, University of Massachusetts Med Center. Dr. Donna Whittaker, Chief, Department of Clinical Support Services, Fort Sam Houston. Ms. Judith Baker, Regional Administrative Director, Federal Hemophilia Treatment Center, Region IX. And on the other table, Dr. Louis Katz, our industry rep. He's Executive Vice President of Medical Affairs, Mississippi Valley Regional Blood Center. Dr. Catherine Manno, Professor of Pediatrics, Children's Hospital, Philadelphia. Dr. Matthew Kuehnert, Assistant Director for Blood Safety, Division of Healthcare Quality Promotion, CDC. Dr. Maureen Finnegan, Associate Professor, Department of Orthopedic Surgery, University of Texas Southwestern Medical Center. Dr. Willarda Edwards, President and Chief Operating Officer of Sickle Cell Disease Association of America. And Dr. Adrian Di Besceglie, Professor of Internal Medicine, Chief of Hepatology, St. Louis University School of Medicine.

Committee members not in attendance at today's meeting are Drs. Ballow, Cryer, Kulkarni, Quinn, and Quirolo. I'd like to thank all the members and TVMs for attending this meeting.

Now I have a little rather lengthy statement to read for the Conflict of Interest. Please bear with me. The Food and Drug Administration (FDA) is convening today's meeting of the Blood Products Advisory Committee under the authority of the Federal Advisory Committee Act (FACA) of 1972. With the exception of the Industry Representative, all participants of the committee or Special Government Employees (SGEs), are regular federal employees from other agencies and are subject to the Federal Conflict of Interest laws and regulations.

The following information on the status of this Advisory Committee's compliance with Federal Ethics and Conflict of Interest laws, including, but not limited to, 18 U.S. Code 208, and 21 U.S. Code 355, Section-N.4 is being

provided to participants in today's meeting, and to the public.

FDA has determined that participants of this Advisory Committee are in compliance with Federal Ethics and Conflict of Interest laws, including, but not limited to, 18 U.S. Code 208, and 21 U.S. Code 355-N.4. Under 18 U.S. Code 208, applicable to all government agencies, and 21 U.S. Code 355-N.4, applicable to certain FDA committees, Congress has authorized FDA to grant waivers to Special Government Employees who have financial conflicts when it's determined that the agency's need for particular individual services outweighs his or her potential financial conflict of interest, Section 208, and where participation is necessary to afford essential expertise, Section 355.

Members of the committee who are Special Government Employees at today's meeting, including Special Government Employees appointed as temporary voting members, have been screened for potential financial conflicts of interest of

their own, as well as those imputed to them, including those of their employer, spouse, or minor child, related to the discussions of (1) Issues related to the implementation of blood donor screening infection with *Trypanosoma cruzi*, and issues related to the potential transmission of *Trypanosoma cruzi* by human cells, tissues, and cellular and tissue-based products. (2) Transfusion-related acute lung injury (TRALI); and (3) Issues related to the implementation of blood donor screening for infection with West Nile Virus.

These interests may include investments, consulting, expert witness testimony, contracts, grants, CREDAs, teaching, speaking, writing, patents and royalties, and primary employment.

Today's agenda also includes several updates. In accordance with 18 U.S. Code Section 208(b)3, a waiver was granted to Dr. Adrian Di Bisceglie for discussion of Topic I regarding the implementation of Chagas Testing, and the discussions of Topic III regarding the

implementation of West Nile Virus testing. A copy of the written waiver may be obtained by submitting a written request to the agency's Freedom of Information Office, Room 12A30 of the Parklawn Building.

With regard to FDA's guest speakers, the agency has determined that the information provided by these speakers is essential. The following information is being made public to allow the audience to objectively evaluate any presentation, and/or comments made. Dr. Richard Benjamin is employed by the American Red Cross. Dr. Benjamin received consulting fees from firms that could be affected by the discussion.

Dr. Celso Bianco is employed by the American Blood Centers. Dr. Michael Busch is employed by the Blood Systems Research Institute. In the past, he participated in a clinical trial. In addition, Dr. Busch has spoken on behalf of a firm that could be affected by the discussion, for which he has received a fee.

Dr. Brian Custer is employed by the

Blood Systems Research Institute. He is the principal investigator on a grant supported by a firm that could be affected by the discussions.

Dr. Eileen Farnon is employed by CDC in Fort Collins, Colorado. Dr. Steven Kleinman is employed by the University of British Columbia. He receives consulting fees from several firms that could be affected by the discussions. Dr. Susan Montgomery is employed by CDC in Georgia. Dr. Ravindra Sarode is employed by the University of Texas Southwestern Medical Center. He is the Scientific Advisor for a firm that could be affected by the discussions, for which he receives a fee.

Dr. Susan Stramer is employed by the American Red Cross. She is the principal investigatory on a study from a firm that could be affected. She, also, is a speaker for an affected firm. And Dr. David Stroncek is employed by the National Heart, Lung, and Blood Institute at NIH. As part of his official government duties, he is the Scientific Advisor

for an NHLBI-funded grant on TRALI.

As guest speakers, they will not participate in the committee deliberations, nor will they vote. In addition, there may be regulated industry and other outside organization speakers making presentations. These speakers may have financial interests associated with their employer, and with other regulated firms. 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. These individuals were not screened by the FDA for conflicts of interest.

Dr. Louis Katz is serving as the Industry Representative, acting on behalf of all related industry, and is employed by the Mississippi Valley Regional Blood Center. He receives consulting fees from firms that could be affected by the discussions. Dr. Katz is also the Medical Director for Scott County, Iowa Health Department, who has a contract with an affected firm. Industry representatives are not

Special Government Employees and do not vote.

This Conflict of Interest Statement will be available for review at the registration table. We would like to remind members that if the discussions involve any other products or firms not already on the agenda, for 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 sponsor, products, direct competitors, and firms that could be affected by the discussions. And that's all I have.

Just a reminder, if everybody could either turn their cell phones off or in the muted position, and thank you for your patience. Dr. Siegal, I turn the meeting over to you.

DR. SIEGAL: Thanks, Mr. Jehn. I want to thank Don especially for this gavel,

which we got after the last meeting. I don't expect to have to use it at this one, but I did want to comment on the degree of appreciation that I had for the committee's process at the last meeting, which was extraordinarily interesting, and I think it worked well. So without any further ado, we, perhaps should start. And I gather we're not going to have an update at this point, so we should just go right into the meeting.

We have a number of issues. The first one is prevention of transmission of *T. cruzi*, and our primary issue, apart from the blood, is whether tissue testing should be done, which donors should be involved if there's a selection, and whether certain tissues are regulated by the FDA and others are not. So Topic I is issues related to implementation of blood donor screening for infection with *T. cruzi*, and the potential transmission of *T. cruzi* by human cells, tissue, cellular and tissue-based products. And the first speaker will be Robert

Duncan, Ph.D. of FDA, who will introduce us to the issues related to implementation of blood donor screening for antibodies to *T. cruzi* infection. Dr. Duncan.

DR. DUNCAN: Yes, there it is. So, FDA, Office of Blood Research and Review, is seeking the input of the Advisory Committee on issues related to the implementation of blood donor screening for infection with *T. cruzi*. And a quick overview of the issues, they will be in the area of donor management, product management, and also, design of research studies in the areas that we feel we need more information before policies could be developed. And those are specifically in the areas of strategies for selective screening, for investigation of the cross-reactivity of the licensed test with other pathogens.

I'm going to read the questions right up front, just to focus your attention on the formal questions we're presenting, as you listen to the rest of my background. And we'll come

back and take these one at a time after the Open Public Hearing.

So the first question - Please comment on any scientific issues that FDA should further consider in developing its recommendations on implementation of blood donor screening for antibodies to *T. cruzi*. Number two - What suggestions does the committee have on the design of research studies to validate a strategy for selective screening of repeat donors? Number three - Please comment on the need for and design of studies to determine whether repeatedly reactive test results for antibodies to *T. cruzi* should be further investigated for cross-reactivity to Leishmania, Plasmodium, Paracoccidiodies Braziliensis, or other agents, when the donor lacks risk factors for *T. cruzi* infection, or a test sample is found negative by other more specific tests. So I'm focusing on just a quick overview of the background looking at the key points that I think will guide our implementation strategies.

Trypanosoma cruzi is a small protozoan parasite that's free swimming in the blood, as you can see in that blood smear from an infected individual. The little purple squiggles are the parasite. This pointer is so dim you can barely see it. The kind of infected individual that we're primarily targeting is a person who has a chronic long-term asymptomatic infection.

At this stage, the infection is very difficult or impossible to treat, with severe symptoms arising late in the infection in about 30 percent of the cases. And those severe symptoms can be debilitating, or fatal.

The infection is primarily acquired in the endemic areas in Mexico, Central America, South America, and current estimates are that about 16-20 million people are infected. There's been active work to reduce the prevalence in those areas, so we can look forward to a declining prevalence, but at this point, it's still at this estimate.

The disease can be transmitted

naturally from the feces of an infected insect that are rubbed into the bite wound, or into other open sores, or liquid around the eyes, but it can also be transmitted, as it's in the blood through congenitally, by organ transplantation, blood transfusion, and some cases have been documented for transmission in breast milk. There's also the possibility of laboratory accidents that cause a blood exposure.

The blood transfusion transmission is recognized as a problem in the endemic areas, and generally, there's testing of blood donors in those areas. And there's a general estimate over time that an infected unit, or a seropositive unit, is estimated to have a 12-20 percent chance of transmitting the infection.

Here in the U.S. and Canada, there have been seven cases of transfusion transmitted documented, five cases of solid organ transplant transmission, and rarely, though sporadic, there's some evidence of natural transmission from an infected insect itself within the borders

of the United States. Seroprevalence in the U.S. donor population has been estimated from past studies to range from .01 percent to 2 percent, depending on the proportion in the study population of immigrants. We'll have, I think, much more current data on this as we look at the results of current blood screening in a later presentation.

There's also, for a long time, been an issue of increased immigration, which is potentially bringing more infected people into this country. And that's sort of illustrated by this listing of the documented cases. In almost all cases, the donor could be identified as having a history of being born or living in one of the endemic areas. Although, I want to be sure to point out at this point that these seven transfusion transmissions, and five solid organ transplant transmissions are just the reported cases. There may be many others that have gone unreported, and that's something we have to keep in mind at all times.

This is not the first discussion we've had in front of this committee on the subject of Chagas Disease. It began in 1989. At that point, the Advisory Committee voted in favor of recommending donor screening for Chagas Disease, if a suitable test were available. The issue was brought up again in 1995 with a presentation of the available license diagnostic tests, and at that point, there was not clarity what would be the proper criteria for acceptance of a diagnostic test for blood donor screening. So later in 2002, we came back with a presentation outlining the regulatory pathway and criteria for evaluation of Chagas tests specifically for blood donor screening. And based on that presentation, a number of industry groups got into the act and resulting at that point in December of 2006, FDA approving the ELISA test system made by Ortho, and that system is currently in use. But I want to make an important point here, that though the blood donor screening test was approved, there is no

supplemental test, so for our purposes in this issue presentation, all of the issues will hinge on whether a person is repeatedly reactive in the blood donor screening ELISA.

With the beginning of testing in January of 2007, I'm giving a few numbers that were current on March 27th, just to give an overall perspective of how the donor screening has gone. There will be more current updates in Susan Stramer's presentation. But the general point is that with about a million donors screened, there were almost 200 repeatedly reactive. And that repeatedly reactive rate very satisfyingly landed right in the same range that we saw in the clinical trial that led to licensure.

There has been follow-up testing on those donors with a more specific radio immune precipitation assay, and based on the results of that unlicenced test, there were 31 that were reactive on the RIPA, 36 at that point were still pending. But using those numbers for confirmed

positives, we could make a calculation of specificity of 99.984 percent, which is, again, exactly consistent with what we saw in the clinical trial, and that's satisfying that we're not generating a lot of false positives.

We can also make a prevalence calculation based on those results of .004 percent. This is a little lower than the earlier estimates, and probably reflects the nationwide screening that's going on. It's not all centers across the nation, but it's throughout almost every state in the union.

I also want to make a point about these results that, it's working out that about 20 to 25 percent of the repeat reactives are confirming with the more specific tests. And we consider that a very good positive predictive value, much better than many of the other disease screening tests when they were first put into use. But this implementation is being guided by voluntary industry recommendations that are listed in the AABB bulletin, which is in your

packet.

So now to the issues that we would like to get some input on. In terms of donor management, we are considering whether blood establishments should test all of their donations for antibodies to *T. cruzi*. Universal screening could mean testing every donor every time they report. We're also considering whether there's a potential for selective screening if a strategy is appropriately validated.

We are considering whether blood donor establishments should defer indefinitely, and notify all donors who are repeatedly reactive by the licensed tests. There's also an issue of counseling of donors, and the question is, should we inform all repeatedly reactive donors about the likelihood and medical significance of the infection, and make referral for additional medical diagnostic testing in that case. And there's also a question about proper medical follow-up for cross-reacting diseases. Specific counseling of repeatedly reactive donors with no

apparent exposure or a negative result on a more specific medical diagnostic test for further medical follow-up based on some considered risk factors for these other parasitic diseases, or other cross-reacting diseases.

We're also seeking input on issues of product management. We are considering whether blood establishments should quarantine and label all repeatedly reactive donations, that being the index donations. There's also the question of products from prior donations by donors who test positive. Should they be retrieved, quarantined, and labeled appropriately?

There's also a question about look-back, or tracing recipients of donations, prior donations from donors who test positive. Should we notify consignees to enable notification of recipients in that case? Another issue in terms of product management is autologous donations. And in this case, we're just considering whether to add Chagas Disease as an infectious disease that would fall under already existing

regulation, which states that a blood donation should be tested, an autologous donation should be tested when allogeneic use is possible, or if these units are to be shipped to other centers where allogeneic use is possible. In any case, any repeatedly reactive autologous donation must be labeled biohazardous.

Other questions in product management include, should there be testing of existing inventory once testing of new donors is initiated? And should there be changes in the circular of information be updated to include *T. cruzi* antibody testing? Also, should there be changes in the biological product deviation report and fatalities reports? In other words, to report release of reactive units, or any fatality that results from a reactive unit.

So in areas where we think further research is needed, one is a possibility of targeted screening of repeat donors. The question being, is it necessary for continued universal screening after the initial test? And

what kind of strategies, or what kind of validation of those strategies might be put forward for retesting selected repeat donors. And this, of course, means donors who tested negative on the initial test, when they come back, do they need to be tested again, what kind of strategies would we come up with that? And there's a much more detailed presentation later that will give some more insight on this question.

Another area where additional research may be needed is a possibility of cross-reactive antibodies of medical significance. And there's already some evidence from Ortho Clinical Diagnostics Performance Evaluation Study that there's cross-reactivity with people infected with Leishmania. There were 100 samples from individuals who were suffering from Leishmaniasis that were collected in a non-endemic area, so there's no possibility that they were infected with *T. cruzi*. Seventy-four out of 100 tested positive on this screening test.

There was some evidence of other pathogen cross-reactivity. One person suffering from malaria, who was from Africa, tested positive on the test. That donation tested negative on the more specific RIPA test. There were also two out of five blood samples from people who had antibodies to Paracoccidiodies, another fungal parasite, but these were collected in an endemic area, and they also tested positive on the more specific RIPA test, so we can't exclude the possibility that the individuals were dually infected, but it raises the question.

So some of the brainstorming that CBER has done, along with parasite experts at the Centers for Disease Control would be to test a panel of serum or plasmid samples form individuals well characterized as infected with Leishmania, test them with the licensed *T. cruzi* blood screening assay, so possible sources are the repository of samples at the CDC, or there are other collections of Leishmania positive individual samples that could be sought in the

U.S., or also, the possibility of acquiring additional samples from Leishmania endemic countries.

As another approach to that kind of cross-reactivity study would be to prospectively follow-up for Leishmaniasis, all donors who are repeatedly reactive on the licensed *T. cruzi* blood screening assays. And they could be followed up for Leishmania serology, for other risk factors for exposure to Leishmania, or other forms of medical diagnosis. And it's also possible to suggest similar studies of Plasmodium or Paracoccidiodies.

So that's the end of my background presentation. We will go forward through the rest of the speakers. The next one, Melissa Greenwald, will present the issues from the point of view of cell and tissue donations. We'll also have a presentation on the current testing experience from Susan Stramer of the American Red Cross, a presentation on the public health impact by Susan Montgomery, and then the potential

strategies for targeted testing of *T. cruzi* infection in repeat donors by Michael Busch and Brian Custer. And that will be followed by the public hearing, a break, and then we'll come back to the specific questions.

DR. SIEGAL: Thank you, Dr. Duncan. Are there any questions for Dr. Duncan before we go on? All right. Then let's proceed. The next speaker is Melissa Greenwald, Commander of U.S. Public Health Service from the FDA, talking about issues related to the potential transmission of *T. cruzi* by human cells, tissues, and cellular and tissue-based products.

DR. GREENWALD: Good afternoon. I am from the Office of Cellular Tissue and Gene Therapies. I'm happy to be here today. Thank you for the opportunity. And I'll start off by just saying that we regulate human cells, tissue, cellular tissue-based products, which we call HCTPs for short, because I can't say that over and over several times. Just an overview of what I'll talk about today.

First, I'll start with a question for the committee, just again to focus your attention on why I'm giving the presentation, give a little background. First, I'll talk about HCTPs themselves, and then a little additional background about Chagas Disease, more specific to our products, just building on what Dr. Duncan has already talked about. I'll briefly review literature that was provided to the committee, just the results of those papers, and then a few final comments.

So the question today will be to please comment on the current scientific data as it relates to the potential for transmission of Chagas Disease by HCTPs. Since I want you to comment about transmission by HCTPs, I'm just going to start by letting you know what they are.

It does encompass a very wide variety of products, and it's defined in the regulations as articles containing or consisting of human cells or tissues that are intended for

implantation, transplantation, infusion, or transfer into a human recipient. So some examples of HCTPs include things like musculoskeletal tissues, cardiovascular tissues, ocular tissues, reproductive cells and tissues, and hematopoietic stem or progenitor cells directly from cord blood, as well as other products.

In order to keep focused, though, I need to remind people about what are not HCTPs. Vascularized human organs for transplantation are not regulated by FDA, and the Health Resources and Services Administration provides oversight for those products. Also, of course, blood or blood products, secreted or extracted human products, like human breast milk, certain bone marrow products are not HCTPs, ancillary products used in the manufacture of HCTPs, cells, tissues, and organs derived from non-humans, as well as in vitro diagnostic products.

Like in blood donors, HCTP donors undergo a screening and testing process. The

donors screening includes a medical history interview, a physical assessment in non-living donors, or a physical exam in living donors, as well as a medical record review. Donor testing should be performed using FDA licensed, cleared, or approved donor screening tests, and we also require that specifically labeled tests for cadaveric donors should be used, if applicable, and available.

So all HCTP donors are currently screened or tested for what we call relevant communicable disease agents or diseases. Those include HIV I and II, Hepatitis B, Hepatitis C, Human TSEs, including CJD and syphilis.

We've also issued some recent guidance that describes some additional relevant communicable disease agents or diseases, including West Nile Virus, sepsis and vaccinia. But today's discussion will focus on the current scientific data as it relates to the potential for transmission of Chagas Disease by HCTPs, and thus, the possible need to test HCTP donors for

T. cruzi.

So we'll move on now to the Chagas Disease background. And as Dr. Duncan already mentioned, in addition to vector transmission of *T. cruzi*, it can also be transmitted vertically. There's been oral transmission through breast milk or contaminated food. It can enter via the conjunctiva from hand contamination, and there's been transmission from blood transfusion and organ transplantation.

I'm going to just briefly go over this cartoon describing the *T. cruzi* life cycle in humans, just to sort of get an idea about what it can do. It starts off as a trypomastigote in the blood, and then it circulates and it invades tissues and cells. Once it's intercellular, it converts to an amastigote form that replicates, and then the trypomastigotes are released into the blood stream where they can circulate and invade other host cells, but the trigger for what causes that release isn't fully described.

There have been no reports of

literature of tissue transmission of *T. cruzi*, but I would like to point out that in the United States, there's not been a requirement for reporting transmission-related incidents to the FDA until May 25th of 2005. The reasons, though, why you could surmise why there have been no reports. Association between the tissue transplant and the development of symptoms may not be recognized, because there's a long time between exposure and symptom development in immunocompetent individuals. The acute phase is generally asymptomatic, as well as the chronic phase, the indeterminate phase.

It also just may be difficult to recognize a tissue transplant transmitted infection in endemic areas where there's ongoing vector exposure. There are some other factors, too, that tissue allografts generally undergo some type of processing, and some methods may remove or inactivate *T. cruzi*. I haven't been able to find any published papers specifically describing any of those methods. It's just that

it seems likely that some of them may remove the agent.

Some cellular products are cryo preserved, and also, some tissue products are frozen. There's been one study that showed a parasite may survive two to three weeks at refrigerator or freezer temperatures, but really survival beyond that time is unknown.

There's really scant information about the tissue distribution of *T. cruzi* infected individuals. During the acute phase, parasites are found in skin lesions at the site of transmission. It's spread through the blood stream, and then lodges in various tissues, but particularly skeletal muscle.

During the chronic asymptomatic phase, the parasite has been demonstrated in muscle, especially cardiac muscle, nerve, and the digestive tract. But there's not been a lot of investigation of the distribution within other tissues in humans during this phase. There is a tendency to look at tissues that have been known

to have clinical manifestations, and probably not too many people lining up to have random tissue samples taken. Parasites have been demonstrated in the affected tissues of individuals who die with cardiomyopathy, megaesophagus, and megacolon.

So now I'll just kind of briefly go through the results of the articles that were provided in the packets, mostly mouse studies. In one study, mice were inoculated with *T. cruzi*, and those mice were then looked at their tissue, at both three weeks, and ten months after infection. The three-week mice demonstrated parasites in skeletal muscle, heart, bladder, peripheral nerve, liver, spleen, adrenal gland, brain, and adipose tissues. Those were the only tissues that were examined in that study.

Over the next ten months, the parasite load decreased about 100 fold, but they did still demonstrate visible parasites in skeletal muscle and bladder. In this study, I'd also like to point out that the stain that was

used, according to the authors, should have been identified viable parasites, as opposed to just looking for pieces of the parasite by DNA.

Mice subcutaneously inoculated in another study with *T. cruzi* demonstrated PCR positivity for *T. cruzi* DNA in ocular tissue and surrounding structures, including corneal stroma, and that's important when thinking about cornea donation.

In another study where mice were experimentally infected by injection, the mice demonstrated pseudocysts filled with amastigotes in less than 1 percent of the evaluated tissue sections, but the IHC methods that were employed in this study demonstrated *T. cruzi* antigens in about 11 percent of the inflammatory infiltrates.

This is an old study. It was from 1988, and the point mostly just being that visualization, direct visualization of the amastigotes is relatively insensitive.

Experimentally infected mice demonstrated *T. cruzi* in sternum chondroblasts,

osteoblasts, and macrophages, as well as fibroblasts. The osteocyte and chondrocyte invasion, that study was rare, but cells within the bone marrow were found to be infected in the study.

I also found a human placenta study. Human placentas were collected immediately post partum, and were experimentally profused, and they were profused for an hour. They were inoculated with a large bolus containing *T. cruzi* trypomastigotes, and profused for an additional two hours. And the study specimens were collected of the tissue, as well as the profusate immediately following profusion, and then tissue specimens were also collected after 24 and 48 hours of incubation. And in that study, *T. cruzi* DNA was identified in cells within all the post inoculation placenta tissue specimens.

So moving on to clinical data - there is a study where individuals who had identified chronic Chagas Disease, who underwent endomyocardial biopsy. Examination of those

tissues demonstrated *T. cruzi* antigenic deposits by immunohistochemical techniques, as well as *T. cruzi* DNA by PCR. And in the histopathology evaluation, they found necrosis, inflammatory infiltrates and fibrosis, as well as a few scattered organisms here and there.

There was another similar study where they were doing endomyocardial biopsies that demonstrated a correlation between the presence of *T. cruzi* antigen and the severity of myocardial inflammatory process. And *T. cruzi* DNA by PCR has been demonstrated in esophageal tissues in persons who died of esophageal Chagas Disease.

So just a few final comments. How do HCTPs transmit infection? The infectious disease transmission by HCTPs is complex, and we know probably less - we don't know more than we know. In cases of known transmission of other infectious disease agent where it's been proven that tissues have transmitted, like HIV, Hepatitis B and C, it's really been difficult to

determine whether or not the transmission occurs because of agent within the tissue itself, or because of agent in blood that is in the tissue.

Also, the infectious dose of *T. cruzi* isn't clearly defined in the literature, but is generally believed to be low. And then what activates the organism to mobilize from the intercellular amastigote stage into blood borne trypomastigotes is also unknown, but it has been demonstrated to occur in persons who were infected via organ transplantation.

So, in summary, *T. cruzi* is found in blood and various tissues. And while much is unknown about the potential transmission from tissue allografts, it's still necessary to make public health decisions based upon the best available information. Any questions? Thank you.

DR. SIEGAL: Okay. Next speaker is Susan Stramer, Ph.D., from American Red Cross. She'll talk to us about the Ortho *T. cruzi* ELISA test system experience.

DR. STRAMER: Good afternoon. Thank you for inviting me to speak to you today. So today what I hope to cover are issues regarding the qualification and implementation of an antibody test for *Trypanosoma cruzi*, or *T. cruzi*. What I hope to cover, or what I will cover, and I apologize if this is hard to read in the back, and there are some changes from the handouts. I will review the clinical study that we participated in, the design and the results. It covered the period of time from the end of August in 2006, to the end of January `07, just the day before we implemented the license test. It covered three of our regions, what is referred to as the Western Division, our Southern California region in Los Angeles, our Northern California region in Oakland, and Arizona region in Tucson. And the results were outlined in morbidity, mortality weekly reports. The citation is given on February 23rd.

Then I will cover our implementation, and we implemented, as outlined by the AABB

Association bulletin 0608. I will review our licensed results, IVD stands for our licensed results from the end of January, the day of implementation, through the middle of April.

The data will be presented not only for the Red Cross, but also for Blood Systems, the UBS Centers, and all of the blood facilities that we both test for. So this represents approximately 65 percent of collected blood in the United States. As I mentioned, it's Red Cross centers, United Blood Services centers, 15 other blood centers, and more than 50 hospitals.

I'll cover the distribution of positives in the U.S., and accuracy of our predictions. I will review test performance of the ELISA, the RIPA that has been mentioned, the unlicenced RIPA. I'll cover a *T. cruzi* IFA, Leishmania IFA, and something we call the Special Protocol.

I'll review look-back results, and lastly, I'll cover donor demographics, including our donor risks, and possible autochthonous

cases, which are those that would indigenous or native to the U.S.

Starting with the IND study, following the request of the FDA and Ortho to expand their clinical studies to include areas where *T. cruzi* antibody prevalence was previously documented, and this was required because the pivotal clinical trial yielded zero confirmed positives of over 40,000 donations tested. The specificity of that study was 99.998 percent. So we decided, at least when we discussed this within the Red Cross, what would be a study of sufficient magnitude. We decided upon 100,000 donations to define the study.

We also said as we tested for Chagas before in many of the same regions and had positives, that is, from 1996 to 1998, if we initiated the study and found positives, we wouldn't stop. That is, we wouldn't stop again, and we would continue testing under IND through licensed test implementation. So when we confronted SOCAL, our L.A. region, we decided to

do this as a division, because of the way we manage processes within the Red Cross, so it included three regions for the Red Cross.

We followed FDA requirements for donor-informed consent, which included a specific signature and date required for each donor that was tested. The requirements were difficult, and other blood centers that we asked to participate in the study along with us declined. They said it was a great study for you to do.

So these are the results of our testing in yellow. This represents all sites. Here's L.A., Tucson, and Oakland. These are the numbers of donors who were approached. The numbers of donors who were consented, or actually were tested. The first red line refers to the repeat reactive rates. We had 63 repeat reactive donations for an overall prevalence based on repeat reactivity of one in 2,300. It had ranged from a high in Los Angeles of one in 1,913, to a low in Tucson of one in 6,000. But what's more important is how many were RIPA positive. And

overall, it was one in 4,655, with a high in Los Angeles of one in 3,827, to a low in Arizona of one in 11,000, or one in 12,000. None of these actually would be considered low numbers.

Here you can see that the percentage of refusals varied, but overall, as was repeated in the pivotal trial, where the same type of consent process was used, about 20 percent of donors refused to participate in the study. And looking at the prevalence of one in 4,655, this meant for the testing of 149,000 donations, we would have missed nine positives.

This is a conclusion that CDC had written in a Morbidity and Mortality following the two organ transplant transmissions in February of 2006. And I included it here because it's obviously applicable to the results of our prevalence study. The prevalence of infection with *T. cruzi* in the United States varies by region. It might now be higher than previously thought, especially in geographic areas, such as Los Angeles County, where a substantial

proportion of blood and organ donors have emigrated from Chagas endemic countries.

So how did we implement? As I mentioned, it was according to the AABB Association Bulletin, which was developed by the blood community, the blood industry, in collaboration with the CDC, and FDA, and released the day after the licensed test was announced.

For component management, components from repeat reactive donors are quarantined and withdrawn from the market within three calendar days. That includes the index donation, any prior end-date donation, and we do product retrieval for prior donations, as long as electronic records exist. We also do recipients tracing and testing of recipients, which I will show.

It includes autologous unit release with the approval of the autologous donor's referring physician. Inventory testing we did not do, but the association bulletin did not recommend it. It said it's up to each facility

to assess their own risk.

Regarding donor management, repeat reactive donors are notified and deferred. Supplemental testing is encouraged, although no FDA licensed confirmatory or supplemental test exists. RIPA, we recognize that is Radio Immuno Precipitation Assay, is the most sensitive test; however, no test for Chagas Disease is 100 percent sensitive. But of all the supplemental tests that exist, RIPA is the most sensitive.

We recommended Leishmania testing on supplemental tests unconfirmed, that is, not the RIPA positives, as was done by Ortho in the package insert that Rob Duncan showed the data of 74 cross-reacting samples. We do them in the unconfirmed samples. We did not mention Plasmodium or Paracoccidiodies Braziliensis, which I had to Google to figure out what that was. It's a dimorphic fungus just like histo or Basidiomyces. But, anyway, for those who wondered what that is.

Donor counseling, including donor

follow-up studies are encouraged. There's no donor re-entry, as we know, because there is no licensed test, licensed confirmatory or supplemental test. We refer supplemental test positive donors to a knowledgeable physician. We refer our positives to their personal physician; that is, the blood center can counsel these donors, but it's best for them to have a personal physician. Their physician is listed in the American Association of Tropical Medicine, and the CDC also on their website has a number that you can call for referrals, as well. Also, for example, in L.A. County, there will be some Centers of Excellence that CDC will set up for counseling and treatment of blood donors who test positive.

Recipient tracing from supplemental test positive donors we also do, and recipient testing is included, using the licensed test. And I took the quote out of the Association Bulletin, "The licensed test for antibody detection has suitable performance

characteristics for blood donor screening, and, as such, may be useful in testing the above individuals. Although, there is no diagnostic test claim on the test, we do use this test for this purpose, and also, for family members, that is children of infected mothers who are concerned. A circular of information and component labels that also you may label, but that will come out with the AABB Circular of Information Committee."

So what are models for testing and implementation? These will be discussed today. I mentioned, we did universal, but we discussed all of these as an organization. Should we test for only those like using a CMV model, immunosuppressed patients? We feel this puts the burden on the hospitals to identify the correct units for recipients at highest risk, and from physician feedback we got, this was not acceptable.

Can we use geographic models? And I'll show you one using the U.S. Census data, and

WHO seroprevalence by country, to try to predict where the highest at-risk areas would be. Can we do a one-time only per donor test method, where only new donors are tested, and repeat donors are questions regarding risk, and only the yes responses are tested? Well, we know that this assumes that the donor understands the questions. And from work that David Leiby has done at the Red Cross before, we know that this isn't always true. The questions may be culturally sensitive, and it assumes no native or autochthonous risk. Of course, an alternate strategy must be validated. Each positive requires knowledge of risk, and when it occurred. And for us, the major reason is that it's logistically complex relative to sample tracking and component management. For us, it's far simpler just to test all.

Also, the financial benefit, at least in our system, has not been that - well, it hasn't been validated in any system, and models we looked at, we didn't see any financial

benefit. And, also, I believe it sends a confusing message to test kit developers. Here there's a test that we've talked about at BPAC for decades already. We're sending a message to test kit manufacturers, here's the test, and now we're questioning whether we want to use it, at least universally.

This is the map of our confirmed positives, that is for the Red Cross and Blood Systems. The blue or green here represent states that have repeat reactive donors. The numbers within each state tell you the number of repeat reactive donors. The states in pink, including this pale pink here, tell you which states had confirmed positives. So we have 265 repeat reactives. This is testing over 1.757 million donations. There were 50 confirmed positives, including two that I'll briefly mention, and 224 that were subjected to RIPA testing. When I say "confirmed positive," I mean RIPA positives.

The two in the brackets here represent four that were not RIPA positive, but

I'm including two of the four here as if they were RIPA positive, because these donors did have risk, and they scored very high signal to cut-off ratios on the Ortho test. There are six states that still don't have repeat reactives. It's a combination likely of no repeat reactives, or states that have not yet implemented testing. So the 50 occur in the 17 states, with 19 in California, 11 in Florida, three in Maryland, two in New York, Utah, and Virginia, and then one each in the remaining states. And Arizona is in parentheses here because it includes one of those two questionable donors.

The AABB, just like for West Nile, as I'll show you tomorrow, has also constructed maps for reporting. And this is the map showing repeat reactive donations, which is, for the most part, the Red Cross and Blood Systems entries. But seven facilities are reporting results thus far, with four sites reporting repeat reactive donors, or 272 total. This goes to 4/24, a week later than the data I showed you for the Red

Cross.

Here are the confirmed positives. Again, the states indicated by intensity of color where more confirmed positives occur. California is leading the pack, followed by Florida, as I show for the Red Cross. But here by zip code, you can see where the confirmed positives, in this case 49, reside. This is by zip code of residence. Of the 48 here, this is of 228 for positive predictive values, similar to what Rob Duncan already mentioned, of 21-1/2 percent.

This is the number of repeat reactives by week, and then the number of RIPA positives by week, just showing that there isn't any particular trend we're seeing, pretty consistent number of RIPA positives by week.

So one question is, do the states in which I showed you confirmed positive donors agree with models based on immigration patterns, and prevalence of *T. cruzi* in those countries? So this is a map we put together. It's called a pliograph, so the color and the height of the

state shows you the risk. So in this case, the metropolitan areas of New York City, Washington, D.C., and Los Angeles show the greatest risk in this geographic model, and by states it would be Florida, followed by Texas, and California. Although we haven't seen any confirmed positives yet in Texas, we are bound to. And for the most part, this model does coincide with what we're seeing, not exactly, but we are seeing the highest rates in Florida, and in Los Angeles County.

This is our algorithm for both the clinical trial and the implementation of the licensed test. If the index sample is repeat reactive, it's sent to QUEST, and QUEST is the reference lab that has been trained and signed off by Ortho to do the radioimmunoprecipitation assays. At the same time, we retrieve the frozen plasma or an index retention sample from our blood collection regions. If a RIPA is positive, we take the plasma and we repeat the ELISA, and we repeat the RIPA. This is an algorithm we use

for all studies that we do, whether it's HIV, West Nile. We confirm the index results in an independent sample that hasn't been introduced in the testing lab.

If the sample is negative by RIPA, in addition to doing the two tests I just mentioned, we also do Leishmania antibody IFA. And in the case, when I talk about follow-up, if a Leishmania donor is reactive, Leishmania does get added to the algorithm for follow-up, so this is donor follow-up, recipient follow-up, and any family members who choose to be tested. And family members I'm not going to address, but we've tested very, very few. The most was one mother with her six children, so that was about our family testing pot. Anyway, so we do Leishmania, again, only if the index Leishmania was positive, and we'll talk about that.

Test performance, I've divided the slide into the clinical trial, versus what we've seen since we've implemented the licensed test. Firstly, for the clinical trial, we had 32 RIPA

positives, of 63 repeat reactives, which, for the two states, Arizona and California that we saw confirmed positives, was a PPV of 51 percent. This is higher than what we're seeing nationally, of course, because these were considered more high-risk areas. The repeat reactive rate was higher, again, because these were high-risk areas, .042 percent, overall prevalence was one in 4,655, specificity was 99.979, or almost exactly what it is in the package insert. For nationwide screening, I mentioned already on the map, together Red Cross and Blood Systems has seen 50 RIPA positives, including the two questionable donors of 224 tested, for a 22 percent positive predictive value, with positives in seven states. Sixty percent of those positives come from two states, California and Florida, actually, both Southern California and South Florida, although we've had one positive in the Panhandle of Florida. The repeat reactive rate has been excellent, .015 percent. I indicate to you that that's the lowest repeat

reactive rate of any test we do. Well, it's about comparable to what we're seeing for HIV, HCV NAT.

Our projected prevalence, based on the percent of RIPA positivity that we're seeing, is about one in 30,000, and the specificity of the licensed test in comparison to the test we used in the clinical trial was comparable. This comes from our process qualification that we did the first week of testing. So the overall prevalence, if we put all of this together, is one in 21,000, with a PPV of 27 percent.

Looking at signal to cut-off ratios, everyone wants to know, well, what S to CO value is predictive. If you focus on the non-reactives, they cluster around one, with a mean of 1.42. The positives, however, have a wider distribution, and we see a range that goes anywhere from .93, which yes, is under the cut-off, to .772. We used a gray zone during the clinical trials and did note that three confirmed positive samples had reactivity under the cut-

off, and Ortho will be making a further cut-off change to the existing assay.

Speaking of those three samples, this slide here shows you an example of difficult samples. Of all the testing we did, I'm not showing you high positives that are easily RIPA positive. I'm showing you problem samples. We had three samples in the clinical trial that were under the cut-off. I'm going to talk about what happens when you freeze plasma, and it's not a good thing, because you lose reactivity. This is a follow-up. This is a serum sample. This is plasma. This is follow-up serum sample. Again, for this donor just under the cut-off, the index RIPA was positive, the follow-up RIPA was positive.

In our case, many donors, actually, any donor who we've got to retrieve plasma from, and a follow-up sample, we will have up to three RIPA results to choose from, performed in two different laboratories. The QUEST RIPA, as I mentioned, and David Leiby and the Holland Lab,

performs in-house RIPA, so that's the plasma RIPA.

As I already mentioned, these samples are already highlighted. The plasma drops reactivity, and then we see repeat reactives that flip-flop on RIPA, as well. With the exception of this one donor who was one of our autochthonous cases, who is a donor who I believe is truly positive, all of these individuals have risk. They all come from endemic areas. In fact, this individual remembered being bitten by a reduviid bug. Each of these individuals did live in the type of housing, sub-standard housing that's characteristic of the transmission of Chagas Disease.

So what we did with plasma, we saw that we were losing reactivity, so we took two tests, Test One, and Test Two. These are both screening tests, one licensed, one unlicenced, and we tested all our frozen plasma to see how much reactivity we lose, because we do lose reactivity. And we haven't seen this phenomenon

for any other marker that we test for, so it's new to us. And I'm not sure why specific IgG would lose reactivity, but from 14 to 31 percent of reactivity is lost in frozen plasma. And the package insert actually tells you, you can freeze/thaw samples five times. This represents two freeze/thaws, actually, so only between two-thirds and 84 percent of reactivity is retained in plasma.

Also, with the encouragement of one of our colleagues, Sylvana Wendel in Sao Paulo, he said to me, besides the RIPA, look at IFA, so we looked at the *T. cruzi* IFA as an alternative to RIPA. The RIPA results here in this column represent the concordance between two results, or the agreement between two results, both positive, or both negative. So we sent a panel of 54 samples to FOCUS, who does a Chagas IFA IgG test, and from that testing, we saw - well, the panel of 54 consisted of 24 RIPA positive samples, but only 11 were IFA positive. We saw 16 discordants, the 11 IFA positives are listed

here. Of the 16 discordants, 14 were IFA negative, but RIPA positive, which is disturbing. Two IFA positives were RIPA negative, and these are both here. Both of these are likely false positive donors. This donor here happened to also react with Leishmania, and I'll tell you more about her. So the overall agreement we found unsatisfactory at 70 percent.

Moving to Leishmania testing, all reactive RIPA unconfirmed samples go to FOCUS for Leishmania, IgM and IgG testing. Initial reactives in the IND went, per protocol, and repeat reactives in the license protocol. The test at FOCUS looks at, I mentioned, IgG and IgM to Donovani, Braziliensis, Tropica, and Mexicana, so two old world, and two new world species.

From our IND, 65 Irs, 36 were sent for Leishmania, including 31 RIPA negatives, 5 RIPA positives, that were sent because Ortho wanted to understand more about some low-level positive samples. In the licensed testing, we sent 104 repeat reactive RIPAs to FOCUS for

testing. And I said they are negative to-date, yes, that's RIPA negative to-date; although, we did see some Leishmania reactivity, that I will talk about.

In both the clinical study and licensed test, we've seen now four Leishmania positive donors, all four are very low-level reactive, multiple species, and we believe all four are false positives. And after the discussion at today's BPAC, I plan to drop Leishmania testing, because we believe it adds no value, and it only adds confusion. And if other studies are planned between CDC and FDA, that's great to look at Leishmania cross-reactivity, because it will stop here.

Our four donors are a 17-year old female donor from California, who was *T. cruzi* RIPA negative, but she was the IFA positive donor at a one to 16 on the *T. cruzi* IFA. Her index was just borderline reactive for L. Tropica, and then when we tested her plasma, that was borderline reactive for L. Donovani. She's an

Asian American. She visited her maternal relatives for two weeks in 1996 in urban areas of Brazil. Although her mother and grandmother lived in Brazil for a long period of time, she has no travel risk for L. Tropica, and follow-up testing was negative for all agents in this individual, so she was negative for Leishmania, and negative, except in the ELISA for *T. cruzi*. She was RIPA negative.

We also tested the mother, because we were interested in congenital transmission of either *T. cruzi* or Leishmania, considering that the mother did live in Brazil, and the mother's mother, but the mother did test negative by ELISA, RIPA, and Leishmania for those markers.

We have an 18-year old male, similar case, reactive only for L. Donovani right at the cut-off, no travel risk. A 71-year old female, again no risk, but reactivity to L. Braziliensis. We haven't followed up that donor yet. And, lastly, a 64-year old female repeat donor with 19 total donations, who did have some low-level

reactivity, this time to Braziliensis and Donovani, but this time it was IgM reactivity. We did talk to the donor yesterday, and the donor absolutely has no travel risk, no infectious disease risk, and no contact with anyone who could have had Leishmania, so we believe these likely represent all false positivity.

We're also doing other procedures, including PCR and hemaculture. And that's being done courtesy of David Leiby at the Holland Lab. And in the IND study, of 16 samples tested for PCR, we did have one positive. And based on the low positive results, we are now using what's called a special protocol, where the regions actually, our blood collection regions actually prepare the samples for both hemaculture and PCR, to hopefully increase the sensitivity. However, even with that, thus far, we've only seen one PCR positive.

Now I'll just talk about look-back and donor demographics, and then summarize. For a look-back in the IND, I mentioned we had 32

confirmed positive donors, a mix of 17 repeat donors, and the remaining first-time donors. For the repeat donors, we had 140 prior donations, or 170 components. From those components, they broke down into whole blood platelets, red cells, or plasma. And the numbers in yellow indicate those transfused. One platelet, 38 red cells transfused, and four plasma transfused. Plasma we do look-back and recipient tracing; however, we don't believe a parasite will survive freezing without a cryo protective agent. But of all the transfused components, we've done look-back, or we've had 11 recipients consent. These were all of the red cell - 11 of the 15 red cell recipients. Our platelet recipient, unfortunately, died 11 days post transfusion, and from a review of the medical records, it was related to his underlying disease, and not acute Chagas.

For the IVD testing, so far we have 171 prior donations identified, but only, thus far, to-date, regions have only told us about 108

components. Of those transfused, they include 6 platelets, 9 red cells, and 2 FFP. Of these transfused components, we have one living platelet recipient, actually non-leuko reduced random donor platelet, but I hate to say unfortunately, but fortunately for the recipient, she did test ELISA negative, PCR negative, and the RIPA is pending. And the other red cell recipients that we have tested, two living, have all been negative to-date, and the same is true for our plasma recipients. So putting this all together from 10 RIPA positive donors tested, that is, the recipients, we tested 11 red cell recipients from 8 donors, and from the remaining 2 donors, 5 additional components, so a total of 16 recipients, and they've all tested negative to-date.

Why is this number so low? I will mention that, but what have other studies shown? We know that platelets and whole blood are likely the components or risk. And from studies that David Leiby has done previously that have

been published, only one out of four platelet components did transmit, which was an inadvertent release in Miami. Kirchhoff published some Mexican data relatively recently on transfusion, four of nine, two whole blood, two platelets. I just showed you, we have zero and only one platelet recipient investigated, so that comes out to 36 percent, probably much lower. We need to test more recipients, so this is very early data.

Why not higher? Again, I told you it's because our numbers are very small, but the donor must be parasitemic, and we know donors are only intermittently parasitemic. The parasites must remain viable, and infectious in the component during processing and handling. And we know that parasites are relatively fragile. And acute infections are most frequently recognized in only immuno-suppressed patients, so most patients who receive blood will probably be unrecognized even if they are infected.

Now going to donor demographics, I've

listed, but I won't review from our IND study what we've seen, for first-time repeat male, female, et cetera. The usual break-outs, countries represented. We do send donor surveys out to each positive donor, and those are completed during donor interview with a trained counselor, so here we have 11 of 15 endemic areas represented by positive donors. We believe here we have probably three, one may be a false positive, but at least three autotoctonous cases.

Similarly, I've listed donor demographics for our licensed test donors. Nine out of ten who we've gotten surveys back, were from endemic areas, and again, we have one who is likely an autoctonous case. He's definitely positive, and has never left the United States.

UBS, we've received information on their donors, so I present the same type of information. Again, the same countries of risk, so they represent. One is unknown, but at least eight out of nine have endemic risk, so if you

put this all together, of 328 repeat reactive donors investigated, representing 82 RIPA positives, 40 are first-time donors, 42 are repeat donors, it's about an even split, 53 males to 29 females, about a two to one ratio of males to females. The vast majority are allogeneic donors, two pheresis, three auto, and one directed donor. And the countries represented include - well, for 33, Mexico 13, El Salvador 7, the U.S. 5, Bolivia 3, Guatemala 2, Venezuela 1, Argentina 1, Brazil 1. So that is a total of 28 of 33, or 85 percent coming from an endemic area, versus our controls, which we also survey and question, which 28 of 28 came from a non-endemic area, all from the U.S., except one from China.

I don't expect you to see this - well, you may in your handout, although, it may be microscopic. This just shows risk factors, and there are risk factors, including endemic country, how long has the donor lived in the endemic country, is the mother born in the endemic country, have you lived in a rural area

of the endemic country, including a thatched-roof house, what floors, have you been bitten by a reduviid bug, so many donors do recall this. And then if you have symptoms, and there's a variety of symptoms that we ask for relating to cardiac or GI symptoms. And then there's a second page of these, and you can look at these at your leisure.

Let me just talk to you about our autochthonous cases, and I will mention four. We have one 61-year old female runner. She's a runner. I call her our runner. She's a marathon runner, she lives in Los Angeles, and she runs daily through Griffith Park. This is the only possible risk factor we can cull out of this donor. Griffith Park is a zoo, and other animals in the park have been demonstrated to harbor *T. cruzi*, including Polar Bears, and quite a few exotic animals. In addition, wild animals in the park harbor *T. cruzi*. In California, there are six species of tryamine bugs that are infected with *T. cruzi*. In addition, 18 mammal species

serve as reservoirs.

She lived in L.A. her entire life in high-quality housing. She's traveled outside of the United States only during a nine-year period of time, where she had a time share in Cancun in a modern high-rise building that's very unlikely to have presented reduviid bugs. And she's not a camper, specifically.

Our next donor probably has risk from, she's a retired Vet. She did live in rural areas of Mexico, where she volunteered as a Vet, and she does recall having been exposed to infectious material, so that one may not represent an autoctonous case.

Then we have a 57-year old female who lives in a rural area of the San Fernando Valley. This area is recovering from fire damage, and I mention this because the autoctonous case that was published in Louisiana was also an area of fire recovery, where the reduviid bugs didn't have enough mammal reservoirs, do being in need of a blood meal, they actually went to humans for

their blood meal, so it's interesting, this is a similar area. Although she does have many pets, and even though this is a fire recovery area, she does frequently seen racoons, possums, skunks on her property, and adjacent property, and she also gardens, and is outside frequently. She's lived in L.A. her entire life in high-quality housing. She did have multiple transfusions in 1971 in California, so it is possible she did get infected from a transfusion. She's asymptomatic.

And then, lastly, we have our Arkansas gentleman, who has lived in the United States his entire life. He's had one one-week trip to Nassau. He's completely asymptomatic. He actually called me. We didn't even have to contact him. I picked up the phone and there he was, so in talking to him, the only possible risk that we could determine was the time he spent in Corpus Christi, Texas, where he slept outside for several weeks. Couldn't tell me why, didn't want to tell me why, and I'm not sure I want to know.

So, in summary, we've seen an overall

prevalence, including all the testing we've done, of one in 21,000. This includes 82 RIPA positive donors from 328 repeat reactives, and 17 states during all testing, including greater than 1.9 million donations. Sixty percent of our RIPA positives come from California and Florida. Let me add that Leishmania after today will not be performed. It adds no value, only confusion.

Our look-back to-date has yielded no positives. Sixty transfused components from 278 manufactured from 38 positive donors, 16 recipients were tested from 10 of those donors, including only one platelet recipient. So this shouldn't be alarming that we haven't seen any positive look-backs yet. Donor demographics, 85 percent show traditional risks from endemic areas versus controls that show no risk. And we've seen five possible autoctonous cases, probably three of which are real, time of infection in each case is unknown. And I thank you for your attention, and I will address any questions.

DR. SIEGAL: Thank you, Dr. Stramer.

Any questions?

DR. Di BISCEGLIE: The Leishmania story is confusing, as you say. What was the rationale behind?

DR. STRAMER: Doing it?

DR. Di BISCEGLIE: Behind doing it. Was there a thought that this was - it might be cross-reactivity; and if so, why? Or dual infection, and if so, why? I just don't quite understand why it was done.

DR. STRAMER: We did it to investigate if we were seeing repeat reactivity, that could not be confirmed, what could be the source of the reactivity in the licensed or the investigational test. It really stemmed out of the clinical protocol, and we just carried it forward during the licensed test. Leishmania is a trypanasome, pretty related to *T. cruzi*, so we just wanted to exclude any possibility of cross-reactivity.

DR. Di BISCEGLIE: A follow-up, if I may, Mr. Chairman. The things that these

conditions have in common is hyperglobulinemia. Were other hyperglobulinemic conditions to auto immune disease, rheumatoid arthritis, Lupus, those kinds of things?

DR. STRAMER: In our repeat reactive donors who were RIPA negative, I don't think -- I mean, they all presented as healthy individuals. We haven't done anything for hypergammaglobulinemia or anything else. We could, but at this point, I think we're going to just drop the Leishmania test.

DR. KLEIN: Susan, I have a couple of questions, just to follow-up on Adrian's question. Has anyone used this test on known positives for Leishmania? I mean, do we know how it performs?

DR. STRAMER: I asked that question of FOCUS yesterday.

DR. KLEIN: I'm sure.

DR. STRAMER: I mean, I probably should have asked it months ago, but it finally dawned on me yesterday -- well, all of the tests

- IFA is very subjective, and these tests are all highly cross-reactive, so if hindsight is 20/20, after the clinical trial, we should have just agreed not to continue it, but at least, we're going to stop now.

DR. KLEIN: Do we know what the seronegative window is for *T. cruzi*? How long does it take for these infected recipients to --

DR. STRAMER: I think the shigoma - I'm probably not the best person to answer this question, but the shigoma appears relatively quickly, I believe, after an individual has been bitten. Do I see David Leiby out there? See, David, you came for a good reason. David, do you want to address the question, what the window period is to acute? You're talking about circulating antibody, parasitemia?

DR. LEIBY: I think probably the best example is the paper we published in *New England Journal* on the case of Miami, where we actually tracked the recipient and were able to demonstrate parasitemia, and serologic

positivity. Now, remember, this is an immuno suppressed individual, but as I recall off the top of my head, the parasitemia was about 47, 50 days was apparent, and we did not get sero reactivity, this is by the Abbott test then to 100 days. It's about 50 days.

DR. KLEIN: So it really isn't known. Is that a fair --

DR. LEIBY: Certainly not well known.

DR. KLEIN: And, finally, the freezing story is, of course, a very interesting one. How were these frozen? They weren't flash frozen, I take it. Has there been any study of freezing and reactivity?

DR. STRAMER: No. Well, David has done some component studies, and he's actually doing now another study regarding freezing and the presence of cryo protective agents, because our red cell reserve, and we've had questions as far as what we should do with frozen components. Just like a red cell, *T. cruzi* is an animal cell bound by a cell membrane, and ice crystals will

rip apart that membrane, the same way it will in a red cell, so freezing an FFP is very unlikely to preserve the agent, but freezing in the presence of glycerol or other cryo protective agents, just like preserving red cells, would be expected to preserve *T. cruzi*.

The literature on freezing is very old and very poor, and even in trying to preserve *T. cruzi* in cell banks, well, maybe 50 to 71 percent of viability was preserved, so it's poor recovery post freezing.

DR. KLEIN: I'm also thinking about the antibody that has been an issue.

DR. STRAMER: There's no reason antibodies shouldn't survive freezing. Oh, I thought you meant the parasite. I mean, this is IgG. I mean, these aren't recent sero converters. I'm not destroying IgM. We just never have seen this phenomenon before. We treat plasma, we repeat every infectious disease agent we do, antibody and NAN, and this is the first time I've ever seen -- you know, first I thought

maybe it's an indication that these are really positives, and it's a labile component in these weak reactive samples that is being destroyed by freezing. We used to see that before when I used to work on HIV antibody tests. But in these cases, the RIPAs continue to be positive, the donor continues to be positive on follow-up. It doesn't affect, as I showed you on the IFA slide, side-by-side with the *T. cruzi* EIA values, it doesn't seem to affect samples with high reactivity, but samples right around the cut-off if frozen, the reactivity will disappear. About 30 percent of the reactivity declines.

DR. KLEIN: The cross-reactivity with Leishmaniasis is clearly a benefit. We don't want to transmit Leishmaniasis either, and if this test were able to detect both, that would be good. But I wondered, has anybody looked at the non-repeat reactive, that is initially reactive, that wasn't repeated with regard to cross-reaction with Leishmania?

DR. STRAMER: The clinical trial that

was our protocol. All initial reactives went for Leish testing.

DR. KLEIN: Oh, okay.

DR. STRAMER: And now in the IVD testing, just the repeat reactives.

DR. KLEIN: I guess I missed the results. Were some of them Leishmania positive?

DR. STRAMER: No, only the four positives we had were all repeat reactive on the Ortho test. And can, although, maybe there is some value in trying to detect Leishmania, I'm not sure this is -- if we need to detect Leishmania, I'm not sure this is the way to do it.

DR. NAKHASI: I'm Hira Nakhasi, FDA. I just wanted to address the question, which was, I think, addressed again by Susan. The cross-reactivity is not unusual because it is the lysate, do you remember this? It's a total lysate, and if you look at the genome sequences between Leishmanias and Trypanosomes, there are a lot of common hemalogies, and so, obviously, that

is the cause of that. And, as you pointed out, that it's good to have the cross - to some extent, you don't want to transmit the whole thing.

The question I wanted to ask Sue was, which you tried to answer to some extent, why do you see by freezing and thawing the loss of antibody? Is it because those samples had very low activity, and you alluded to that, and that could be that somehow you lost reactivity?

DR. STRAMER: That's the variability of samples around the cut-off, but some of these did lose considerable amount of reactivity. Now, I'll have to go back and look at equally high numbers, let's say of HIV weak positives, or HCV weak positives that we've collected, and plasma collected the same way, try to repeat the same studies to see if we actually do see that across other pathogens. I've just never encountered it in all the work we've done. And IgG is stable, I mean, it's easily frozen.

DR. NAKHASI: Yes, that's what I

thought.

DR. STRAMER: So when we saw reactive samples with .2 or .4 S to COs, and this was in multiple tests, not just Ortho's test. We looked at another test, I want to make that clear. It's related to the sample, and not the test; although, the RIPA maintained reactivity. So I think we just need to further evaluate this.

I was hesitant upon even putting this in the presentation, because I didn't want to focus on this topic, but it is an interesting finding.

DR. NAKHASI: Thank you.

DR. SIEGAL: Do you have a question?

DR. SZYMANSKI: Yes, I would like to ask you about irradiation of the products, because I understand that that might inactive the *T. cruzi*. And how much irradiation you need for that?

DR. STRAMER: I don't believe irradiation inactivates the parasite. I believe in the case that was just published, the Rhode

Island case, wasn't that an irradiated --

DR. SZYMANSKI: That's right.

DR. STRAMER: But I don't believe irradiation touches the parasite.

DR. SZYMANSKI: Okay. And these antibodies that lost activity, they were all IgGs?

DR. STRAMER: Pardon me? Yes. Yes. Well, the test probably has the capability of detecting IgM, but we're not -- if that's your question, we're not detecting early sero converters. These are IgG frank positives, who have been infected for considerable periods of time.

DR. SZYMANSKI: Okay. Thank you.

DR. SIEGAL: Any other questions?

DR. TOMFORD: Could you say what's happening when your repeat reactive is positive, or you go on to your RIPA test, and it's negative, what is happening there? In other words, your --

DR. STRAMER: What is the

significance of an ELISA repeat reactive, that doesn't confirm? One frustration for testing in blood donors is most blood donors are going to be negative, so these tests have extraordinary specificity. But even with the extraordinary specificity they have, most of the reactives we have will be false positives, with the exception of maybe HBSAG. That's the test we use that has the highest positive predictive value. But we know it's because the tests are designed also to be very, very sensitive, and as Hira just mentioned, they're produced in cell lines, or the recombinant antigens. We do pick up cross-reacting antibodies. What the nature of these cross-reacting antibodies are, are subjects that have been looked at by many, many individuals, and never really identified conclusively. That's why the package inserts lists potentially interfering substances, ANA, other antibodies, hypergammaglobulinemia, other conditions that may cross-react or interfere on the test. But as far as what is the nature of the false positives we

see in this test, who knows? Antibiotics, other viral infections. I mean, we see this with every other agent.

DR. TOMFORD: Secondly, I'd be a little careful about talking about the sensitivity of the *T. cruzi* to radiation, because that requires a lot of research that really hasn't been done.

DR. STRAMER: Yes. In those studies that have been done, and certainly, radiation, intense methods will vary, but there has not been one published to-date yet that has shown reduction in titers.

DR. SIEGAL: And we're running out of time, so please, if you have other questions, make them quick.

DR. KUEHNERT: I just had a quick question in follow-up, as far as the repeat reactives. As you pointed out, the RIPA is not a gold standard, so if you have a repeat reactive that's positive and RIPA negative, you have -- the first positive is a false positive, but it

could be a true positive.

DR. STRAMER: Yes.

DR. KUEHNERT: And I wonder, did you investigate further as far as the donor's history to see if this could represent a true positive that was missed by RIPA confirmation?

DR. STRAMER: And this links to your question. And, actually, the RIPA is not a gold standard. It's not 100 percent sensitive. And we do expect that in some of the RIPA negative samples, that there truly may be truly antibody positive samples. There's just really no way to know at this point, but I believe the majority of them are false positives.

But, Matt, to answer your question, I mean, we did the IFA, and the only two uniquely IFA positives we had had no risk factors. We tested in follow-up. They were negative. The RIPA positives, I showed you that the vast majority of them, 85 percent of them, do have risk factors, if you consider living in an endemic country, or the type of housing that

we're talking about to be a risk factor, bite by a reduviid bug, your mother had Chagas Disease. So we've had all of those in our positives versus no risk in our RIPA non-reactives. And what more can we do for our non-confirmings? I mean, it's just the survey data that we've collected that indicates that 28 of 28 had no risk.

DR. KUEHNERT: So the answer is you picked an IFA that you thought would be reasonable to compare against, but then you had two that were positive, where the RIPA was negative, where you had that particular discordance. And those two did not have a travel history.

DR. STRAMER: Correct. That was one of the 17-year old girls, the Asian American whose mother lived in Brazil, blah, blah, blah.

DR. KUEHNERT: Okay.

DR. SIEGAL: Last question.

DR. KATZ: Yes. Sue, and you might need help from Mike or David, and/or David. There's more look-backs than what you have

referred to here, and I wonder if we can just get some of that additional data into the record now in terms of look-backs that have been done previously, and the test methods that were used to accession the donors that were subject to look-back.

DR. STRAMER: Well, in the studies that David has done before the `96 through `99, and he'll correct me if I'm wrong, but zero of 19 were positive. I believe there was a red study, Mike, correct me, or Steve, correct me if I'm wrong, there was zero of 17 was the number, and whatever I presented today. So, it depends if you can find -- it depends what your denominator is. Is this all components, is it just platelets? So what I just gave you for the zero in 19, zero of 17, and my zero of 16, that's all components.

DR. KATZ: Well, I bring that up because it may give rise to thoughts about other more selective screening strategies; for example, screening platelet donors all the time, but not

red cell donors all the time since we don't transfuse whole blood in this country any more, or mostly. That's less of an issue. But I think it's very interesting that in the U.S., in North America, excuse me, in all the cases reported for which we have identified the component, the transmitter was platelet, not a red cell. Correct?

DR. STRAMER: Well, two of the seven received multiple components. I mean, it wasn't -- they received platelets, many, many components. Five clearly have platelets. Well, one, I think the first one didn't have a component listed. I mean, is unknown. Four out of seven were clearly platelets.

DR. KATZ: Let me say it the other way. We have not definitively implicated a packed red cell in the transmission.

DR. STRAMER: Right. That's right. Anything other than a platelet.

DR. SIEGAL: Okay. Thank you very much.

DR. STRAMER: Thank you.

DR. SIEGAL: All right. Our next speaker is Susan Montgomery, DVM, MPH, from the Centers for Disease Control, talking about the public health impact of donor screening for *T. cruzi*.

DR. MONTGOMERY: Thank you. I'm going to speak to other health considerations, really more from the donor's perspective, and just as a very quick review to introduce my talk, the acute phase of this infection lasts about four to eight weeks, often asymptomatic. People are usually infected as very young children in endemic countries, and are not even aware that they've been infected. Then they move into a chronic phase, which can last from years to decades. About 60 to 70 percent of people may go lifelong without developing any disease from this infection, but those who do develop disease, can have severe cardiac disease or gastrointestinal, very few of them have both.

Any parasitic treatment is most

effective during early infection. There are indications for treating people in the indeterminate or disease phases to try and reduce the morbidity. And I think it's important, also, to remember that there are supportive treatments that can be very beneficial.

Yes, screening does make the blood supply safer. It is going to identify infections, and ideally, these donors are directed to seek care and get care, and the look-back investigations are going to potentially identify transfusion transmitted disease. However, there are impacts in other ways that I'm going to address, starting with the donors, their families, and communities, but also, the healthcare providers, and the public health systems.

These donors are essentially acting as sentinels in their communities. They may be women of child-bearing age. Identifying those women and getting them treated is more important to potentially reduce congenital transmission.

Also, we now know that here are children who need to be screened and tested. The family members and friends of these individuals may have been exposed to similar risk in endemic countries, as well. They're just not donating blood, so they wouldn't come to our attention, otherwise.

Again, these donors, and I'm speaking specifically of the immigrant population from endemic countries, they don't know they're infected. They're donating blood because they believe they're healthy. And, as you know, blood centers are actively recruiting donors from the Hispanic communities. Most of their infections are acquired in the endemic country, not autochthonous; although, as we've heard, there are risks for autochthonous transmission, as well.

Just to give you a sense of the magnitude, of the U.S. foreign-born population, from Latin America there are 33.5 million people, more than half of them are from Latin America, most from Central America. They tend to live

more in the west and the south states, more likely to be aged 14 to 64 years, which is the blood donor population. And, also, likely to be lowered level of education, lower income. These are data from 2003, probably the numbers are even greater now.

The donors perceptions of Chagas Disease are important to consider. People who come from endemic countries, if they have lived in rural areas, may already know about Chagas Disease, and there is a stigma attached with Chagas in many of these countries; for instance, in Brazil, many types of employment, people are actually screened for Chagas Disease, and are ineligible if they turn out to have it. There also is a perception that really there is nothing that can be done if you have Chagas Disease, and that's probably related to the poor availability of drug in these countries, and also, that access to care, particularly in rural areas, is very limited.

The emphasis has been on vector

control, not on patient treatment from a public health perspective in PAHO and WHO's control programs. In the U.S., these are potential barriers that we are considering for people and donors, patients, barriers to seeking care. One of them being that likely, this population is under-insured, or has no health insurance at all. There are obvious language barriers for many of them. Immigration status is a concern. They will not want to bring themselves to the attention of a U.S. government agency, for instance. And there may be employment concerns. Many immigrants are employed in day labor or have jobs where they're concerned about taking time off from work, and this is true not only for immigrants, but for anyone working in the U.S. now with limited insurance. And the disease potentially limits their ability to work.

U.S. Healthcare provider perceptions are also likely a barrier to getting appropriate care. Awareness of the disease is very limited in the U.S. Healthcare providers, and their

training may be minimal and outdated. Chagas Disease is really considered a tropical disease, and so more the tropical disease specialists would know about this. And there have been changes in standard of care. There's increasing evidence that treating people even in the chronic phase has benefit. Advances in cardiology mean there are better supportive treatments, and we now have a much more aggressive approach to treating mothers and reducing the risk of congenital transmission.

There are barriers to providing care in that insurance coverage may not allow the full extent of evaluation that would be indicated. There is no gold standard diagnostic test, and I think some of the issues have already been discussed in relation to the screening assay, but certainly, the screening assay specificity questions make this very difficult. If a physician sees a donor who has received a letter from the Red Cross saying that this person has screened positive for Chagas Disease,

interpreting that in the clinical setting becomes very complicated. Is this truly someone who has Chagas, and now I'm going to request anti-parasitic drug, which is not always tolerated well. These are important questions to the physician, and not easy to answer because we really don't have many testing choices. Also, because of the chronic nature of this disease, it's important to maintain long-term follow-up. If this person is going to develop cardiac disease 20 years down the road, getting that person to see regular evaluations can be a challenge, as well.

For the public health departments, most state and local health departments certainly have very little familiarity with Chagas Disease, and the kinds of disease manifestations associated with it. Chagas Disease is going to rank very low in a public health system's priorities. And as a result, very poorly funded, there's a lack of resources, state laboratories do not have capability for testing for Chagas

Disease. The clinical laboratories, commercial labs that offer Chagas testing use in-house IFAs. They are not -- they're obviously a CLIA approved test, but we have no -- at CDC, we have no real feeling for the sensitivity and specificity of that testing.

There's also a lack of resources in the health departments for providing care, and for actively doing follow-up on family members, getting the children of infected mothers tested, and issues like that. Really, one of the biggest barriers, though, has been that the donors do not seek care themselves, and so they never come to the attention of the health department. Then, again, there are language barriers. Many of the immigrants do not speak English well, and there are likely political barriers, as well, tied to their immigration status.

I thought I would briefly outline the response that we have planned to try and address these many barriers. Obviously, we want to increase awareness and knowledge of Chagas

Disease, and actually, especially in the immigrant community, because as -- although, in rural areas, many people are aware of Chagas Disease, in the endemic countries in the urban areas, there's actually little awareness. And many of the people who come to the U.S. have moved from a rural area into an urban area, maybe as a very young person, and then come to the U.S., and have not become aware of Chagas Disease in their home country.

There are issues with cross-cultural communications, and also, to emphasize that this is a health problem, and not a political issue, not to be tied to their immigration status or concerns related to that.

We also hope to inform blood bankers, healthcare providers, and the public health systems about Chagas Disease, and we're actively in communication with state health departments now doing that.

CDC is the only source for anti-parasitic drug. The two drugs that are used

against Chagas Disease are both not approved by FDA. They're available in other countries. We have Nifurtimox under IND. We are in the process of getting Benznidazole, so a physician who wants to treat a patient actually has to contact CDC to get this particular drug. We're also hoping to establish public health surveillance for Chagas. This disease is not reportable in the U.S. It is now reportable in one state, in Arizona, but not in any other jurisdiction.

We're emphasizing the health communication education aspects in our response. As I said before, we're coordinating closely with state health departments, but we're also coordinating with the blood collection agencies in trying to ensure that appropriate donor counseling and referral practices are in place.

We've been updating our web pages and have them translated into Spanish, but it has become apparent that most of the population we'd like to reach is probably not on the net, so those pages are there, but they're not being