use for the treponemal survival time in stored blood. There are data in the literature but all of it is not very consistent. We also didn't know the proportion of transfused blood components with storage times that would all survival of treponemes. Most people feel that the current storage procedures will not allow prolonged storage of treponemes but that, in some parts of the country, fresher blood is transfused, blood that has not been stored as long. This may vary and we really didn't know the proportion of storage time of the different blood components or of the different units that are used across the country today.

We also did not have data on the component-specific treponemal densities. I think that there are some people who feel that treponemes can survive in platelets, some people who feel that they can't, because of different reasons. And so we felt that we really couldn't come up with good specific estimates for this.

So this is by way of saying that the estimates that we came up with have a lot of uncertainty in them.

[Slide.]

This is the first estimate we came up with which I think I was questioned about so I have done a subsequent one. But since this wasn't a memo that we sent to the FDA, I am going to walk through this.

The first assumptions that we made were that, for each donation, there would be two components. So the 46 bacteremic donors would result in 92 components. The risk from transfusion of these components would range from 0.001 from stored, if it was a stored component, to 0.05 if it was a fresh component. These estimates are not really based on hard data so I can't really provide specific background information to substantiate these.

But if we were going to use these estimates, then we would have a low of less than one transfusion-associated case occurring per year to a high of 4.6 transfusion-associated cases per year.

[Slide.]

After talking with a variety of people in the blood-banking industry, we decided to come up with the worst-case scenario to estimate transfusion-associated cases in the absence of screening. I think this is because the blood-bank industry would assume, and this is what I was told, that if a unit had treponemes in it, that we should assume 100 percent risk.

So we came up with these assumptions. We were assuming inadequate refrigeration or storage, if there was a blood shortage and there was a very short storage time, so inadequate to kill the treponemes.

And then we assumed that there were between 1 and

1.5 risk components per donations. Here we are assuming that, perhaps, some of the components would not contain spirochetes and would not be, therefore, at risk and that if you got one of these units, your risk of getting a transfusion-associated syphilis would be 100 percent.

So, using this estimate, the 46 donors per year, 100 percent risk, and 1 to 1.5 components per donation, one would come up with between 46 and 69 cases a year. So, again, I think we could plug different numbers in here and, again, our assumptions are not very precise, but, just to walk you through some of the process that we used.

[Slide.]

So, in summary, recent changes in surveillance have allowed identification of syphilis cases detected through blood banks or plasma centers. Again, we can't distinguish those. We estimated that, from 1995 to 1998, syphilis screening by blood banks and plasma centers resulted in 927 cases of early syphilis being detected.

Some parameters that we need to estimate the risk of transfusion-associated syphilis from these donors are not really available. Survival of treponemes in the different blood components under current storage conditions will determine whether these cases pose any real risk for transfusion-transmitted syphilis in the absence of screening.

DR. HOLLINGER: Thank you.

Questions? Dr. Linden?

DR. LINDEN: I have one question. Could you please clarify what you considered to be "fresh" because fresh blood, per se, isn't really transfused anymore. What time line did you consider to be fresh and what was stored?

DR. MARKOWITZ: In our first assumption, where we used the fresh, stored by 0.001 and fresh being 0.05, I think that that was probably not a good use of those words. We meant like less storage and more storage. But, in talking with the American Red Cross, it was our impression that there are some units that are transfused relatively soon after they are obtained.

But I can't really speak to that issue. It was suppose to be a range.

DR. HOLLINGER: Do you have a comment about that, Jeanne?

DR. LINDEN: I am still not sure what relatively soon means because I am thinking platelets versus red cells. You are talking three days, two days, one day, five days?

DR. MARKOWITZ: That is why I presented this second analysis where we use a new terminology called inadequate storage because I think that we don't really know how long is long enough to kill the treponemes. So I presented that first slide where we used the fresh and the

stored terminology because we had communicated that previously to FDA and I wanted to walk through how we came up with those calculations.

But I think it is better to use this term inadequate storage because it is my understanding that the data are not absolutely clear what adequate storage is.

DR. HOLLINGER: I thought Dr. Cable, in his article, said something to the effect of five days, or three to five days or something like that, seemed to be sufficient to eliminate the infectivity of syphilis in blood.

Paul?

DR. SCHMIDT: I think those are all very old experimental data when they first started putting citrated blood on the shelf. That was all refrigerated. Into this, you have to build the fact that platelets are stored at room temperature so it is not only a question of time. But that variable I don't think anybody has ever looked into.

I had a question. As you mentioned early on, not all positive serologic tests in donors that are reported to health departments are followed up. We find, for example, that there are certain parts of town they like to follow up and other parts of town they don't based on what they usually think syphilis is due to.

Wouldn't, then, your estimates based on what health departments report to the CDC be lower than the

actual? In other words, there are blood-donor cases that don't get into that system.

DR. MARKOWITZ: I think there are several things that could bias it to be lower than I reported. In addition to what you pointed out, some patients who were referred into the SDT clinic for evaluation if they have a positive serologic test for syphilis, they might be coded, because this is not totally standardized, as source of report being the STD clinic rather than the blood bank.

So I think that is actually a larger issue than the one you brought up. Most of the cases that don't get followed up, there is actually a set algorithm they are supposed to follow. In cities, where they have a lot of syphilis serology to follow up, they focus on high-risk age group, women of reproductive age, and things like that so that they would actually follow up people--much older people, for example, they would decide not to follow up.

DR. HOLLINGER: If I recall Roger's data, it would appear that about 5,000 cases were positive by the FTA ABS in the American Red Cross. If you then double that to 10,000, that is pretty close to the 10,000 or 12,000 that you are reporting per year. I think you have reported 927 per month. Is that right--or 927 per year?

DR. MARKOWITZ: The 927 was over the four-year period.

DR. HOLLINGER: Over a four-year period?

DR. MARKOWITZ: It was a four-year period. That was a four-year period; yes.

DR. HOLLINGER: Sorry. Okay.

Dr. Mitchell?

DR. MITCHELL: So you are saying that your estimates are from 0.09 cases per year to 69 cases per year?

DR. MARKOWITZ: I see you are looking at the earlier estimate. I would say it is from 0, yes, from 0 to 69 cases per year.

DR. MITCHELL: But, in fact, we have seen 0 cases.

DR. MARKOWITZ: This is in the absence of screening. What we are saying here is this is what we would see in the absence of screening.

DR. MITCHELL: Okay.

DR. CHAMBERLAND: Lauri, just another area of potential misclassification in the surveillance database, classification of stage of disease, would it most likely be done stage at presentation and evaluation to the Department of Health as opposed to the stage of disease, let's say, for purposes of discussion, when someone was presenting to blood bank because, depending on the interval of time, and I don't know if you collect data on interval of time between source of report, meaning the time the patient was initially tested, and their definitive evaluation at the health

department.

So is that another potential source?

DR. MARKOWITZ: Yes; that has come up again as to how good are these classifications. I think that, if anything, there will be people misclassified as early-latent cases where they may have been primary, secondary, at the time of donation because of the time lag between donation and full evaluation by the health department.

DR. CHAMBERLAND: Just one other question about the surveillance system. I wanted to ask you if the implementation of the electronic surveillance system was uniform in the country or, for example--because, certainly, distribution of cases is not uniform with the focus in the Southern states.

So is the electronic surveillance system--how is that implementation faring in those parts of the country where you see the most cases because then that factors into this estimation factor.

DR. MARKOWITZ: I don't know the answer to that, how it was roled out. I don't know that actually. But I do know that, in 2000, all except four states are reporting electronically. But, in 1995, during the '95-'98 period, many fewer states were reporting electronically

DR. TUAZON: If you look at your estimate of potentially infectious donors, you have the premise that the

primary and secondary are 100 percent bacteremic. In fact, they are probably not so that is actually an overestimate because I think the secondary or disseminated disease, those are probably 100 percent spirochetemic but the primary actually may not be.

So, in essence, your estimate of 0.09 is probably an overestimate.

DR. MARKOWITZ: Yes; that could be. We based that on maybe congenital syphilis.

DR. TUAZON: Because we really don't know the--

DR. MARKOWITZ: We don't know. We can't say for sure. I could have put a range in there as well in terms of the percent that were bacteremic.

DR. TUAZON: But even those who are bacteremic, we really don't know the infectious dose, how many spirochetes would be capable of causing disease.

DR. MARKOWITZ: Right. And that is why the estimate of 0.001 and 0.05, that was supposed to represent all of these different factors, the density of the treponeme in the component. We didn't really know what to put in there and it was really more of a guesstimate. We sort of got together and said, what can we really use here? But there are a lot of different factors that go into whether or not a unit is actually really going to be a risk unit.

DR. ORTON: I am Sharyn Orton from the Red Cross.

I had a lot of conversation with Lauri and I think maybe I can shed a little bit of light on the worst-case scenario risk factor assessment that she did.

When we discussed the old papers which I went back to, even some of the whole-blood studies that were done as far back as 1927, the only thing that those papers really do is discuss an inoculated concentration of treponeme and the length of time it takes before that inoculation in a unit of whole blood is no longer viable or no longer causes rabid infectivity.

All of those authors state two things. One is that we do not know what the concentration is in a person who is truly infected so you cannot necessarily extrapolate a certain concentration of inoculated material no longer being viable at five days to necessarily what happens in a human.

The second thing is that they do not state that the refrigeration kills the organism. What they state is this is the length of time that it is viable. There has been some extrapolation to room-temperature platelets, but I could find nothing in the literature that actually has a comparison of the organism live, inoculated live, into a component at room temperature and then rabid infectivity studies being done.

The next part that I just want to mention is when

it comes to platelet concentrates, and this actually will impact slightly the study I will be presenting to you, we have, in the transfusion medicine field, always been concerned about platelets because of room-temperature storage for the reason I stated before.

However, the oxygen tension of the platelet bags is dramatically higher than anything a spirochete can withstand for even periods of hours. The literature clearly states that anything above 3 percent oxygen tension, the organization will be dead in a matter of hours.

In the current bags we use, the oxygen tension is almost 16 percent. So when I talked to Lauri, I didn't feel that a platelet is a large risk, if any risk at all, and that I agree that, with primary syphilis, we don't really know the concentration but we have to assume the if a human is spirochetemic, a red cell has 100 percent transmission risk.

So that is basically where we got those. They are worst-case-scenario figures but I think they more clearly represent if a donor walks in and they are spirochetemic, it is closer to 100 percent than 0.1 percent for the transmission. That is where those figures came from.

DR. HOLLINGER: Yes; please?

DR. WILLIAMS: Alan Williams, Red Cross, Holland Lab. Many times, hospital blood banks are structurally

affiliated with clinical laboratories and, because of donor testing, may have the best access to syphilis serology. Can you, in fact, rule out that some of the reports made by blood banks might represent non-donors?

In addition, have you considered the fact that autologous donors may also be tested by both hospital and community blood centers and are known to have higher levels of infectious-disease marker rates, but would not represent a threat to the community blood supply.

DR. MARKOWITZ: We don't have data on any of that because the only thing we get is really this one piece of information that says source of report. So, unless we went back and interviewed, talked to the surveillance people in the state, I think in the time frame that would allow them to remember these cases to be able to follow up on them, I don't think we would be able to distinguish those.

DR. KLEINMAN: Steve Kleinman. I know probably each state does this differently but it might be a useful thing if, if the case is reported to the health department from a blood center or a plasma center, if they could include, as part of their evaluation actually asking these people whether they have been recent blood donors. That might be something that you could prospectively collect over the next year in evaluations done at state health departments.

Because I agree with what Alan said, and from the limitations you gave, we really don't know whether these people--from a coding form, we really don't know for sure whether they were active blood donors or whether they were just an anomaly of the system and the type of reporting.

DR. DODD: Roger Dodd. One other thought here is that we now are required to defer, for at least one year after clinical syphilis, anybody who presents with a history of syphilis, or we have to ask them about a history of syphilis. Potentially, some of these may be reported back without even entering the blood system.

DR. MARKOWITZ: I don't know if there is a mechanism. You are saying that they wouldn't even donate, blood then. But there is a legal requirement for reporting positive serologic tests for syphilis. So that would be much more likely to be reported than if somebody just said they were a case. I don't think there is a mechanism for those patients to be reported.

DR. HOLLINGER: As there is for hepatitis which is very much underreported.

Yes; go ahead.

DR. BIANCO: Celso Bianco, America's Blood Centers. The other part of the equation that would be interesting. You said, in the absence of testing, this would be the risk. But just following what Dr. Dodd just

said, we ask our donors for any history of venereal disease and will defer them for a year if they reveal any history.

So that also would reduce the number of individuals that ultimately, without testing, would be part of your estimate.

DR. MARKOWITZ: I would assume that these cases that made it through and got reported to CDC, I would have assumed that they would have been asked that question and would be deferred so they wouldn't even get into the system; isn't that right?

DR. BIANCO: Possibly. Yes.

DR. HOLLINGER: Dr. Simon?

DR. SIMON: I thought I understood that there were two issues here and from the discussion, I just wanted to clarify. We are dealing with, really, two issues, one is the safety of the blood supply and, if we stop testing for syphilis, will that be impacted, and then whatever public-health benefits we may be supplying by screening the people who come into our centers.

So, even if we decide the blood supply is safe, there is another issue, do we have a public-health benefit; is that correct?

DR. HOLLINGER: Certainly, the blood supply is the issue right now before the committee about the testing.

DR. SIMON: So we are not dealing with the issue

of whether we are performing a public-health benefit by doing this testing?

DR. HOLLINGER: We are dealing with it, but I don't think it is part of the agenda, as I understand it.

Paul?

DR. SCHMIDT: I would hope that we are dealing with the public-health aspects unless the public-health authorities are willing to put up some money for the blood centers to do some of their screening.

DR. SIMON: Maybe Dr. Markowitz can respond to that. Does the CDC and the public-health establishment see a benefit from the reporting they are getting from blood and plasma centers because, actually, in plasma centers, the testing is done for the safety of the recipient because all those products are frozen and it was retained in order to get that information on the recipient and send them for treatment if they were positive.

DR. MARKOWITZ: That is a separate issue, is the benefit for the patient versus a global public-health issue. One of the reasons I showed the slide that had the information on the percent of all of our cases that identified through this kind of screening was to illustrate that it is actually a small percentage.

But it is still something. 1 percent of our early-latent cases were identified through this mechanism.

We have not developed a formal stand on this on whether or not CDC feels that this is a vital part of syphilis surveillance. We don't consider it--we have many other ways that we are trying to enhance syphilis surveillance because of the syphilis elimination initiative that was started last year. There have been a lot of additional efforts being made to try to locate and treat all syphilis in the U.S.

So we don't feel that this should be a major component of the syphilis elimination effort. But it is not that it is not helpful. It is. But it is not a major component.

DR. HOLLINGER: Dr. Boyle?

DR. BOYLE: Just a point of clarification. Early in your presentation, did you say that 50 percent of all new cases are coming from 26 counties?

DR. MARKOWITZ: Yes.

DR. BOYLE: So, with 3,000 counties in the United States, basically 1 percent of counties were responsible for 50 percent of all new cases.

DR. MARKOWITZ: And they are not the same counties every year. That is one of the tricky things.

DR. BOYLE: Okay. Thank you.

DR. MARKOWITZ: I think there are several endemic areas, which is mainly the South, that occur for the bulk of all cases, but then, every year, there are different

counties that are having outbreaks.

DR. HOLLINGER: Dr. Katz?

DR. KATZ: I am a clinical infectious-disease doc so I have to have several jobs to make a living. One of them is I run an STD clinic at the health department so we get the referrals, and my blood center sends in approximately two reports of RPRs for every ten we get from the plasma centers, locally.

I don't know if that holds up nationally, but I think that that is important data that we probably need to have for this type of decision.

DR. HOLLINGER: Thank you.

We are going to take a ten-minute break and then we are going to come right back and start again.

[Break.]

DR. HOLLINGER: We are going to continue on. The next speaker is with the American Red Cross NAT Donor Study. Sharyn Orton?

American Red Cross NAT Donor Study

DR. ORTON: I would like to thank you for inviting me here today to present this data.

[Slide.]

I would also like to thank everybody who was involved with the American Red Cross through the American Red Cross ARCNET Program. This is an epidemiologic group of

four blood centers that do quite a bit of our surveillance work.

I would also like to mention Dr. Hsi Liu who is also here today and who will be speaking shortly from the Centers for Disease Control and Prevention who collaborated with me on this study.

[Slide.]

The scientific question that we started with was do blood donors with confirmed positive syphilis tests have evidence of circulating T. pallidum and, if so, what is the prevalence. Theoretically, these confirmed positive syphilis tests should represent antibody detection to either current or past infection.

[Slide.]

Our hypothesis was the confirmed positive syphilis tests in blood donors do not represent current infection. How did we arrive at this hypothesis?

[Slide.]

There is quite a bit of anecdotal evidence from blood donors who have been notified of confirmed positive syphilis tests. One of the medical directors at the Red Cross pointed out that the largest number of phone calls they get from donors who receive counseling letters complaining have to do with syphilis tests.

In addition, a later study that I did, a survey of

donors, both cases and controls, I got numerous, dozens, of phone calls from these individuals.

Secondly, there is evidence in the literature that in low-risk populations, most, if not all, positive results represent antibody from previous disease or biological false-positive reactivity. This goes quite to the predictive value of the test in this population.

[Slide.]

Next, we know that conditions associated with biological false-positive test results can affect all of the tests currently in use for screening of donated blood and these tests, as Dr. Dodd pointed out earlier, for the Red Cross, are the PK-TP, the FTA ABS and the RPR.

[Slide.]

In order to do our study, we made a few assumptions. Our first assumption was that an individual with spirochetemia is not likely to present as a blood donor, or, associated with that, a blood donor is not likely to present with spirochetemia.

The reason that we made this assumption was that syphilis is a rare disease in the United States. In 1998, the CDC reported an incidence of 2.6 per 100,000 population. This incidence in whites is actually 0.5 per 100,000.

Peak spirochetemia, from the literature, occurs primarily during the secondary phase which almost always

presents as acute symptomatic disease with fever, lymph adenopathy and macropapular rash.

[Slide.]

In addition, there has not been a documented case of transfusion-transmitted syphilis in this country in over 30 years despite the fact that spirochetemia may occur during the primary phase and this phase may be asymptomatic and may be seronegative early in the course of the phase, and transfusion-transmitted syphilis would likely result in a secondary-phase syphilis that should be recognized.

[Slide.]

So our goal was to determine if there was any evidence of circulating T. pallidum in the blood of donors who are PK-TP reactive, FDA-ABS-positive by specific detection of DNA or RNA as a surrogate measure of potential infectivity.

[Slide.]

Our sample size that we used was a target sample of 100 PK-TP-reactive FTA-ABS-positive blood donations. 50 of these would be from donors who were subsequently RPR-positive and 50 from RPR-negative donors. We went ahead and were using existing platelet concentrates from these donations.

Now, this study was started several years ago and, at that time, it was the first of the studies that I did in

syphilis, based on the literature and the concern by transfusion-medicine experts about platelet transfusion temperature storage, we decided to use the platelet concentrate.

As I mentioned to you earlier, we now know that, based on oxygen tension, this product is not likely to be a product that is going to have viable treponeme. So there our concern was were we using a component that was really a legitimate component to use for this test.

There is evidence in the literature that T. pallidum spirochetes are likely to segregate with white blood cells. This goes back to some work that has been done as far back as 1978.

On this slide, you can see the, in the preparation of the platelet concentrate, the yields of white cells are relatively high in the platelet concentrate, only one log lower than in whole blood when you talk per ml. So we felt that, while whole blood would have been a preferable component, we don't have whole blood available to us because of the component preparation and we did have the platelets available.

[Slide.]

The PCR testing that we did for T. pallidum, we did two different tests. Actually, the CDC did two different tests. The first was a specific DNA test using

the polA gene target. This is work that Dr. Hsi Liu did for us. They used capillary electrophoresis and a fluorescent-detection system. It is read on an ABI 310 genetic analyzer and the sensitivity of the test was 10 to 25 organisms per 100 microliters of platelet concentrate extracted.

[Slide.]

The second test that was used was a multiplex PCR test which included testing for T. pallidum. It uses a 47 kD basic membrane protein-gene target for T. pallidum. It is sensitive to ten organisms per 100 microliters of platelet concentrate extracted. In the event that this test would come up positive, another confirmatory test would have been done with individual PCR which is sensitive to one organism.

[Slide.]

The RT-PCR procedure that we used is a 16S rRNA template for reverse transcription of production of cDNA. We used detection by Southern Blot or, more recently, an Agilent Biolanalyzer. This particular test can be quite sensitive, down to 10^{-3} organism equivalents. We consistently considered a one-organism per 140 microliter platelet concentrate extracted for the control as an acceptable run.

[Slide.]

For the DNA, both assays included internal and

external control samples. The positive external controlled samples were provided by my laboratory and were diluted to 50 organisms per 100 microliter from stock T. pallidum, Nichols strain, cultures from Sheila Lukehart's lab at University of Washington.

RNA-positive controls diluted to 10^{-1} genome equivalents per 140 microliters were prepared from the same stock samples. All assays also included negative controls.

[Slide.]

Ultimately, we tested, as I counted yesterday, 101 of each. 101 samples tested negative for T. pallidum DNA by both assays and 101 samples tested negative for T. pallidum RNA. I do want to point out that, in all, more than 101 samples were run. There was an overlap of about 30 samples that had both tests done.

[Slide.]

There were some study limitations, the first being that the optimal sample for detection of T. pallidum is fresh blood for two reasons. The test procedures work better with fresh blood and the concentration of organism is higher in fresh blood.

Also, because we can never prove a negative test result, in a pilot study of this size, with a sample size of 100, and all-negative test results, there is up to a 3 percent chance that there is an incorrect interpretation of

no evidence of infectivity.

[Slide.]

There are differences in findings between this study and a study that is going to be presented today by the CDC and also from some of Dr. Markowitz' information. The study that is going to be presented later has to do with syphilis-infected individuals. I want to point out that the differences in the population here is that these are blood donors. The prevalence in disease in the two populations is very, very different and, therefore, the predictive value expected for the tests is very different.

As Dr. Dodd mentioned results of a case-control study that I did, approximately 50 percent of blood donors with confirmed positive test results report a previous history of syphilis greater than one year prior to the donation that we were addressing.

It turns out about 30 percent of them are repeat donors who have had a previously seronegative. So it appears that we also see some intermittent seroreactivity in those panels.

[Slide.]

In conclusion, we did not demonstrate circulating T. pallidum DNA or RNA in platelet concentrates of PK-TP-reactive FTA-ABS-positive blood donors in this pilot study. This data is not consistent with a diagnostic model.

[Slide.]

Further, it is unlikely that the blood of donors with confirmed positive syphilis test results is infectious for syphilis.

[Slide.]

Last, I would like to thank all of the ARCNET staff, also Dr. Chen from CDC and Sheila Lukehart from the University of Washington. Thank you.

DR. HOLLINGER: Thank you, Dr. Orton.

Dr. Mitchell?

DR. MITCHELL: On one of the later slides, you reported that 50 percent of the blood donors reported a previous history of syphilis. Does that mean that 50 percent did not report a previous history?

DR. ORTON: Yes; that is correct. What we did in this case control is we identified all confirmed positive donors in a time period from four regions that surveys were sent to. We did not have 100 percent response rate. So, of the ones that responded, it was 50 percent. And, yes, 50 percent of the confirmed positives reported no previous history of syphilis; that's correct.

DR. HOLLINGER: Ms. Knowles?

MS. KNOWLES: You said this was an ongoing study, but it sounds like it is now completed?

DR. ORTON: It was completed as of Tuesday.

DR. HOLLINGER: Dr. Schmidt?

DR. SCHMIDT: Confirming from the old literature, DeGowin and Hardin published a book immediately after World War II. Dr. Hardin was the blood officer for the European theater where they collected a lot of blood by civilians, but civilians and soldiers. The quote from that is, "Wasserman-negative blood is more dangerous than that which reacts in the test."

I don't know about the Wasserman test, but the other thing, for the Red Cross, he says, "Physical examination should be performed on perspective donors to detect primary and secondary manifestations of the disease. The male genitalia should be examined particularly for chancres but, in most clinics, the female genitalia are not inspected because of the difficulty of finding the lesions."

DR. ORTON: I think you would have to pay your donor staff a lot more money if you want to do that.

DR. HOLLINGER: Dr. Epstein?

DR. EPSTEIN: How many of the samples tested were from donors with a positive RPR. You said the target was 50.

DR. ORTON: Yes; the first group that were tested with both DNA tests, there were 50 RPR-positives. Even though there was some difference in the actual samples of the RNA, we did 50 as well.

DR. EPSTEIN: Is it fair to deduce that about 25 of them lacked a history of prior treatment?

DR. ORTON: In these individuals?

DR. EPSTEIN: Of the actual 50 that were RPR-positive, how many lacked a treatment history?

DR. ORTON: We have no information on the individuals from the samples for the DNA and RNA testing. The case-control study was done on an entirely different group of individuals so I have no information on the RPR-positives. Are you talking about the case-control study, how many were RPR-positive?

DR. EPSTEIN: No. What I am trying to get at is that if we think that the bacteremia may be in the fairly acute untreated patients, with respect to donors, then the piece that matters here is what percent of RPR-positives who lack a treatment history were in the tested cohort.

DR. ORTON: I have no information on the actual donors themselves from the tested cohort.

DR. HOLLINGER: That would have been very important, wouldn't it? It seems like that is a very important piece of information. You have done this study to look at this. I would think you would want to know that.

DR. ORTON: At the time, we knew absolutely nothing. So we didn't know if we would even find positives. So this was, indeed, a pilot study just to find out whether

we would detect any DNR or RNA at all.

DR. HOLLINGER: But we still don't know anything because we don't have the information that is critical.

Dr. Nelson?

DR. NELSON: Isn't the perinatal data persuasive? These perinatal cases are extensively investigated with regard to treatment, with regard to stage, and there is some transmission in the latent stage so that, irrespective of what the DNA results are, it suggests that transmission is possible during the latent stage in the absence of treatment. Isn't that true?

DR. HOLLINGER: Dr. Kleinman?

DR. KLEINMAN: I have two questions, Sharon. The PCR assays; you didn't tell us anything about their validation. I don't know much about T. pallidum strain differences but if they have been substantiated across different strains, if, in fact, they have been applied to cases of secondary syphilis and do, in fact, yield positive results, as you might expect them to, is that going to be presented by the CDC speaker? Or, if not, can you tell us something about it?

DR. ORTON: The RNA procedure that we do at the Holland Lab was a procedure that was very extensively validated by Sheilah Lukehart. It is used on spinal fluid. She also used it on whole blood, a variety of stages, of

phases, as well.

Hsi, if you want to comment.

DR. LIU: This is Hsi Liu from the Center for Disease Control. In terms of the PCR, I would say that we have performed extensive sensitivity and specificity studies on the DNA polymerase I target that we used for detecting T. pallidum.

The reason we selected DNA polymerase I is that this is a relatively conserved within T. pallidum. The target we selected, later on, Dr. Markowitz will talk about the two unique features. But I would just stress that not only the sensitivity and specificity and, also, would stress that we can detect approximately one organism per PCR reaction, which is correlated very well to what Dr. Orton's present, approximately 10 organisms per 100 microliter of the sample.

Did I answer your question?

DR. HOLLINGER: Do we have any knowledge at all about how many organisms are present in an ml of blood in various phases of infection?

DR. LIU: This is a question probably in the mind of all the committee members, how many organisms are there. The answer is we do not know. However, because the sensitivity of our test is close to about 10^3 to 10^4 organisms per milliliter of blood, and later on you will see

that we were able to detect some of the organisms, therefore, therefore, there must be that many organisms in the blood circulated in the patients.

Does that answer your question?

DR. HOLLINGER: In a way. In the plasma industry, because of the development of infections, periodically panels have been made, seroconversion panels, and things like this. Are similar kinds of things available for syphilis seroconversion? Do we know, for example, in the plasma industry, what is the number of patients who have some sort of seroconversion from RPR-negative to positive, during follow up?

DR. SIMON: I don't know. We haven't collected that data.

DR. HOLLINGER: But the tests are done on all the individuals who have plasma donations; is that correct?

DR. SIMON: It is done on all donors when they initially present the first time. And then it is done on all donors every four months in conjunction with their serum protein electrophoresis.

DR. HOLLINGER: What number seroconvert?

DR. SIMON: I don't know. We have not collected that data. I guess it would be interesting. But we do a screening test and then a confirmatory test and the donors are reentered. We are allowed to reenter them if the

confirmatory test is negative. And the ones who are confirmed positive are reported and referred for treatment.

DR. HOLLINGER: Steve?

DR. KLEINMAN: My other question, Sharon, was you have made some statements about platelet concentrates and oxygen tension, and I wonder--it is such an emphatic statement that you made that we don't have to worry about platelet concentrates because of the oxygen tension. Then I have two questions. Is the oxygen tension in platelet concentrates today substantially higher than when they were first made years ago?

DR. ORTON: Yes.

DR. KLEINMAN: Secondly, is this medium the same as applying oxygen tension to other preparations? If we haven't done the experiment, how do we know for sure with this?

DR. ORTON: It was actually 1985--I have a reference, Steve, I can get you--there have only been two references since the early '70's that I could find, both addressing the oxygen tension in the platelet bag regarding spirochetes. They are both in 1985. I don't have them right at my fingertips.

So is there no risk? We don't know if there is no risk. But I guess what I wanted to point out was we have been really focussing on platelets because of room

temperature. I suspect that they are extremely low risk versus a red cell that is refrigerated.

Actually, Dr. Liu made a very interesting statement to me yesterday. He said, "Through all of this handling of components, what is the first thing we do right away when we are collecting these components so we can do the testing is we stick them on ice so that we will organism to do our DNR and RNA. We certainly don't leave them at room temperature."

So, intuitively, along with, like I said, the oxygen tension--yes; it has gone from less than 10 percent in the early '80's to over 60.

DR. KLEINMAN: You also mentioned, I think partly in passing, that there is some evidence that the spirochetes segregate with white cells.

DR. ORTON: That's correct.

DR. KLEINMAN: I wondered how good that evidence really is.

DR. ORTON: Dr. Liu, can you answer that as well?

DR. LIU: At the end of the presentation, we will have some proposed experiments, but I will just let you know the data we have collected so far--at CDC, we have separated blood components into plasma and buffy-coat fraction after we spike the whole blood with organisms. We were able to detect an organism in all the fractions even though, for the

buffy coat, for example, you do a ficoll-hypaque and, and, after that, you wash, like, three times and you can still detect organism in that fraction.

The worst fraction that we used was the serum which is put in the refrigerator for--actually, at room temperature--for overnight and then refrigerated. That has very, very little organism in the serum fraction.

DR. HOLLINGER: Thank you. Thank you, Dr. Orton.

The next speaker is Alan Williams on the REDS Study of syphilis screening as a surrogate test.

REDS Study of Syphilis Screening as a Surrogate Marker Test

DR. WILLIAMS: Thank you, Blaine.

[Slide.]

I was asked to provide some information about the value of serological tests for syphilis in the context of their surrogacy for behavioral-risk values. What I will do is provide a couple of slides just to review data from the literature primarily from correlation of marker rates between syphilis and other markers but then spend some time on a study from the REDS Survey Program in which we actually looked at risks at blood donors and were able to correlate them with both anti-hepatitis-B-core and serologic tests for syphilis, both of which have been arguably associated with behavioral risk in donors.

[Slide.]

Both anticore and STS, as I will refer to it, result in a substantial loss of donors for anticore. The current prevalence is 0.45 percent in the donor population and we loose approximately 40,000 donations per year. For the PK-TP, it is 0.18 percent. We loose about 16,000 donations per year.

That is the current situation. In fact, historically, we have lost many more donors due to the anticore test who, at some point, might be able to be reentered as active donors.

Both reactivities exhibit marginal predictive value for the specific infection in populations such as blood donors who have low infection prevalence. And the surrogate value for behavioral risk detection for these two markers is speculated, but largely unknown.

[Slide.]

Specific to syphilis, the correlation of serologic tests for syphilis with HIV and other infection markers in risk populations has been known for many, many years and, in fact, in the early days of HIV, syphilis positivity was felt to be a fairly strong predictor or likelihood of developing AIDS or having an HIV-positive test result.

In the blood-donor situation, there have been two major studies, also describing test reactivity over labs,

the study by Herrera et al. from the CDC published in Transfusion in 1997 and by John Aberle-Grasse in our group at Holland Laboratory, both showed strong evidence of correlation between STS and HIV as well as hepatitis and other markers.

However, in both papers, these correlations were then extrapolated to the likelihood of STS predicting a window-period HIV infection and, due to the rarity both of incident HIV and the shortness of the window period, which, in fact, at that time was described by anti-HIV testing, it was determined in both studies that the predictive value of STS would be less than one window-period case per year.

As I mentioned, that was based on anti-HIV as a screening test. One would expect that, with the advent of p24 testing and now NAT testing, the that the predictive ability would be cut in half or even lower than that.

But this summary was reflected in the NIH consensus statement on infectious-disease testing for blood transfusion and was generally accepted by that group as representing an absence of surrogate value for this test.

[Slide.]

You have seen some of these dates before. I will only mention it to the effect of saying that, in 1938, the STS was indicated for syphilis purposes and then the test was retained for its surrogate value in the early '80's.

This was then dismissed at the NIH Consensus Conference and there was some question about residual value for syphilis and we are debating both issues today.

So just in case this pendulum swings back, we took the opportunity of the survey research program in REDS to collect some further data which, I think, shed light on the surrogacy value.

[Slide.]

So the objective of this program was to use established measures of blood-donor risk behaviors to assess the value of anticore and syphilis as surrogate indicators of parenteral and sexual risk in the blood-donor population. I think anticore is going to prove to be useful as a comparative measure in this study.

[Slide.]

REDS has conducted several large-scale anonymous blood-donor surveys. The first one was done in 1993 followed in 1995 and this most recent data that I will show is from the 1998 donor survey. In addition to the five REDS sites located in Baltimore-Washington, Detroit, Southern California, San Francisco and Oklahoma City, for this survey we added the New York Blood Center, the Blood Bank of San Bernardino and Life Blood Blood Center in Memphis, and the whole study is coordinated by the Medical Coordinating Center in Westat.

Obviously, many people are involved with these studies. I can't name them all but we are certainly grateful for the cooperation.

[Slide.]

In terms of methodology, this was first established with the 1993 survey. We used an anonymous mail survey sent to donors approximately four to six weeks after inactive donation. The donors are all allogeneic donors and are selected to be over 18 years of age.

We have a very highly quantified database for all of the REDS sites in our coordinating center and we can use this as a sampling frame to do a very well-structured sample for this survey. The data I will show you is from a monthly probability of sample of donors, April through October of 1998.

This comprised a total of 92,500 sample donors at the AIDS sites. In this survey, we had a 57 percent survey-response rate. This is slightly lower than we have had in the past and we attribute it to the fact that this questionnaire was getting rather long because we were trying to build a lot of additional things into it.

So we have had response rates up as high as 76 percent in the past.

[Slide.]

In addition to getting data from the survey form,

itself, we have the ability to tap into other data available from the sampling frame and pre-code the surveys. Even though they happen to be anonymous, you can take something like an existing laboratory test result and pre-code the survey. In the time frame in which we need to get these out, we can only deal with the initial screening-test result. The confirmatory tests are not yet available. But, for anticore, the screening test is the only test that is available and for STS, the PK-TP has at least a fairly high level of confirmability with the FTA ABS, so we are certainly in a better position than we would be with some of the other viral screening tests.

So we pre-coded our outgoing surveys into those that were anticore-positive and no other markers, STS-positive and no other markers, any other marker and totally seronegative.

We over-sampled this group to get a better representation. We actually did not sample. We used the whole population of anticore and STS-positive donors that were available.

[Slide.]

The questionnaire, itself, captures demographics about the donors, information about the donors and history and experiences. There is an extensive behavioral-risk assessment which goes beyond the actual screening done at

the blood center, but the questions are formulated so that we can actually reproduce responses to the questions that would be asked at the blood center.

This survey has been applied to multiple investigations. The one I am describing today, surrogate value of syphilis and anticore screening, or using it for studies of incentives to blood donors, hemochromatosis and studies of HIV test seeking.

[Slide.]

One concept that I think most of you have heard before that is critical here is what we are calling deferable risk. Deferable risk, in the context of a study like this, is risk factors that have been self-reported by an individual responding to a survey that, if identified at the time of blood donation, should have resulted in that donor's deferral.

[Slide.]

There is a long list of screening questions. The latest count is some thirteen questions associated with potential infectious-disease risk and the range from male sex with males since 1977 to ear and other body piercing, obviously different levels of specificity.

[Slide.]

Our results stratified by the testing we obtained, the overall deferral estimate for the negative donors is

2.9 percent. Now, this is a little higher than the estimate published for the '93 study, and this is largely due to the addition of additional questions at the blood center including things like incarceration, birth in Africa, and ear piercing.

But our background rate here is 2.9 percent. The rate for deferrable risk in anticore-positives is 8 percent, STS-positives, 13.7 percent and other, 11.5 percent. We did the appropriate odds ratios and then subjected these to a logistic-regression model adjusting for gender, age, race, ethnicity, education, center and first-time donor status. You can see that the adjusted odds ratios really did not vary too much after doing that.

[Slide.]

This, in fact, is a little bit of a teaser because if you look at the next slide, one thing that is built into our overall deferrable-risk measure are two questions related to history of syphilis. So, clearly, we had to correct for those. One; "In the past twelve months, have you had a positive test for syphilis?" This is a group of blood donors that has been found positive at the blood center and, presumably, have been notified of that test results so that creates a very muddy situation.

The other question; "In the past twelve months, have you been treated for syphilis or gonorrhea?"

[Slide.]

If you exclude those two questions and take another look at the deferrable risk, the negative value becomes 2.7 percent, the anticore value, 7.3, STS, 4.7 and for any other markers, 11.5 percent with the appropriate odds ratios.

Interestingly, once you do the adjustment for the same variables, anticore stays relatively close at 2.5. The STS variable becomes non-significant at 1.3, and the other variables stay significant at an adjusted odds ratio of 3.6.

[Slide.]

Now, as I mentioned, there is a wide range of risk behaviors that the blood center asks about. So we wanted to pick some of those that we felt were, perhaps, the most important and look at the specific values here.

So we looked at the proportion of MSM and injecting-drug-use what I am calling "burden" associated with anticore and syphilis test-positivity. What you can see is the columns add up to a total burden of MSM risk in the active panel population that we are surveying.

For MSM, 94 percent comes from the seronegative group, 3 percent from the anticore, 0.3 from the STS and 2.6 from other test markers. You can see consistently the anticore group is a little bit higher. For all the risk levels, the STS remains rather low in proportional

contribution.

I think one observation that sort of validates our method of looking at this is you see a fairly high value for the other tests related to IDU and even sexual contact with an IDU. We think this probably reflects hepatitis C emerging as a test result. And that would make sense.

[Slide.]

So, in summary, when controlled for first-time donor status and demographic factors, anticore-positive donors have a 2.6-fold higher level of reported deferrable risk than seronegative donors and the value of anticore, as a surrogate, needs to be considered in the context of other variables that have elevated levels of deferrable risk.

Now, we see differences between males and females and first-time and repeat donors at a magnitude of about 2. CUE panels have a deferrable-risk odds ratio of about 13. And HIV-test-seeking donors an odds ratio of about 8. So I think you need to keep the magnitude of some of these odds ratios in mind when assessing the relationships to things like anticore positivity and incentives and some of the other variables that we determine.

[Slide.]

To summarize the STS data, when controlled for first-time donor status, demographic factors and history of syphilis, STS-positive donors do not report a higher level

of deferrable risk than seronegative donors when those STD questions are removed from the formula.

[Slide.]

The results of the study indicate that when measured directly, STS does not appear to have value as a surrogate predictor of behavior risk in U.S. donors.

Thank you.

DR. HOLLINGER: Thank you, Alan.

Dr. Boyle?

DR. BOYLE: Alan, how did the response rate vary by the four strata?

DR. WILLIAMS: I don't have the figures with me, but they were lower in the seropositive group. They traditionally are.

DR. BOYLE: A lot, lot lower or just somewhat lower?

DR. WILLIAMS: Probably a half to a little bit less than a half.

DR. STUVER: So are those categories, then, mutually exclusive? In other words, the STS positive, they were only positive for that screening marker?

DR. WILLIAMS: That's correct.

DR. STUVER: What was included in the other lab reactivity category?

DR. WILLIAMS: Any screening test used by the

blood center would be initial reactives for HIV, for HTLV, hepatitis B surface antigen, anti-HCV, any other screening test that was available immediately found on collection.

DR. HOLLINGER: Dr. Mitchell?

DR. MITCHELL: When we are looking at HIV, we talk about the test-seeking behavior. Is there any evidence of that with regard to the syphilis or RPR? Have you looked at that at all?

DR. WILLIAMS: We have not looked at that. The test-seeking behavior analysis that we did was based on the '93 data and we found a prevalence of 3 percent of respondents who had sought testing in the past year in a blood-bank setting.

We have correlated that with deferral risk but not with the test positivity. We didn't have that as a variable at that time and we have not done the '98 analysis on that yet. That is good point.

DR. HOLLINGER: Alan, you make a comment about--you didn't show a slide on this, but you said if parallel molecular studies continue to show an absence of T. pallidum in STS-positive donor, the requirement for STS testing of donated donor should be removed.

Can you tell me sort of how much parallel molecular studies would you need to feel comfortable with that?

DR. WILLIAMS: I suspect the way to really hone on the issue is to define the donors with seropositivity that would be expected to have--if any group has active infection, try your best to define those clinically and by questionnaire and do the nucleic-acid technology on that group.

We were limited to, essentially, a convenient sample of platelet samples in first looking at this because it was a first shot. Our presumption is that probably most of the infection that is real is remote and that about half of it is not real. But probably the ideal would be some sort of a collaborative study between the CDC field sites and a laboratory capable of doing infectivity studies to look at those subjects specifically.

DR. HOLLINGER: Thank you, Alan.

I think, Dr. Kleinman, you have a few comments, also, on the REDS study, too?

DR. NELSON: I don't know if it is in the database that Alan presented, but you asked about the plasma centers. I am interested in repeat donors, what is the frequency of incident RPRs. Are those data available?

DR. KLEINMAN: I am going to present that for REDS. I don't know about the plasma centers.

DR. NELSON: Oh; okay.

DR. KLEINMAN: As an independent analysis in REDS,

we decided to look at the frequency of PK seroconversion in REDS donors. This is going to be presented at this year's AABB and I am sorry I don't have any slides now. The analysis is preliminary.

But we looked at four of the REDS centers from 1995 through 1997. They all followed the same testing protocols, screening by PK-TP, on the Olympus, 7,100, confirmation by FTA and then the RPR for the confirmed positives.

We found 103 donors who went from PK-TP-negative to PK-TP-positive, FTA-positive, on a subsequent donation. When we calculated that as an incidence rate, we got 15.5 per 100,000 person years.

The mean time from negative donation to positive donation was six and a half months in that two-year database. When we took at look at the demographics of those donors and compared them to PK-negative donors, the positive, if you will, potential seroconverters, although I am not sure that is the right interpretation--they were more likely to be greater than 36 years of age, more likely to be black or Hispanic, have lower educational levels and be born outside the U.S.

Of these factors, race and age remain significant in multivariable analysis. Interestingly, about three-quarters of the seroconverting donors, when tested by RPR,

were negative. And one-quarter were positive, similar to what Roger presented for the overall donor set. This is quite confusing to us because we would expect, if they were truly recent infection and they had not yet been treated, or if they had only recently been treated since their coming in within six months, that we might expect that they would still be RPR-positive.

So it brings up a couple of alternative explanations for these people, that some portion of the dataset might be persons who are now false positive on both assays since they both are T. pallidum assays, and from a comment that Dr. Orton made earlier about her experience in her case-control study, it also brings up the possibility that, at first screening, some of these people were false negatives, that, in fact, their old infection that intermittently becomes positive.

So that is really as far as we can go now. Just to recap, now, of 103 of these individuals in these four centers, REDS collects about 9 percent of the blood in the U.S., 8 to 9 percent. So you might say if this were done nationwide, we would find about, in a two-year period, 1,000 serological conversions and only some portion of these represent new infection. Others probably represent false-negative or false-positive tests.

So that is as far as I can explain the data. But

the demographic data do indicate that these people fall into categories that you might expect from clinical syphilis case reporting which is at least being non-white and having lower education level. So we have some confusion of explanations.

DR. HOLLINGER: Thank you, Steve.

Questions of Steve? Sherri?

DR. STUVER: So the overall mean between the negative and the positive is 6.5. That is what you said?

DR. KLEINMAN: Yes; 6.7.

DR. STUVER: Did you look at whether there was a difference in the mean time for the RPR-positives versus the RPR-negatives?

DR. KLEINMAN: Yes; in fact I have that data. The RPR-positives were 8.2 months and the RPR-negatives were 6.1. I don't have the confidence intervals. I don't know if those were different numbers. Probably not.

DR. HOLLINGER: You don't know if they have been treated or not, you said?

DR. KLEINMAN: No; these are just a review of database information so all we have is the demographics. We don't have the samples, so we can't do any further testing to elucidate this. These are just historical data reported by the blood centers which we retrospectively, as they became interested in syphilis in the last couple of years, decided we should go back and look at.

DR. HOLLINGER: Thank you.

Yes?

DR. NELSON: What about the geographic distribution? Does it follow Lauri's map?

DR. KLEINMAN: Yes--well, none of the REDS centers are located in the South. One of the centers is Baltimore-Washington, D.C.

DR. NELSON: Baltimore has the distinction of, in 1998, being the city with the highest incidence and prevalence of syphilis which has now been shifted to Chicago.

DR. KLEINMAN: Ken, I am glad to hear about your claim to fame, but actually we did look at that, and the Chesapeake Region was not higher than the other three regions, so, to that extent, we did have some geographic information and it didn't follow the clinical-case distribution.

DR. HOLLINGER: Mary?

DR. CHAMBERLAND: So my sort of variation on that question is were they, then, uniformly distributed among the five REDS centers?

DR. KLEINMAN: Unfortunately, I don't have that data with me and I don't remember. I don't know if it was uniform, but I don't think we saw--we suspected that we might find a peak in Chesapeake, so I specifically remember

that we looked at that and didn't find that.

DR. MITCHELL: Were these repeatedly reactive? Did they come back for further donations?

DR. KLEINMAN: The donors that we included were-- the ones that I gave you did not come back for further donations, I think, in general, although a few might have. We did have a few other donors who went from negative to positive but then returned again and were negative, so we excluded those. We figured those had enough evidence to be false-positives since they were not consistent. But I don't know, within this dataset, whether some of these people, I think, did have multiple donations.

But most of them were one-time negatives to one-time positives, lost-to-follow-up, no further information.

DR. MITCHELL: Also, you said that the prevalence of foreign-born was higher?

DR. KLEINMAN: Yes; in the basic analysis, it was higher, but in the multivariable analysis, that dropped out as a risk.

DR. MITCHELL: Okay; because I was wondering whether you would be able to distinguish something like yaws from syphilis with the test.

DR. KLEINMAN: We could probably do more demographics by country or origin, U.S. versus non-U.S. I don't have that data, though.

DR. CHAMBERLAND: Just another question to help sort through this, whether or not some of these represented at least, on a previous donation, false-negatives, and I am assuming that most of these are from repeat donors. Because of the repository capacity of REDS, do you have the ability to go back and pull samples from even earlier donations to see if there is any--

DR. KLEINMAN: We haven't looked at that. Unfortunately, the years in which these data were calculated, we wanted to wait until people were well into the PK which started in 1993 but really the protocols were more established in 1995. Our repositories are general repository collections go from 1991 through 1995 and scaled off. So we might have some previous donations from a few of the--and we only put about 15 percent of our donor samples in the repository.

So I think we, unfortunately, while we might get a few samples and it is worth looking for, it probably wouldn't help that much.

DR. HOLLINGER: Dr. Nelson?

DR. NELSON: In some studies we have done in drug-using populations in Baltimore, we have found some fluctuations of the so-called treponemal tests and even differences between different laboratories. So I think that both false-negatives and false-positives are a reality with

these.

DR. KLEINMAN: Yes; my sense is these data are not as useful as we thought they would be at first because I think the explanation for them are across the gamut, new infections, false-negatives, false-positives. I don't think we will have any way to sort out what proportion were due to each.

DR. NELSON: We found, since this cohort that has been actually followed every six months since 1988, people who the tests--with or without treatment, there is a tremendous fluctuation. When we got the SCD records, who also had another set of data done in the different laboratory, we found that there was surprising--I mean, some of it was pretty concordant, but there were much higher rates of discordance, both in the treponemal and the non-treponemal--particularly in the treponemal test.

DR. KLEINMAN: I was certainly surprised, and I don't know if this would hold for people on the panel, but you learn about syphilis diagnostic testing and read the textbooks, and it seems fairly straightforward. And then, when you actually go and talk to people who have experience and see that the tests perform with a lot more fluctuation than you would be led to believe.

So I think I am less optimistic about the ability to interpret these data than I was when we began.

DR. HOLLINGER: Does the Red Cross have any data on seroconversion in repeat donors?

DR. DODD: Dr. Katz advised me, just say no. It is buried in a huge database. We haven't analyzed it, to the best of my knowledge. But we can do that.

DR. HOLLINGER: Before we finish today?

The next; again, Dr. Markowitz is going to talk about the Maricopa County STD study.

Maricopa County STD Study

DR. MARKOWITZ: Thank you.

[Slide.]

One of the things I want to say before I present these data is I am going to present data on the amplification of DNA polymerase gene T. pallidum from the whole blood of persons with syphilis. This study was conducted during the molecular subtyping of T. pallidum during an outbreak investigation of syphilis in Maricopa County, Arizona.

We were looking at a new subtyping scheme that had been developed in our lab using amplification techniques of the arp and tpr genes. It was not really designed to look at this issue of the safety of our blood supply, and so there is really, as Sharon pointed out earlier, some very basic differences between our study population and the study population that was presented earlier.

We actually decided to look at this only after we had discussions with the FDA related to syphilis screening in blood bank.

[Slide.]

As background, I wanted to show you the epicurve of syphilis in Maricopa County since 1988. There was a major increase in cases in 1990 that coincide with the national epidemic of syphilis, then cases decreased and then began increasing again in 1996. They have continued to have this increase through 2000 despite declines in the rest of the country.

Maricopa County, in case people don't know, is basically Phoenix, the Phoenix, Arizona area.

[Slide.]

So our study population was persons attending the Maricopa County SDT clinic who either had signs and symptoms of syphilis or who had a sex partner with infectious syphilis. These people are required to be investigated by the health department.

[Slide.]

We used the following case definitions for this; an incubating syphilis is a person with significant sexual exposure to infectious syphilis but who, themselves, have no signs or symptoms of syphilis and are non-reactive on RPR and MHA-TP. For primary syphilis, it was someone with a

genital ulcer who had a positive dark field. This is a definite case. The STD clinic had the ability to do dark-field examinations.

Secondary was rash and/or lymphadenopathy with reactive serologic test for syphilis and latent was someone with no signs or symptoms but with reactive serology.

[Slide.]

Data was collected on exposure to syphilis and clinical data from the medical records, serologic testing. 5 to 10 mls of whole blood were collected in tubes containing EDTA, were stored at 4 degrees and then were shipped to CDC for analysis.

[Slide.]

Prior to amplification for the arp and tpr genes for subtyping, the samples were screened using a polymerase chain reaction to amplify the DNA polymerase gene, polA. Primers were designed based on the unique region of polA and were used to amplify 378 base-pair product.

Appropriate positive and negative controls were used for each set of replications. The samples were kept at 4 degrees until analyzed by agaros-gel electrophoresis. To validate the polA PCR, we confirmed testing using two additional targets, the arp and the tpr genes.

The choice of polA was made because it is a highly conserved housekeeping gene for T. pallidum. The gene

target has unique properties and it contains four additional inserts in sequence and it is high in cysteine content.

[Slide.]

The additional targets that were used for molecular typing that were also used in this study include the acidic-repeat protein, which has multiple repeats and can be used to distinguish among clinical strains, and the tpr gene, which is a multiple-gene family and is also used to distinguish among clinical strains for our subtyping scheme.

[Slide.]

These are our basic results. Of 32 blood specimens that were obtained, polA was amplified from thirteen, or 41 percent, and seven, or 22 percent, were positive by at least one additional target, either the arp, tpr or both.

[Slide.]

This slide outlines the clinical stage of persons from whom PCR was done and for whom we had either polA amplified and those who had at least two targets. As you can see, polA was amplified from persons in every stage of disease. We had eight people who were incubating--that is, people who had contact but no evidence of syphilis clinically or serologically, and polA was amplified in four of these individuals.

There were seven cases of primary and polA was amplified in one, one case of secondary and that person had polA, and twelve latent cases and polA was amplified in seven. There were four persons that were included that were suspected syphilis but actually turned out to have other ulcerative STDs diagnosed, and polA was not amplified from any of those individuals.

We were able to get additional targets on fewer. Part of the reason for this is that we went back later to look at these samples, so the conditions may not have been as good. We had two incubating, one primary. So both the primary and the secondary were able to be--we had greater or equal to targets for both of those. Latent, three. And, again, none of the persons with nonsyphilis ulcers.

[Slide.]

This slide outlines the range of polA amplification by the serologic tests. For those that were nonreactive, RPR nonreactive, polA was amplified in three of these. Rpr titer, one to one to one to four, four out of seven, and those with greater or equal to one to eight, six out of 14. For the MHATP, the treponemal test--well, these were at ones that were not done. Fourteen were not done. Of those that were reactive, nine out of fourteen and, of those nonreactive, one out of four.

[Slide.]

Our conclusions; in this study, T. pallidum DNA was amplified from whole blood; samples from persons known to have untreated syphilis or exposure to syphilis; and the viability of T. pallidum that yield the DNA from these samples is really not known.

But the data do suggest that potentially infectious spirochetes are present in blood during incubating primary, secondary and latent stages.

[Slide.]

I mentioned this earlier, but there are two fundamental differences between our study and the ARC study. First, there is a major difference in the study population. Persons in the study were patients with untreated syphilis, seen at STD clinics or those who were recently infected. And then, the ARC study, this was a donor population and there was not actually good history on the treatment or the disease status of any of those persons.

However, they were likely to have late-latent or treated syphilis. There are also differences in the blood component, as was mentioned earlier. We looked at whole blood and the ARC study looked at platelets.

[Slide.]

The last slide here, I just wanted to mention there is one other study that looked at this issue. This study was recently published in 1999. It was conducted in

Italy and it has results somewhat similar to ours. In that study, sera was used instead of whole blood. They looked at patients in different stages of disease as well as treated patients and they tested patients by a commercially available nested PCR kit which they purchased from BioLine which is produced in Turin, Italy.

The DNA extraction and amplification were performed according to the manufacturer's specifications. I have collapsed some of the categories from the paper for this slide, but, basically, they had twenty seronegative subjects. None of these were PCR-positive. They also had twenty subjects who were previously treated, had syphilis and were documented to have it previously treated, and none of those were PCR-positive.

They also looked at six patients with PNS syphilis and six of those were PCR-positive and nine patients with latent syphilis, and six of those were PCR-positive. So, at least there is one other study that has found results fairly similar to what we found.

DR. SCHMIDT: In your methods and case definitions, you told us the incubating people had a nonreactive RPR, the secondaries had a reactive. What about the primaries, the seven primaries? Was their RPR positive?

DR. MARKOWITZ: That wasn't part of the case definition. Serology was done--I actually should have

brought those data, but, actually, the definition required they have a positive dark field, dark-field examination of their ulcer.

DR. SCHMIDT: But do you know the answer for the seven?

DR. MARKOWITZ: Not all of them were positive.

DR. SCHMIDT: Okay.

DR. MARKOWITZ: Because, actually, if you look at the serologies, there are more negative serologies than can be accounted for. So some of them were RPR-negative, as we know occurs in primary syphilis.

DR. HOLLINGER: The one that was positive, PCR-positive, was what? Do you know that? Do you know the reactivity?

DR. MARKOWITZ: No; I did not bring a line list of the data so I don't have those broken down.

DR. MITCHELL: In the Italian study, you grouped together the primary and secondary whereas we were expecting in the secondary phase, they should always be viremic. Do you know the differences? Do you know whether all of the secondary stages were--

DR. MARKOWITZ: No; I have the paper. They also lumped primary and secondary. The way that they broke it out, which I think addresses your question, was they looked at it by serologic status. They looked at the latent by

serologic status and the primary and secondary by serologic; that is, they did not break it out by primary and secondary.

DR. HOLLINGER: Dr. Nelson?

DR. NELSON: You raised the issue that the PCR-positive might not always equate with infectivity but has anybody done rabbit inoculation of PCR-positive samples? I mean, that is the model.

DR. MARKOWITZ: I was asking Dr. Liu or Sharyn Orton to address this.

DR. LIU: Actually, if you just wait for a few minutes, I will present some of the proposed studies.

DR. HOLLINGER: Dr. Tuazon?

DR. TUAZON: In your PCR results, those with the two targets positive, are those the same ones with the polA-positive, or these are different?

DR. MARKOWITZ: No; they are a subset of polA. So everyone who is polA--we wanted to see, just to make sure there were not issues, other issues, that could have accounted for the polA positivity. So all those that are greater than two are a subset of the polA-positives.

DR. HOLLINGER: Just for my understanding of the polA and the other targets, is there a reason why some of the polA are positive, for example four are positive, two are just--the other targets are seven and three. Why--

DR. MARKOWITZ: Again, Dr. Liu may address these,

but I think they are harder targets to amplify because they are larger targets. Hsi, do you want to--

DR. LIU: I believe you just answered the question.

DR. MARKOWITZ: Okay.

DR. HOLLINGER: Thank you.

DR. LIU: Actually, I will just make it very clear that the polA target is only about 400 base pairs. However, the other two targets are over 1,000 base pairs, and are relatively difficult to amplify.

DR. HOLLINGER: Thank you.

Did you have a comment? Please?

DR. NAKHASI: Hira Nakhasi from FDA. I am looking at the data from CDC and from the Red Cross. The data which is presented here which is the number of positives incubating, primary, basically they are seropositive; it that right? The number comes from where, the seropositive? The eight, seven, one, twelve--

DR. MARKOWITZ: Let me go back--

DR. NAKHASI: PCR in whole blood by syphilis disease stage.

DR. MARKOWITZ: Did you ask if those are serologically--

DR. NAKHASI: Yes. They were selected on the basis of serologically positive.

DR. MARKOWITZ: No. I put up our case definitions and the incubating were not seropositive by definition. By definition, incubating syphilis is someone who has been exposed to infectious syphilis but doesn't have any evidence of infection yet. And, for primary syphilis, in this situation, we did not require a positive serologic test if they were dark-field positive.

DR. NAKHASI: My question is where is the number coming from, and then, when you have PCR-positive, only 50 percent in some cases, or one in seven, or seven in twelve, does that mean that what the Red Cross found out that, even though they were antibody-positive, but they could not detect any treponemal DNA.

DR. MARKOWITZ: We did not detect--if I understand your question, we did not detect treponemal DNA in everyone who was--

DR. NAKHASI: Antibody-positive.

DR. MARKOWITZ: Was antibody-positive. I don't know if you can get the slide back up there but, for example, we did detect it 100 percent of the secondary cases. There was one secondary case, that person. But, in the latent cases--

DR. NAKHASI: It was seven out of twelve.

DR. MARKOWITZ: Yes; so the remaining ones, we did not detect.

DR. NAKHASI: And they were antibody-positive.

DR. MARKOWITZ: Yes; they were antibody-positive.

DR. NAKHASI: So, therefore, it doesn't differ that--we had projected there were differences but I don't see that many differences because of the fact of--the more cases are antibody-positive and then only a few are DNA-positive. So what they found was they also found--in their case, they found none, whereas you find some cases.

DR. MARKOWITZ: If we could go back to the slide.

[Slide.]

You are talking about this slide here?

DR. NAKHASI: Yes; I think so. Yes; that is one.

DR. MARKOWITZ: So you are saying seven of twelve latent, for example, all of those were positive, had positive serologic tests for syphilis, so five of the latent cases who had a positive serologic test for syphilis, we were not able to amplify polA in five out of the twelve.

DR. NAKHASI: Okay.

DR. LIU: I think I can clarify this a little bit if you are looking for whether there is a direct relevance between the serological test and the DNA amplification, I think my answer would be you will not be able to see 100 percent correlation.

DR. NAKHASI: Thank you.

DR. SEN: I have one question. The ARC tested the

16S ribosomal RNA--

DR. HOLLINGER: Could you state your name, please?

DR. SEN: Yes. Keya Sen from FDA. They tested the 16S ribosomal RNA ARC which is very high copy number RNA and at the DNA level, too, there are several copies. So do you have plans of testing the 16S ribosomal RNA, a third target, with those samples. Maybe you will see better correlation.

DR. LIU: Actually, Sharyn would be the person better to answer that question, but in terms of our studies, we did not use the 16S RNA for the test. Number one, is that we are not using the test in the laboratory. Number 2, there were some specificity problems with test. And we have not been able to evaluate the specific test.

Thank you.

DR. HOLLINGER: Thank you. Dr. Liu, I think we are going to go on, then, with the CDC-proposed studies. Dr. Liu is going to start off.

CDC Proposed Studies

DR. LIU: Thank you very much.

[Slide.]

I would like to clarify that. I will not be able to propose a study to answer all the questions that we have talked about today, but the following proposed studies are what we think is pertinent.

[Slide.]

CDC proposed to study four major questions. Question No. 1, is which blood components contain treponemes. We are already starting this study at this point. Which fractions of the blood are infectious? What is the prevalence of donated blood with treponemes and what is the concentration of treponemes in the blood?

[Slide.]

In terms of which blood components may contain treponemes, all these studies were done in collaboration with ARC. What we propose to do is to spike whole blood and then separate it into fractions in the American Red Cross using their methods and perform either PCR or semiquantitative PCR which is developed at CDC at this time to determine whether we can detect organisms in the blood.

[Slide.]

In terms of what is infectious, this is a very tough question and we propose to spike whole blood as before, separate it into fractions and then perform the rabbit infectivity test which will answer the question we brought early on. But I would like to be cautious that the rabbit infectivity test is very time consuming and expensive, and we will not be able to do a very large portion of this test.

[Slide.]

Furthermore, a very important question is what is the prevalence of circulating T. pallidum in donated blood. We propose to expand the ARC study that Sharyn has mentioned early on to regions of higher syphilis incidence. Unfortunately, or fortunately, in the United States, the cases of syphilis are declining so we really think that now would be the best, and maybe the only time, to perform the study in this country.

Additionally, this will be collaborating with all those sites including another location that is in South Africa which has a very high prevalence rate of syphilis. The method is very simple. You obtain blood and then perform PCR which will be able to detect the DNA of the organism.

[Slide.]

Then we also propose additional studies including to test the different stages of syphilis and then these will be validated with STS, the serological tests. And we will extract DNA of CDC and then do either PCR or semiquantitative PCR to answer those questions.

It is a long meeting. I am the last presenter, so I think this will be all. Are there any questions? Yes, please?

DR. McCURDY: You were talking about using discarded blood for your spiking studies. Is that outdated

blood or fresh?

DR. LIU: In the original slide, I believe, the committee members received a piece of paper outlining the studies. No; we are not using discarded blood at all. Actually, Dr. Orton and I have already started the study and yesterday we separated some of the blood fraction, and then I believe we used fresh blood.

DR. McCURDY: Because, if you are going to separate it into components, you will get different results if it is stored rather than separated fresh.

DR. LIU: We are using fresh blood, number one. And, number two, in terms of the storage problem, unfortunately, it is going to be really complicated because the different components will be stored a different length of time. We will not be able to answer all the questions, but we will design a study to answer part of the questions, like, for example, platelets we may just leave at room temperature for one hour, two hours, or maybe a day and then test for the DNA.

However, remember that whether we can detect the DNA or not has nothing to do with infectivity. So it has nothing to do with the transmission of disease.

DR. McCURDY: I might point out, also, that whereas random donor platelet concentrate from whole-blood donations are often, if not usually, stored for a couple of

days and may be subject to oxygen tensions that would destroy the treponema. Pheresis platelets are much more likely to be given fairly promptly and a certain number of them, at least, are drawn for a particular patient.

So those may carry a different risk than the random donor platelets.

DR. LIU: Yes; I agree with you.

DR. HOLLINGER: Dr. Schmidt?

DR. SCHMIDT: I don't understand number three, expand the Red Cross study to areas of higher syphilis incidence. In the United States?

DR. LIU: We are thinking of the United States because right now there are really not too many places have a high incidence.

DR. SCHMIDT: In the hopes of collecting a donation from a person with infectious syphilis. Why not go to Maricopa County, Arizona, where you have all of this stuff and give them $25 and you would have a unit at all of these different stages. To go to South Africa to do this is just--I don't understand.

DR. LIU: The reason being because the question really is related to whether the person who donated the blood in the blood bank has a high risk. If you are talking about the people in Maricopa County, these are patients with syphilis, active syphilis or latent syphilis. We have

already shown that we can detect organisms there.

DR. SCHMIDT: But then why do we want to do that again?

DR. LIU: Pardon me?

DR. SCHMIDT: What about this person being a blood donor for the Red Cross is different as far as their bugs from somebody in Arizona?

DR. LIU: The question is quite straightforward. Whether we are interested in detecting organisms in the blood or whether we are interested to study whether people donate blood in the blood banks are the high risk. To answer the first question, we pretty much show that if you are in the high-risk group, like the Maricopa County studies, there are organisms floating in the blood. Whether these are infectious or not, we do not have the data right not to support that.

In terms of the Red Cross study, it is 100 percent for the purpose of the safety of our nation's blood banks. That, if you want to do it, for example, for the committee to determine whether serological tests should be used or not, this is the only time in my presentation--that will be the only few places we can do that study.

Does that answer your question?

DR. SCHMIDT: No. But you have your mind made up.

DR. LIU: Oh, no, no. Not at all. I do not have

any mind set at all. But if you can explain, or maybe someone can help me to explain. Sharyn?

DR. ORTON: Dr. Schmidt, I think what Dr. Liu meant by doing studies in areas of higher incidence, what we did was I mapped what counties had the highest incidence of syphilis in the country and where we happened to coincide having blood donors come from.

The idea was, rather than just taking a random sample of blood donors who test positive for syphilis is go into areas where we do know that the incidence is higher and look at the components from those donors who test positive for syphilis. I think that is what he was talking about.

If we are going to find a donor who has evidence of syphilis by DNA or RNA PCR, we are more likely to find it going into counties where the incidence of syphilis is higher and, therefore, look at those corresponding Red Cross sites in those areas.

I think that is what he meant.

DR. LIU: Thank you very much, Sharyn.

DR. STRONCEK: But I think the question is, are blood donors in the U.S. that test positive by the FTA assay, are they infectious or not, then I agree going to counties in the U.S. where there is a higher incidence might be worthwhile, but if South Africa is not using exactly the same blood donation screening and testing information, it is

totally irrelevant because there are different factors.

If you really think that this study is impossible, you are not going to find blood donors positive, then I guess you are saying that we should just get rid of the syphilis testing.

DR. LIU: Unfortunately, I cannot make that decision as to whether we should abandon the syphilis test. That is up to the committee to decide. We are here to present the current data we have and also present the potential studies.

The study in Africa will answer some biological questions. It may not be directly relevant to the safety in the blood banks. However, they do answer some questions.

DR. HOLLINGER: Dr. Simon?

DR. SIMON: I had one question. I don't know if Dr. Liu is the right person, but I don't believe it has come up, unless I have missed it, it seems to me, in my memory, that when the FDA in the '80's was looking at abandoning the test, that one of the concerns was that the positive STS, or the RPR, would be passed; in other words, that the recipient wouldn't get syphilis but would have a positive test and that would have certain recriminations.

What is the situation regarding that?

DR. LIU: I think you are right. I am not the right person to answer that question. Anyone?

DR. SIMON: In other words, do you transmit a positive test result as a passive factor of blood transfusion and then you have a problem that someone has a positive test.

DR. LIU: Dr. Orton will answer your question.

DR. ORTON: I do know that there have been some papers. I don't know the exact figures off the top of my head or how long ago they were that did talk about when seropositive units were transfused into somebody, the length of time that they remained seropositive and the titers that are seen.

It is not seen for long. The titers are not high. But I don't remember that being a particular concern. Maybe back then when it was done more routinely as a routine test in the hospital or something, but I don't, in recent years, remember that being an issue with transfusing those units.

DR. SCHMIDT: That was done experimentally in Dick Walker's study which Cable refers to where he intentionally gave STS-positive blood and he followed that and observed that and did discuss it.

DR. SIMON: In those days, people, of course, had to have it for marriage and so on.

DR. HOLLINGER: Dr. Tuazon?

DR. TUAZON: Do we have information on the false-positivity of PCR such as in Lyme disease or other

treponemal infections?

DR. LIU: You are talking about the PCR we are using at CDC?

DR. TUAZON: Right.

DR. LIU: I have not clarified in great deal, early on when someone asked me about the specificity and also the sensitivity of the test. To test for specificity, we have performed the PCR test on relatively high concentrations of DNA in organisms including almost all the spirochetes, including Borrelia bergdorfii causing Lyme disease, and leptospirosis, many different serotypes and other nonpathogenic treponemes.

The test that we are using at this time is only positive on pathogenic treponemes including the three subspecies.

DR. HOLLINGER: Dr. Nelson?

DR. NELSON: I think international studies are not irrelevant to this issue because I think the real issue is if you have some--the problem is we have got so many, like, false-positives previously treated, et cetera, in the U.S. blood donors that there are places where there are a lot of true-positives.

The issue is when a blood bank processes the specimen, is it still infectious. There may even be places where blood is not screened and that would also be an

interesting population because there are ethical issues, certainly, but if one could identify what the rate of transfusion-transmitted is, there may be international blood banks in Africa, Asia, somewhere, where screening is either not done or not routinely done or something where you could actually get some real data on transmission.

I would think that an international population might be able to contribute some very important information on this although this kind of study would be difficult. I assume that, in South Africa, donors are screened and, if they have a positive result, they are not transfused. Is that correct?

DR. LIU: I believe that South African blood donations should be screened. I do not know that as a matter of fact, but I believe they do. And you were right that, in my view, it is very important to study this organism not just including in this country but also expand it to other territories.

We have to realize that this organism is very difficult to study. It grows very, very slowly and we know very little about the biology of these particular organisms.

DR. HOLLINGER: Are they screened in Thailand?

DR. NELSON: Yes.

DR. NAKHASI: Dr. Liu, when you mentioned that the experiments to test the biological infectivity in the

rabbits will be expensive or it was a difficult situation to do that, I was thinking is it possible, instead of doing a DNA PCR, one can RNA PCR which will tell you whether, in this case, the bacteria is replicating, which will give an indication that it is infectious, as compared to DNA which could be just a piece of DNA lying around.

DR. LIU: I believe that both RT PCR--we are talking about RNA PCR, RT PCR.

DR. NAKHASI: Yes.

DR. LIU: RT PCR and the regular DNA PCR pretty much answer the same questions. In theory, the RT PCR should be more sensitive than the DNA PCR. Right now, like I said, we have not been able to evaluate that.

DR. NAKHASI: I don't agree with your earlier assertion that DNA and RNA will give you the same answer. The RNA will give you whether it is replicating because if the parasite, or in this case the bacteria, is lying there and not doing anything, which will be just the DNA. If it is replicating, you know, because it has to make the proteins and all those things, the RNA will be there.

So if you see an increase of RNA, that is an indication which is the case with HIV and other things, you do HIV RNA PCR which you gives you an indication whether it is infective.

DR. LIU: Let me sort of readdress your question a

different way. I believe you are concerned that DNA, if we detect DNA, it could represent dead organisms because DNA is either dead and they can stay there. However, RNA poses the same question because the RNA we are using right now is 16S ribosome RNA and they are also very stable. Even after the organism is dead, it can stay in the blood stream.

DR. NAKHASI: Yes; I agree with that. Thank you.

DR. HOLLINGER: Steve?

DR. KLEINMAN: Just to comment on the need for international studies. It seems to me that if you want to answer the blood safety question in the U.S., then, really, the issue is whether you have zero tolerance or low-risk tolerance. We already know that even if units are infectious, even if that is proven by international studies, it seems sort of obvious from the estimates that the number of cases transmitted per year would be very low in the States.

So if you start with the assumption that if we can document that the cases are likely to be above zero, we won't change the policy. Then I don't think you need to document the extent of the problem. I am not sure what the implications of that are, but I think you could define the need for studies by the policy parameters you are going to set.

You can say if there is even one case of syphilis

that would be transmitted per year, based on eliminating the test, that would be unacceptable an we would want to keep the test. I think you go down a potentially different track than if you say, "Gee, well, we would consider dropping if it is only five cases per year."

Then you might have to actually document the number of cases per year in the States. I guess my comment is more relevant to doing the studies at the high-incidence centers within the U.S. So I am not sure we need to document--do we need to document how frequently it might occur in the U.S. or just that it is biologically plausible that transmission could occur if we dropped testing.

DR. NELSON: I think it is useful to understand the biology. For instance, for infectious viruses, there are multiple steps to try to decrease the risk. I don't think anybody would suggest that we should tolerate a few cases of syphilis just to get rid of the test. But the questions are really different; how can you process the blood to decrease the risk or what is the highest--I mean, there have been issues related to are platelets safe or are they hazardous.

We really don't know and I think there are possible ways to get some answers to the question. This is very difficult because there isn't a good animal model and the culture of the organism isn't feasible. So I think that

well-designed international studies might yield some important and useful data.

DR. HOLLINGER: Thank you.

I am going to go on, Paul, to the open public session and get that finished. We will come back. We will have time to talk. There are two people that have asked to talk. The first one is Dr. Katz from the AABB.

Open Public Hearing

Presentation

DR. KATZ: Thank you, Blaine and committee members. We have distributed a written statement that has been amended. So if you were going to fall asleep because you have already read it, stay awake at least, perhaps, towards the end.

The serologic test for syphilis has been retained in the United States for two ends, as we have heard; prevention of transfusion-transmitted syphilis and as a surrogate for risk behaviors associated with HIV infection. Transfusion-transmitted syphilis has not been recognized in the United States for more than 30 years and, in fact, in '85, as you have heard, and FDA committee recommended elimination of the STS for blood donors. This recommendation was not implemented due to the concerns about surrogacy.

The reasons for the disappearance of transfusion

syphilis are multiple including the declining incidence of infectious syphilis in this country to historically low levels and donor-deferral policies in the blood centers that reduce the presentation to donate of those at risk for infectious syphilis.

Storage of red blood cells at refrigerator temperatures is probably an important contributing factor as well as the improved oxygenation of platelets over the last fifteen years. Still, there are transfusion of fresh red blood-cell components and platelets stored at room temperature may be a risk as well.

Receipt of antimicrobial therapy by those ill enough to require transfusion support may be important in preventing either infection or recognition of transfusion syphilis. From a biological standpoint, it must be emphasized that the spirochetemia associated with the majority of transfusion transmissibility of T. pallidum often occurs before the serologic test for syphilis is positive and I think that we can extrapolate from that that spirochetemic donations are being transfused with some frequency in this out as we speak.

At the NIH Consensus Conference in January 1995, it was concluded that, and I quote, "The current blood-storage conditions would not appear to provide an adequate margin of safety against transfusion-transmitted syphilis

should the donor-screening test be eliminated. Further information concerning T. pallidum survival under blood and platelet storage conditions and the application of molecular techniques to assess the presence of T. pallidum DNA in serologically positive units would allow better assessment of this question."

Data presented at the AABB national meeting and again today, by Orton et al., based on PK-TP-positive FTA-ABS-confirmed donors using two PCR methods found none with detectable DNA. On the other hand, we have heard the data from CDC that suggest that, particularly in early syphilis, spirochetemia can be demonstrated by sensitive nucleic-amplification testing.

Regarding the value of STS as a surrogate for other transfusion-transmitted diseases even prior to the implementation of sensitive NAT assays for HIV and HCV, the Consensus Development Conference concluded that surrogacy was no longer an issue.

We are aware of the CDC data suggesting that there are early syphilis cases being reported among blood and plasma donors and, unfortunately, as the CDC investigators have stated, they are unable to segregate paid plasma donors from volunteer whole-blood donors in their data. We view this as a serious flaw in the context of discussing the STS.

Taken against a background of historically low

infectious syphilis rates in this country and the failure of clinicians to recognize even isolated cases of transfusion-associated syphilis, we are not sure of the applicability of the CDC data to the volunteer whole-blood-donor sector.

Ramsey and Sherman reviewed FDA-reported blood-component recalls in the United States from 1990 through 1997. Of an estimated 241,800 components recalled, 57 percent or almost 140,000, were for incorrect syphilis testing. These were primarily a single large recall of units where weakly reactive serologic tests for syphilis results may have been called negative. This recall was classified by FDA as a class III recall, not likely to cause adverse consequences.

With these points in mind, AABB believes the serologic test for syphilis could safely be eliminated based on the absence of recognized transfusion-transmitted syphilis in over 30 years despite the undoubted transfusion of some components from seronegative spirochetemic donors.

We have listened to the presentations this morning and understand the concerns that have been raised and would support further studies to document the absence of transmissible T. pallidum in whole-blood components and apheresis platelets if the committee is not, at this time, willing to endorse discontinuation of the serologic test for syphilis.

Thank you.

DR. HOLLINGER: Thank you, Dr. Katz.

Dr. Bianco, from America's Blood Centers?

Presentation

DR. BIANCO: I am amending the printed statement that was distributed to the committee and the audience in the sense that we favor the elimination of the requirement. However, we recognize that a lot of information was provided today and that some studies are likely to add information, particularly in terms of studies of survival of the treponemes in blood and the infectivity studies. They are the most critical piece of information missing here for a final decision.

However, the basis for favoring the elimination, the primary reason for this position is the lack of documented cases of syphilis transmission by transfusion in decades. As we heard from Dr. Schmidt, he saw the last case in 1966; the declining incidence of syphilis in the U.S. population; effective deferral of individuals in risk behavior through medical history and direct questioning including deferral of individuals with a history of venereal disease in the past year; and the poor survival of spirochetes in stored components which will be further studied.

I should also add that indirectly NAT testing for

HIV and HCV is contributing to prolonged storage because we cannot easily release products before 36 or 48 hours after the collection of the product. But no platelet is transfused today with less than 36 hours of life on the shelf.

Even if syphilis was effectively transmitted by transfusion, which I think it is not, the assay currently used for screening of blood donors is, unfortunately, worthless. PK-TP and the similar assay, HMA-TP, are specific assays that detect antibodies to treponemal antigens.

Individuals who had syphilis remain positive for their lifetimes, even after effective antibiotic treatment and cure. Thus, nearly all those reactive for the AIDS assay are immune and do not transmit the disease. For example, it is not uncommon for blood donors to reveal that they were infected and were treated during World War II or Viet Nam and continue to have a positive PK-TP antibody test.

Actually, CDC recommends the use of reagent-type tests for screening in healthcare settings including STD clinics. These tests do detect active infection and turn negative upon appropriate treatment. Unfortunately, these assays generate a large number of false-positive results and are not automated for use in blood-donor screening.

MHATP and, similarly, the PK-TP are appropriate for confirmation of positive reagin test results to eliminate the false-positives. So I think that even if it were transmissible, the screening in the manner that we do today is inappropriate. It creates a population of stigmatized individuals that had a infection 30, 40, 50 years ago and that represents no danger to the blood supply.

In summary, we recommend that the studies that were proposed today be conducted and we believe that if those studies provide some assurance that the treponemas don't survive, that the test should be dropped.

Thank you.

DR. HOLLINGER: Thank you, Celso.

I know the American Red Cross did not have a statement, but is there any feeling from the--is anyone here from the American Red Cross that would like to make a comment

DR. DODD: I think it is very simple. The American Red Cross position has been ably presented by the last two speakers. Our feelings are entirely consistent with the AABB and ABC position. Thank you.

DR. HOLLINGER: Thank you, Roger.

Are there any other comments from the public that anyone wishes to make a statement at this point? If not, I am going to close, then, the public portion of this. Why

don't we go ahead and put the question forward, and then we will have any additional discussion that is needed.

Questions for the Committee

DR. RUTA: Dr. Hollinger, members of the committee, thank you very much. We turn to you again to ask you for advice about the information that you heard this morning. Just briefly, to put things in context, we raised the issue of syphilis testing at this time because last year the FDA issued a proposed rule to update the requirements for donor testing and also a second rule of notification

We received comments on those two rules and we had a public meeting to allow people to give oral comments. As part of the rulemaking, we solicited comments specifically on the utility of testing for syphilis. We received five comments supporting the elimination of syphilis testing and two opposing the elimination of syphilis testing.

We asked for data and, this morning, you have heard some data presented by Dr. Williams and Dr. Orton of the American Red Cross. I wanted to thank them for submitting data.

We also became aware of data that our colleagues at CDC had which we thought were of value considering and so we invited them to come and present it. I wanted to thank our colleagues from CDC, Dr. Liu, Dr. Morse and particularly Dr. Markowitz who gave three talks this morning.

Now we turn to committee and ask for the committee's advice. The first question we have for the committee is do committee members agree that current scientific data are insufficient to warrant discontinuation of donor testing for antibodies of syphilis.

Shall I continue with all the questions or would you want to take them one--

DR. HOLLINGER: If you would, please.

DR. RUTA: If the answer is in the affirmative, if so, committee members are asked to comment on the adequacy of the additional studies as proposed to resolve the value of testing for antibodies to syphilis in preventing the transmission of syphilis through blood transfusion.

Finally, the last question is do committee members believe that donor testing for antibodies to syphilis should be retained as a surrogate marker of deferrable high-risk behavior even if it is proven that such testing is no longer useful for prevention of transfusion-transmission of syphilis.

DR. HOLLINGER: Thank you very much.

Committee Discussion and Recommendations

DR. HOLLINGER: So we are going to deal with the first question first. Is there anyone who has some comments to make at this point. With a couple of double negatives here, basically the first question says, if you vote yes,

you are voting for continuation of testing, the current testing that is being done.

Paul?

DR. McCURDY: I was wondering, as the morning went on, if the pathogenesis of some of the manifestations, particularly secondary syphilis and the skin manifestations had to do with antigen-antibody reactions as it does for many of the infectious diseases because, if that is the case, then a fair number of patients who are getting transfused with fresh components, platelets and others, are immunosuppressed and may not have any of the manifestations of secondary syphilis that were illustrated by Paul Schmidt in his case of some time ago.

I am not quite sure how one would approach that but the neonate is immunosuppressed and they get fresh red-cell components. And marrow-transplant patients and oncology patients are immunosuppressed and they get lots of transfusions including lots of platelets, both pheresis and random-donor platelets.

DR. HOLLINGER: Dr. Tuazon, since this is your specialty here, would you comment?

DR. TUAZON: For the secondary stage, it has been documented, when you have the skin lesions such as shown by Dr. Schmidt, you could biopsy those lesions, show the spirochetes. So there is active spirochetemia during the

second stage of the illness. It is just like bacteremia.

DR. McCURDY: But the manifestations do not relate to the development of antibodies that attack those spirochetes when they are in the skin.

DR. TUAZON: I am not sure that is any data on that, antigen-antibody reaction, but the classic presentation of secondary syphilis is actual invasion of the spirochetes through the systemic organs to the blood stream.

DR. HOLLINGER: Maybe a question here; patients with HIV, with AIDS, who are immunosuppressed and they acquire syphilis, they would have the same manifestations as anyone--

DR. TUAZON: They do, and they are much more difficult to treat because of their underling cell-mediated immune depressions. It is harder for them to eliminate the syphilis. I was just telling Dr. Schmidt of my one experience where I had an HIV guy who had disseminated syphilis that did not respond to two weeks of high-dose, 20 million units of penicillin, and had to be treated for six weeks to get rid of the skin lesions that were steaming with spirochetes.

DR. HOLLINGER: So those with maybe CD4 counts of less than 200, let's say--

DR. TUAZON: No, no; this guy had actually a good CD--

DR. HOLLINGER: I understand that. In answer to the question about immunosuppressed people, if they have a low level, less than 200, less than 100, and they get syphilis, they have chancres, they have the usual manifestations?

DR. TUAZON: No; I think that stage that you are talking about is the secondary stage because that is the one that would mimic the transfusion-transmitted disease.

DR. HOLLINGER: Dr. Nelson?

DR. NELSON: There are a couple of case reports, or several, in the literature about atypical clinical appearance of syphilis in patients with HIV and immunosuppression including people with secondary manifestations without--who are RPR-negative who do not have antibodies where the organism has been found by biopsy and finding spirochetes.

In most of these cases, antibodies appeared later. They eventually appeared. But maybe agammaglobulinemic would be--I don't know if there are such cases, but AIDS patients are immunosuppressed but it is complex. It is more cellular, et cetera. But I think the question you are raising is whether or not some blood component, or blood recipients, might have, in fact, been infected and it was just not clinically recognized because of their clinical state.

I think that is a relevant question. I think the other issue is many are on antibiotics and they may have been infected but not recognized for other reasons.

DR. HOLLINGER: Dr. Schmidt.

DR. SCHMIDT: In the question as presented, is the question really do committee members agree that current scientific data on the transmission of syphilis are insufficient because otherwise you are asking both questions in the first question. I want to make sure everybody, since it is presented negatively already, we are going to double twist this here.

DR. NELSON: We could just vote yes.

DR. HOLLINGER: Paul?

DR. McCURDY: There is one other issue that occurred to me in light of these questions. Yesterday, we were asked very definitely to separate the plasma side from the whole-blood side. It would seem to me that the multiple steps that go through infractionation and inactivation and such would make it extremely unlikely, even if a very infectious unit got in, that it would carry through to the final derivatives.

I guess my question is should we, here, separate the plasma from the whole-blood side as well.

DR. HOLLINGER: We don't have data, even, from the plasma section about whether there is seroconversion.

Dr. Simon?

DR. SIMON: I just wanted to reiterate, and I hope my FDA colleagues will correct me if I misspeak, but my understanding is that the syphilis requirement for testing plasma donors is strictly for the donor's benefit. We can actually shift product from donors who have tested positive for fractionation into final product so that, with the frozen product, there has been no concern about transmission of syphilis.

So I agree with you that we can separate the two issues and, if there is not felt to be a public-health reason to continue to test the plasma donors, it would appear that we could drop that testing at this time because that is not a test done on the unit. That is a test done on the donor initially every four months.

DR. RUTA: I think that is not actually quite right. If someone in the source-plasma setting were to test positive on the screening test and then confirmed positive, then those units could only be used as controls for the syphilis test. I think what you are referring to, if someone tested reactive on the screening test and then were shown to--that that were a false-positive or that they had been treated, then they could be reinstated.

DR. SIMON: Yes; they can definitely be reinstated.

DR. RUTA: But the confirmed-positive units are not allowed to be used for fractionation.

DR. EPSTEIN: I think that point is that since the donor is only tested once every four months that positive units might have been collected, shipped and used pending the next test.

DR. SIMON: Oh; right. Okay.

DR. EPSTEIN: But a known positive unit is restricted to in vitro use.

DR. SIMON: Yes. I should clarify; once you have identified the donor, then those units cannot be shipped. But there is no look-back required.

DR. HOLLINGER: So just answer Dr. Schmidt's question, and this is regarding transfusion. The question really is do the committee members agree that current scientific data--

DR. SCHMIDT: I am asking do you want to insert data on the transmission of syphilis. In 1a), are just talking about the validity of the test in preventing or not preventing the transmission of syphilis. Are we excluding HIV from this first question?

DR. HOLLINGER: Yes.

DR. RUTA: Yes.

DR. HOLLINGER: That is the second question. I think that was fairly understood.

John?

DR. BOYLE: I have been doing some math. My math may be wrong because I have pulling things together. If it is at any point, please point it out to me, but what I heard from the Red Cross is positive tests lead to the exclusion of about 16,000 donors per year. They represent half of the blood industry and its 32,000 donors who are testing positive to the STS after having denied any experience on the screening questionnaire.

According to another set of information, 50 percent of those who had tested positive upon reinterview admitted that they did have a history of syphilis. Now, if those two pieces actually agree with each other, then, by dropping the screening test, we are reintroducing 16,000 people per year donating who do have a history of syphilis at some unknown state in that process and we have other information put up here saying that, at various points in the history of the disease, that they, indeed, even in the late latency stage, can transmit by blood.

If all those pieces are correct, then I would be concerned that part of our success in the last 30 years in not having any of these things was having this test in place.

DR. HOLLINGER: I think everything you said seems to me, as I hear it, is probably correct except that the

issue of "can transmit in blood." I don't think we know. That is the issue that is before us.

DR. SIMON: Also, these are people presumably--because the question says "in the last twelve months." So they can come back as donors. So these are people who presumably were successfully treated.

DR. KATZ: The donor question is regarding venereal diseases during the past twelve months.

DR. WILLIAMS: The 16,000 estimate also is a U.S. estimate, not a Red Cross estimate.

DR. HOLLINGER: Thank you.

DR. McCURDY: There is also one other question about the public-health significance of screening. At one time, in the past, many places abandoned screening for serological tests for syphilis, like hospital admissions and that sort of thing. And I guess my question is what proportion of the cases that ultimately are reported to the CDC, cases of syphilis, are detected by screening versus the proportion that are detected by either clinical symptomatology or contact tracing or something along that line.

DR. HOLLINGER: Do you know, Dr. Markowitz? She will be back in a few minutes?

DR. LINDEN: Didn't we have that data in her presentation?

DR. HOLLINGER: I don't know how to interpret that but there were, again, the 927 that they got from the blood donors. That represented four years--Dr. Markowitz?

Why don't you rephrase the question, Paul, again?

DR. McCURDY: I am asking the question about the relative value of screening a population for syphilis versus detecting cases by either clinical manifestations and clinical suspicion or contact tracing or that sort of thing. My impression is that screening is pretty inefficient.

DR. MARKOWITZ: Are you saying from a public-health point of view?

DR. McCURDY: Yes.

DR. MARKOWITZ: I think all of these 927 cases would not have been picked up clinically because they were not picked up by the questions that were asked when they went to donate blood. I think, in many other settings, there are a fair number of syphilis patients that are only picked up through screening.

DR. McCURDY: In the 50's, and 60's, at the City Hospital in Washington where it was commonly--every patient that was admitted commonly got a serological test for syphilis, very few--not zero, but very few--of the patients that were detected that way were not already known to the rapid treatment center at that hospital.

So screening didn't pick up very many new cases in

that relatively high-risk population.

DR. MARKOWITZ: Well, maybe I misunderstood your question. I think there are better ways to try to detect syphilis cases. But I think that these cases would not have been picked up, these 927 cases. They may have presented months later with a different manifestation and been picked up, but, unfortunately, we don't have all of the data on these 927 cases. We were not able to go back and really find out a lot of detailed information about them.

But, from what we do know, it appears that they would not have been picked up clinically.

DR. MITCHELL: I think that the question is whether other screening--do they still do premarital screenings in most states and do those pick up a large percentage of new cases of syphilis?

DR. MARKOWITZ: Some states have eliminated premarital screening, but, actually, I don't have those data. So it is not done universally. One place where we do screen is in jails and detention centers. That has been a major component of the syphilis elimination effort, to pick up cases who have not been previously treated in those facilities.

DR. MITCHELL: So are you saying that when you screen in a high-risk population, you are likely to get--let me see; are you saying that screening in low-risk

populations does not provide a significant portion of the number of new cases that you see?

DR. MARKOWITZ: Yes; that is true. Screening in low-risk populations does not provide a large percentage of total cases that are reported. The vast majority of the cases that are reported are through SDT clinics, people that come in for care, the vast majority.

DR. HOLLINGER: Yes? Go ahead.

DR. EPSTEIN: While we have Dr. Markowitz, I have two questions. Your estimate for the cases identified through blood-center screening were 36 primary-secondary and 785 early-latent. But the Red Cross data, which were on whole-blood-donor screening, which should have been only even a subset if some of your data also came from source-plasma screening, which were based on only 500,000 per month, average monthly figures, if multiplied by 20 to give us a yearly estimate, would have estimated 8,500 FTA-positives of which 2,000 would be RPR-positive.

So there is, at the very least, a ten-fold discrepancy between the estimate through the surveillance system and the directly applicable actual report data from the Red Cross. I wonder whether you had noted that and whether you have thought about that in terms of your estimate of the potential risk of stopping donor screening.

And then a second question; you showed a slide

which went by rather quickly on the percent of antibody-positives that had positive PCR. I wondered if you could simply repeat what those findings were, perhaps show the slide again, if that is possible.

DR. MARKOWITZ: The first question is that the Red Cross found--extrapolated by many more positives that we found. That doesn't really surprise me because we were not reporting positives. We were reporting how many, actually, of the positives turned out to be cases that got reported.

DR. HOLLINGER: Actually, with disease.

DR. MARKOWITZ: With disease. We did a small study in Chicago last year to just evaluate their surveillance system, and it was something like only 5 percent of the positive serologic tests for syphilis that come in to the health department ultimately get reported. The rest of them, they determine, are old follow-up titers that people are getting serial titers done to follow treatment and they are determined not to be cases.

There is a huge amount of work that gets done just to wade through all of these serological tests that come in to the health department and to find out which ones of those are actually cases. So that does not surprise me at all. Health departments, the labs in Chicago, for example, 43 labs are required to report every month to the health department.

There is just a huge amount of data coming in so that is consistent with, I think, what goes on.

DR. EPSTEIN: The second question?

DR. MARKOWITZ: The second question? Can I see that slide? Do you just want me to go over the data?

DR. EPSTEIN: Yes; I would just like you to restate what percent of seropositive had positive PCR.

DR. MARKOWITZ: It varied by the different tests and, if I can get a hard copy--can you get that slide back up? Is that possible? Oh; I have it here. Of those who were RPR-positive, one to one to one to four, four out of seven, or 57 percent, were polA-positive. Of those who that had a titer greater than or equal to one to eight, six out of fourteen were polA-positive.

And then, of those that were MHA-TP-reactive, nine out of fourteen were polA-positive. Of those that were MHA-TP-nonreactive, one out of four was positive.

DR. HOLLINGER: Thank you. David?

DR. STRONCEK: My understanding of this is we have a disease that is not transmitted with blood. There is pretty good data to suggest that, at least not at this time. We don't know why. We don't know if the donors just don't have it. We heard data on one test, the PCR comparison that was done on inadequate samples. And then there is some thought that the processing of blood may inactivate the