fact, didn't occur. I mean that was quite surprising to me and I think to them, as well.

So, I think you are correct, it must be quantitative. There is some level of virus within a preparation, that may overwhelm the antibody that is present. That is the way I would interpret the data that have been presented.

Referring to the question as to red cell aplasia, just to make it clear, there is transient aplastic crisis, which is an acute self-limited, although in some instances severe, pure red cell aplasia, but it will get better within a week or two when the patient makes antibody.

That appears to occur only in individuals who have a shortened red cell survival, so that the cessation of erythropoiesis has that consequence.

Now, the chronic pure red cell aplasia that appears as a hematologic disease is a very rare diagnosis. I can't give you an incidence figure because it's too low, it has just not been measured. It is rarer than aplastic anemia.

I think that there are not, to my knowledge, there are not documented cases of pure red cell aplasia due to B-19 in which there is not an underlying immunological deficiency, and the only reservation I would have is that sometimes that immunological deficiency is subtle if it's

congenital, but those are extremely rare.

So, I would not consider that a high risk, and I think that in reference to Dave's comment, even if a donor parvovirus-positive, informing that donor on the off chance that he would be the first one to develop pure red cell aplasia is probably not necessary since examination for B-19 is part of the evaluation of pure red cell aplasia anyway.

So, one would have the scenario, the patient would present to a hematologist, have a bone marrow performed, and then a B-19 test would be done anyway if he were found to have pure red cell aplasia. I would think that is purely hypothetical in any event.

DR. HOLLINGER: Do you know if any IVIG lots or conventional immune globulin lots have been looked at for parvovirus B-19 nucleic acid?

DR. YOUNG: I think the answer is yes, I think you can find it.

MS. YU: I am from the FDA. My name is Mei Ying Yu. There are published papers that IGIV, there are B-19 DNA present. We don't know the infectivity. This is measured by PCR. There are quite a few references.

DR. HOLLINGER: Do you know what the titers were?

MS. YU: I think they are relatively low. I think when they are measured, they are also--this is John Salana and Phil Miner's paper--I think they are relatively low

titer in IGIV and IG compared with the plasma pool. I cannot remember exactly, but it is 10^3, 10^4 per mL.

Again, it depends on the manufacturer, different manufacturers. I think they did look at several manufacturers. There are differences based on the different manufacturing procedures.

DR. HOLLINGER: Thank you.

Dr. Stroncek.

DR. STRONCEK: I have got another question for Dr. Young. Are there no circumstances where you think it would be important to tell the donor that they were acutely infected with parvovirus? It is a very important issue for me anyway to maintain the trust of the donors. How long is the viremic phase, would it be conceivable a woman could have the viremia and then be thinking about becoming pregnant, and would it be best for her to decide not to, or is that not an issue?

A lot of your data suggest that there is still a lot of questions as to exactly what does parvovirus cause, and I have a concern that maybe we haven't tested enough to know exactly what the full implications of this infection are.

DR. YOUNG: Taking the second comment first, obviously, we don't, we are not absolutely confident of the full spectrum, although I hope I conveyed my skepticism that

as the boundaries of parvovirus disease become extended, the data become less and less reliable.

For the first, obviously, you can detect virus by PCR for weeks, months, and many, many months after an acute infection, but there is no evidence that, for example, in a pregnant woman, would result in the typical problems in mid-trimester. I mean, of course, you never say never, so I can't tell you that it is absolutely impossible, but the well documented, and there are many well-documented cases of hydrops fetalis. When infection is known, it is known during the mid-trimester, in other words, it is not an infection, the woman doesn't remember getting fifth disease before she became pregnant and then develop the hydropic infant later.

I think that it is unlikely, and as long as pregnant women are not part of the donor pool, as they are not, they are the only category I can think of that would concern me in terms of notification.

DR. HOLLINGER: Dr. McCurdy.

DR. McCURDY: There is something that I think goes through a lot of the discussions that we have, this one and a number of others, and that is that most of the surveillance that has been done I think for B-19 has been passive surveillance, and most of the surveillance for a lot of other diseases has been passive surveillance.

Sometimes when active surveillance is added at a later time, you get a vastly different picture. I can remember when passive surveillance suggested that post-transfusion hepatitis was about a half a percent or maybe 1 percent. A few years later, active surveillance showed that it was really at that time 10 to 15 percent.

I think there are a number of other instances. I think that parvovirus tends certainly not to produce any significant disease, at least in most people, and I am perfectly willing to accept that. I have long suspected, however, even before I knew parvovirus was involved, that normal individuals may have aplastic crises like patients with sickle cell disease, but people with red cell survival of 100 to 120 days don't miss a week of no red cell production. It would hardly be measurable in the blood hemoglobin level, for example.

DR. HOLLINGER: Any other comments? Yes, Dr. Nelson.

DR. NELSON: I wonder if Dr. Young could comment on the genetic diversity of B-19, in other words, are there any problems with the DNA, the quantitation of the DNA based upon genetic diversity or is this a very conserved region, and that is not a problem?

DR. YOUNG: Kevin Brown can also comment on this. Certainly the studies have been done have suggested there is

very little variation in B-19 isolates worldwide, and the genome probably doesn't vary by more than a few percent.

The only exception, as I mentioned, the subject of this one publication V-9 virus, which was found in a patient with transient aplastic crisis and which was not detected by PCR.

So, I think that there is the possibility that there are other similar parvoviruses of the erythrovirus type that will not be detected by most of the current PCR strategies.

DR. HOLLINGER: Also, Dr. Young, do you think there is a difference between the disease that is produced when you have associated antibody? The issue often was that in patients who were given hepatitis B immune globulin and got infected, they probably had some of a modified disease, didn't seem to become carriers, but had a modified disease in a great way, and I am wondering if this is the same thing, if you had a single unit, that this might be completely different if they didn't receive other units with antibody in there or something like this.

DR. YOUNG: There is no question that there is a difference with B-19 as to whether there is antibody present or not. As I showed in the normal volunteers, if there is no antibody, then, you have a viremia that dominates. There is fever, but you don't have the typical fifth disease

symptoms, and when there is antibody you get fifth disease because it's due to immune complexes.

But I think beyond that, it is really difficult to say, and having looked over the VITEX data, certainly, some of the recipients who seroconverted didn't have typical fifth disease symptoms, but I don't think we can speak more to that because, you know, you are recognizing the disease and then saying, well, I think maybe there is parvovirus, and much as Paul said, it is rather passive.

So, one looks for parvovirus in the setting where you have parvovirus typical symptoms, and it is hard to know how to interpret the absence of that sort of correlation since it has not been looked for, but I think it is certainly possible, I just don't know.

DR. HOLLINGER: Dr. Boyle.

DR. BOYLE: Just two quick questions. It states there are no confirmed reports of transmission by IG or IGIV. Would it also be fair to say that there are no cases in the literature of red cell aphasia in immune-deficient patients on IVIG or IM?

DR. YOUNG: In fact, we treat those patients with IVIG, so I don't know any cases in which pure red cell aplasia has been induced by the administration of IVIG. I have always assumed that that is protective.

DR. BOYLE: The second question is, is that two of

the risk factor that were mentioned were pregnancy and immune deficiency.

Is there any multiplicity of risk factors? If those two occurred at the same time, would it increase the overall risk factor?

DR. YOUNG: Again, you are talking about two very rare events, you would have to have a pregnant immuno-deficient woman, so what you would predict is that a pregnant immuno-deficient woman might develop both pure red cell aplasia and also transmit virus to her fetus.

I actually think that that has occurred because there are actually cases in the literature of multiple pregnancies resulting in hydrops in pure red cell aplasia, in a pure red cell aplasia woman, but we have never been able to document that that was due to B-19.

DR. HOLLINGER: Colonel Fitzpatrick.

DR. FITZPATRICK: On the immune-suppressed patients, we have got a lot of literature on CMV, but I don't recall a lot on B-19 disease in immune-suppressed patients.

Is there an incidence rate?

DR. YOUNG: No, it is far too low to actually warrant an incidence rate, but as I mentioned, the pure red call aplasia that occurs, I don't think it is susceptible to the virus. I mean everybody gets the virus. When they get

it, those patients who cannot mount an effective antibody response have a risk which is unknown. I don't know that it's 100 percent, but a risk of persistent viral infection in the bone marrow and chronic pure red cell aplasia.

We know that that can occur in a number of settings of immunodeficiency from congenital, Nisalot syndrome, cytotoxic chemotherapy in a leukemic child during consolidation, and in AIDS patients, but I don't know, for example, whether that might occur in someone who is on 40 mg of corticosteroids for a month. We just don't have that much information.

DR. HOLLINGER: We have a question before the committee, so I think we ought to deal with it.

I will just read it. Does the committee agree that pending a policy on screening of whole blood donations, FDA need not require studies to validated the clinical effectiveness of NAT or B-19 DNA under IND for plasma for further manufacturing?

Maybe, Tom, you could just briefly tell us what are the implications of a yes or a no answer here.

DR. LYNCH: Maybe. It is kind of hard to give the committee a real sense for how high the licensing standards are for an in-process control. These are not trivial hurdles to leap.

I did provide a small appendix that really was a

laundry list of the kinds of things that we look at, both for an in-process control and a donor screen, so all of those things would be in place up to the point where we would ask a sponsor to go and screen 200,000 donations.

I think in this case, there is very little doubt that screening a large number of donations from the general public is going to generate a lot of positive results, and they are going to be legitimate positive results, and I think that information is not going to add a lot to the confidence that the test is performing adequately over and above the preclinical evaluation of the performance of the assay using known samples, dilution panels and clinical samples that have been set aside for exactly that purpose.

So, what I would envision is, in the near future, a number of submissions coming in to the agency to implement testing of plasmas in incoming raw material. We will have to determine some performance characteristics of those assays. I don't assume that we are going to get a one size fits all PCR submitted that everybody is going to adopt, so we are going to have to look carefully at the context in which the testing is performed, the size of the pools, the sensitivity of the assays, what sort of cutoff or level of sensitivity and depending we should ask for.

Dr. Young and Dr. McDonough, I think, both mentioned that we have a benchmark established through

clinical trials with the solvent detergent plasma, and provisionally, pending the continuance of that trial, we believe that plasma that has a fairly constant level of antibody present in it, and levels of B-19 below 10^4 genome equivalents will not transmit the virus to recipients of that plasma. That needs to be verified fully by the clinical trials, but that is an example of how a rational threshold can be set.

How we will do that for the manufactured products is not entirely clear yet. We are working on setting criteria or standards for making those determinations now, but I have a feeling that we are going to have to exercise some flexibility depending on the nature of the product, the nature of the manufacturing process, and the nature of the test.

DR. HOLLINGER: So, this is a less stringent process here than the clinical trials, which require a lot more.

DR. LYNCH: I think there is a lot more overhead associated with performing a clinical trial. In some cases, you may be useful information out of the trial, and therefore you may say it's more stringent because your informational demand is higher, but we think actually the stringency is equivalent in this case because there isn't a whole lot of additional information that we anticipate would

be produced by going through the exercise of performing a clinical trial.

We think that we are going to get the same level of confidence, same level of assurance of the performance of the test - sensitivity, specificity, reproducibility in preclinical validations as we would in the clinic. So, in that sense, it is just as stringent.

DR. BUCHHOLZ: I have a comment or a question about the question. I have been impressed in the rather nice distinction between the concept of donor screening and in-process control, and I think that that is a very nice intellectual construct in terms of helping us think about this, but by phrasing the question the way it is phrased, and using that word screening whole blood donations, does FDA mean to imply that the rationale for this test being used in the situation of whole blood or its components might, in fact, be viewed differently than it would be during plasma? That is, is the implication here that this would be a blood screening test and subjected to different requirements for whole blood than it would be for plasma?

Secondly, given the whole issue of whole blood, is it appropriate for this committee, in a second question perhaps, to provide some guidance to FDA on the whole issue of what the relationship of this test should be to whole blood donation?

DR. HOLLINGER: Dr. Epstein.

DR. EPSTEIN: On your several questions, yes, we are saying that the debate whether there should be routine screening of donors can be separated from the question of a manufacturing process control, and it is therefore implied that the standard for approving it as a donor screen might indeed be different. So, the answer is yes to your first question.

On the second point, which is--I am sorry, can you repeat the second question?

DR. BUCHHOLZ: Whether it would be appropriate for the committee to, at this time, make a recommendation to FDA.

DR. EPSTEIN: No, we would argue--well, the committee can do as the committee pleases, but the FDA is not asking the committee for its opinion on parvovirus B-19 screening of the blood supply. We think that more data would need to be examined and we think that the whole question of whether it is feasible to do selective screening of a small number of units to protect patients at risk would have to be developed as a strategy before we would bring that question to the table.

I think that it was Dr. Bianco who hinted at that possibility, and we would want to develop that issue before we brought the question to the table. So, I think that what

we are saying, to come back to your question that was answered by Dr. Lynch, the practical implication of a vote in the affirmative is that FDA would not ask manufacturers to validate B-19 testing as a donor screen, therefore, we would not be requiring that positive minipools be traced back to identify the positive unit, we would not be requiring that such identified donors be notified, we would not be requiring any kinds of lookback tracing of recipients of prior units.

So, in other words, we would be dissociating the testing of the minipool from any issues related to medical interventions related to the donor or the recipient of a component, and we are asking, in sharp distinction to the way we looked at the issue for HIV, HCV, and ultimately HBV, where we said I am sorry, you have asked us whether we can implement this as a process control, but we think you are screening donors and should be screening donors, in this case, we would be saying okay, if you are going to screen minipools and simply pitch positive pools with the proviso that you do identify and date untransfused units and remove them, that we will not further regard this testing as donor screening.

That is what a vote in the affirmative means. I guess one more statement I would like to make. I think that where some confusion is coming in is that if a manufacturer

implements parvovirus B-19 testing as part of the manufacturing, and then seeks to make some kind of effectiveness claim regarding parvovirus safety of the final product, that might very well require additional clinical studies of that final product.

We are just saying that the studies would not be studies to validate the assay per se.

DR. HOLLINGER: Yes, Joel.

DR. VERTER: Dr. Hollinger has commented that I haven't been vocal enough today, so, unfortunately he took a little of my steam out of my question because I was going to comment--and I will comment--that after five years on the committee, this may be the last question I get to say this on--but I think you took what probably could have been a simple question and made it so complex, that it's hard to figure it out.

You know, we have rewritten your questions many times in these five years, but I appreciate your clarifications, I understand it. I have only one question. What happens to the pools that are identified as positive?

DR. EPSTEIN: The pools are virtual pools. You have a set of units that may or may not go into manufacturing. You pool samples from them, you test that pool of samples, and then you decide whether you can or cannot use the units themselves.

So, you don't actually have a manufacturing pool.

DR. VERTER: I understand. So, what would happen to--

DR. EPSTEIN: So, if you test the minipool of, for argument's sake, 20 units, samples from 20 units, and you get a positive result, the proposal is that all 20 units would then be discarded, and the question for FDA is whether to take the point of view, no, you have to find the actual positive unit and furthermore tell the donor and furthermore do lookback, et cetera, and we are saying no, we are proposing to the committee our willingness to accept it as a process control, pitch the positive pool, you are done.

DR. VERTER: Given what has been said and what little I know, I may be pretty naive here, doesn't that suggest that an awful lot of units are potentially going to be tossed?

DR. EPSTEIN: No, because as has been said, the frequency of high titer positive units is fairly low, about 1 in 10,000, and the master pools would be pools of 100, so, you know, it is not that high.

DR. HOLLINGER: Dr. Stroncek.

DR. STRONCEK: A couple of things. One, the danger of a high titer pool, my understanding, it has been looked at pooled samples, but many of these samples that come from single donor units like NB red cells that would be

transfused, and we really don't know the difference in clinical problems and a high titer versus low titer pool.

The second thing is, is there a timeline where this testing has to be done, because if it's stretched out long enough, and the pools are positive, all these red cells would be transfused, so you are saying that they must get rid of them. So, we are making the implication that it's important not to transfuse these units, which I believe is really relevant, because most patients that are transfused at my institution are immune-suppressed, yet, you are not giving any time restriction, and red cells only last 42 days, so many of these might be transfused.

DR. EPSTEIN: The vast majority of red cells associated with donations for which there was a positive minipool would have already been transfused under the current scenario.

The committee could, if it wished, advise us that we should encourage companies that implement this to do testing in a time frame such that in-date units might still be identified. The current scenario, however, is that because these are mainly recovered plasmas, tested many, many days later, that there are unlikely to be any in-date units on the shelf.

But I do agree with you that we could apply some regulatory pressure that the testing be done as soon as

possible to permit the interdiction of high titer units on the shelf, but I think that that would be a question above and beyond the issue of whether to further validate it as a donor screen because it is already our policy that there should be retrieval and presumably destruction of in-date units.

We are just at the moment not changing the scenario of testing that exists, which is that it is delayed testing, but I accept your point that we might be missing here an opportunity to interdict some percent of the high titer positives that didn't happen to get used in the time it took to do the testing.

But this is going to be delayed testing because it is mainly recovered plasma being tested, at least in the current environment.

DR. BUCHHOLZ: Jay, did you mean to imply, when you talked about the minipool of 20 units or however many go in, that that pool would be tossed, that, in fact, there did not exist an option to identify the individual unit or if that were done, that that triggered some other sort of requirements to then do something above and beyond what you would do if you simply threw all 20 units in the pool out?

I wasn't clear.

DR. EPSTEIN: We are being asked to accept a proposal that if a minipool test on 20 units, what was

called the primary pool, is positive, that the manufacturer would discard all 20 units whose samples constituted the minipool and would not do any further testing to identify the actual individual positive donation.

DR. BUCHHOLZ: So, the manufacturer would actually be, in essence, prohibited from doing that.

DR. EPSTEIN: Wouldn't be prohibited, and it would be physically possible. I think the underlying question is whether there is an obligation to do it. You know, it does change cost and logistics to require that they test further. This was the very issue that we dealt with--

DR. BUCHHOLZ: It would still remain an option to identify it further.

DR. EPSTEIN: Yes, it would.

DR. BUCHHOLZ: And not prohibit or not fall into some other category is one went ahead to do that.

DR. EPSTEIN: No, we would not be prohibiting further testing. The question is whether we would accept it if it were not done. Now, again, it could be the sentiment of the committee that should testing be performed in a time frame where the likelihood of untransfused units exists, then, it becomes more important to identify the unit, but again, the alternative would be to pitch all the components from all 20 donations.

It still is a small number because remember you

are only going to hit positive pools at the rate of about 1 per 100, you know, if it's 1 per 10,000 in pools of 100, less than that when you get down to the pools of 20.

DR. HOLLINGER: Dr. Chamberland.

DR. CHAMBERLAND: Jay, just based on your last couple of comments, I think I want to get a clarification for myself.

I think when I initially saw this question posed, I took it to be sort of a generic or a generalized question, but your last comment seemed to relate it very specifically to the one firm that has actually approached FDA and has a very specific protocol or procedures in place with a specific number of units constituting a minipool, et cetera.

I also heard Tom Lynch say that you expect there will likely be other firms that come to you, and their procedures may be different. There may be different numbers of units in a minipool for that particular end product.

So, just to clarify, are we voting on really the general question, not the specific VITEX procedure?

DR. EPSTEIN: Yes, you are voting on the general question because we sort of have a watershed decision to make here. We have a proposal in front of us to accept, if you will, a manufacturing supplement, the implementation of minipool testing for B-19.

We can either accept it based on preclinical data

or reject it on the grounds that it is insufficient because a clinical trial is needed to validate the assay. What I think Dr. Lynch has argued, and others have appeared to support, is the concept that those additional studies would not lend value to the assay for its purpose in controlling the virus titer for the manufactured product.

We are just asking whether the committee concurs with that view. Now, if it is a strongly held view that the clinical issues pertinent to the donor lookback, you know, possible infections in recipients are such that we should not take that point of view, that would then be a vote in the negative.

DR. HOLLINGER: I am going to call the question here. The question is up there on the screen.

All those who in favor or vote affirmative on this question, please raised your hand.

[Show of hands.]

DR. HOLLINGER: Any opposed?

[Show of hands.]

DR. HOLLINGER: Two.

Abstaining?

[No response.]

DR. HOLLINGER: Would you read the final vote, please.

DR. SMALLWOOD: The results of voting. There are

11 yes votes, 2 no votes, no abstentions. There are 13 members eligible to vote here.

The industry representative agreed with the yes vote. The consumer representative--

MS. KNOWLES: Would also agree.

DR. SMALLWOOD: -- would also agree.

DR. HOLLINGER: Thank you.

David, do you have a comment you want to make presumably about what you just said a minute ago?

DR. STRONCEK: Yes. I am in favor of this process to streamline the approval of this testing, but I have reservations about ever having any knowingly parvo B-19 red cells in inventory, transfusing them into a marrow transplant patient or other immunosuppressed patient. It would just not be acceptable, and I believe that the testing should be done as quickly as possible, and if a plasma is found to be positive, the red cell unit should be recalled as quickly as possible.

DR. HOLLINGER: Just a comment to that. Do we know that red cells, which have less plasma in them, are as much of a risk as getting 220 mL of plasma?

DR. EPSTEIN: I don't think anyone knows that.

DR. HOLLINGER: I think we will move on then to the next agenda item. It says here we are supposed to go to lunch. [Laughter.]

The next item is on Antigen/Antibody Testing for Malaria. It also is informational. We will start out with an introduction and background by Dr. Syin.

III. Antigen/Antibody Testing for Malaria - Informational

Introduction and Background

Chiang Syin, Ph.D.

DR. SYIN: Thank you, Mr. Chairman.

[Slide.]

Today I will take the opportunity to outline the current status of malaria diagnostics development.

The known history of induced malaria starts approximately a century ago when scientists in various countries confirmed the infectivity of blood from malaria patients to susceptible persons.

Early in this century the rise of blood transfusion was accompanied by an increasing incidence of transfusion malaria, the first case of which was documented in 1911. Over the years, many tests have been developed for malaria diagnosis primarily for patient management or epidemiological studies. However, there is no FDA approved test to date to screen donated blood for malaria risk.

Instead, blood establishments have to rely solely on donor questioning to identify prospective donors at risk for transmitting malaria. In light of recent progress in

the field of malaria diagnostics, especially in the area of rapid diagnostics based on antigen capturing assays, we feel it is appropriate to share with you what have learned about these tests.

In today's session, you will hear a series of presentations on malaria antigen/antibody testing. I will present a brief background on malaria.

[Slide.]

It will be followed by Ms. Freddie Poole, a scientific reviewer from our sister center, CDRH. She will present the regulatory perspective on the antigen detection assays for malaria.

The malaria rapid diagnostic devices currently are undergoing field study in several endemic regions, which will be presented by Colonel Robert Gasser of the Walter Reed Army Institute of Research. He is the principal investigator of these clinical trials sponsored by the Department of Defense.

Dr. Phuc Nguyen-Dinh of CDC will then speak about their analysis on transfusion transmitted malaria based on data collected from 1963 to 1998 in the United States, and their current thinking on malaria tests intended to prevent the incidents of transfusion transmitted malaria.

[Slide.]

As you can see, malaria once considered close to

eradication in the sixties, has re-emerged as one of the major threat to the world health.

Globally, over 40 percent of population, as shown in red, are currently living under the shadow of malaria with an annual mortality of 1 to 2 million.

It is estimated that there are more than 100 million of new infections each year.

Increasing development and spread of drug resistance in malaria parasites, most noticeably Plasmodium falciparum, has posed a great challenge for us to contain malaria resurgence.

[Slide.]

There are four species responsible for all the human malaria infections, namely, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium falciparum. Plasmodium vivax and Plasmodium falciparum are the most prevalent and Plasmodium falciparum is responsible for most of the deaths reported in malaria.

The immune status of infected host obviously plays a role in the development of malaria, however, there are several distinct characteristics on each species that I would like to point out to you.

First of all is the incubation period, which is the incubation period, which is the interval between infection and the time that symptoms first appear. In

general, with the exception of Plasmodium falciparum, the other species have all been documented to have long incubation period.

Vivax and ovale malaria are also known to relapse, which is caused by latent hepatic parasites, reinitiate a new infection in blood. The infection by Plasmodium malariae may last for many years. In some cases, it has been documented as long as 50 years. The severity of the infection due to Plasmodium falciparum, as you can see from the slide, falciparum could have high parasitemia, and it can also be seen in a very short period of time due to the parasite tend to sequester in the infected patient.

[Slide.]

I am going to show you the life cycle of malaria parasite. Most of the people probably know this. Malaria infection in host is initiated by female anopheles mosquito injecting sporozoites while taking a blood meal.

The sporozoites quickly travel into liver and invade the hepatocytes and transform into schizonts through the incubation period.

As the reach maturity, the hepatocyte will rupture and they release thousands of merozoites into the bloodstream. This is the stage causing all the symptoms and all the merozoites as they invade a red cell. They will through cyclic replication. This is also the major stage.

The malaria tests have been targeted into this stage.

[Slide.]

Our current policy on the donor deferral for malaria risk was established in July 26, 1994, in FDA's memorandum to registered blood establishments.

Within this memorandum, it states: Person who are residents of non-endemic countries are deferred for one year after return from the malarious area, provided they have been free of symptoms suggestive of malaria.

The second item is the persons who have had malaria are excluded for three years.

The third item is immigrants, refugees, citizens, residents of malaria-endemic countries are excluded for three years after leaving the malarious areas, provided they have remained symptom free.

We have subsequently revised the donor questionnaire and included the definition of residents, which is five years or more, and an additional requirement of three-year deferral for former residents of an endemic area who traveled to endemic areas.

This has been included in a new draft, "Guidance for Industry - Recommendations for Donor questioning regarding possible exposure to malaria," as presented to BPAC meeting during June, and we expect the guidance will be issued later this year.

[Slide.]

In general, over the last 35 years, according to what the data shows, the risk of transfusion transmitted malaria in the U.S. remains very low. It's about 1 case for every 4 million units collected. So far we have documented 91 cases resulting in 10 deaths between 1963 and 1998.

All four Plasmodium species has been documented. It is interesting. Even though falciparum and vivax are the most prevalent malaria in patients in general, donors, malaria actually has occupied an equitable position as the cause of transfusion transmitted malaria.

[Slide.]

In addition, we have tabulated the last five years of error/accident reports received by FDA from blood and plasma establishments for malaria risk.

This is provided by our Office of Compliance and Biologics Quality.

On the list, "Post donation information" and "Gave malaria history but not deferred" accounted for most of the incidents.

You can see the total number in the last five years, it is going through the increasing trends, and Fiscal 1999, we only have nine months data collected between October 1st to June 30, 1999.

We estimate based on the nine months' data, you

can see this number is going to be over 1,500.

[Slide.]

In addition, we also need to carefully examine the issue of blood supply. What I am showing in this slide is the list of major donor deferrals in 1998, ranked numerically and compiled by America's Blood Center.

Celso Bianco has kindly provided this slide to show that among major risk factors for transfusion, malaria actually has the second highest deferrals with over 15,000 donors out of 4 million donations collected by ABC being deferred due to malaria risk, which is shown in this highlighted area.

It's about 0.38 percent of total donors for ABC.

Based on this projection, Dr. Bianco also estimates of all U.S. donors based on their data suggests that close to 50,000 donors will be deferred in the U.S. donor population.

The potential impact on the nation's blood supply cannot be overlooked.

Again, a good screening test for malaria may help to resolve this issue.

[Slide.]

I am going to show you the last slide in my presentation.

Let me summarize current malaria diagnostics as

shown in the slide. I won't go into the details since most of them will be discussed by the other speakers. Microscopic examination of Giemsa-stained thin or thick (preferred) smears is considered the gold standard of malaria diagnostics.

The reading of the smears by a well-trained microscopist could confirm malaria infection, the species involved, and the parasitemia. However, this is a labor-intensive process that requires a high degree of expertise for proper identification. Therefore, it is considered not suitable for blood screening purpose.

QBC system, a Class III device cleared by CDRH under 510(k), is a test developed by Becton Dickinson based on acridine orange staining. In addition to the above mentioned disadvantages for microscopy exam, QBC cannot differentiate among Plasmodium species.

Other tests such as PCR and serological tests are mainly developed by many laboratories for research uses. Most of the current commercial development is in the rapid diagnostics arena. These tests are all antigen capturing assays targeting either histidine rich protein 2 or lactate dehydrogenase in Plasmodium falciparum. The antigens for other species have not been identified by the manufacturers.

What I am going to do is I am going to stop here and turn it over to Ms. Freddie Poole of CDRH. She will go

over the regulatory prospective of CDRH on malaria diagnostic tests.

Thank you.

Presentation

Freddie Poole

MS. POOLE: Thank you, Dr. Syin.

[Slide.]

At the Center for Devices and Radiological Health, in vitro diagnostic devices are regulated under the authority of the Food, Drug, and Cosmetic Act. The Food, Drug, and Cosmetic Act was amended in May 1976 to add medical devices, in January 1990 to add the Safe Medical Devices Act, and in 1997 to add the FDA Modernization Act.

Before I go into the specifics regarding Malaria Antigen Detection assays, it is important to understand how devices are regulated at the Center for Devices and Radiological Health.

In vitro diagnostic devices are classified under the Act in Section 513(a). They have three classes - Class I devices and Class II devices usually can be compared to another device or found to be substantially equivalent to a legally marketed device.

[Slide.]

For Class I devices, general controls can provide reasonable assurance of the safety and effectiveness of the

device, for example, good manufacturing practices, registration and listing, and recordkeeping by the sponsor.

For Class I devices, the diagnostic use of the test result is not represented as being used to support life or as having substantial importance in preventing impairment of human health, and the diagnostic use of the test result does not prevent a potential unreasonable risk of illness or injury.

[Slide.]

For Class II devices, general controls are insufficient by themselves. Special controls are required to provide reasonable assurance of the safety and effectiveness of the device. The type special controls that we use are guidance documents and the labeling regulations which are found in 21 CFR 809.10.

For Class I and Class II devices, manufacturers submit a premarket notification or a 510(k) as we call it, as described in Section 510(k) of the Act.

[Slide.]

Class III devices, on the other hand, usually have test results that are critical in the diagnosis of the disease. The test results presents a risk of misdiagnosis, which leads to illness or injury. Class III devices must therefore be supported by valid scientific evidence, usually in the form of well-controlled clinical trials.

A premarket approval application, or a PMA as we call it, is submitted for Class III devices.

[Slide.]

Malaria antigen detection devices or devices for the detection of Plasmodium antigen or antibody are of substantial importance in the diagnosis and treatment of a life-threatening illness. CDRH has therefore determined that these devices are Class III devices requiring a PMA.

[Slide.]

Microscopic examination of thin and thick blood films has been considered and still regarded by CDRH as the standard reference method for diagnosing infection with Plasmodium species.

The only device cleared by CDRH is the QBC malaria system. The QBC malaria system is a qualitative screening method for detecting Acridine Orange stained Plasmodium species using a fluorescent microscope. The QBC was classified as a Class III automated differential cell counter in 1982. The QBC tube at that time was approved for the quantitative determination of white blood cells, granulocytes, lymph, and monocytes. This system, however, does not differentiate or identify Plasmodium species.

[Slide.]

FDA is aware that malaria antigen detection assays have been developed that capture Plasmodium falciparum

antigen from a blood sample. There are a number of devices described in the literature that detect Plasmodium falciparum and some Plasmodium vivax.

The literature contains reference to monoclonal and polyclonal antibodies raised against specific antigens, a heat-stable Pf9 and a histidine-rich protein PfHRP-2. Assays use an IFA-IHA, ELISA Methodologies have also been described, as well as DNA probe for falciparum. However, none of these assays have been approved by the FDA.

A PMA for a malaria antigen detection test must therefore contain sufficient information to demonstrate that the device is safe and effective. At CDRH, review criteria have been developed to guide manufacturers in conducting clinical studies that would yield valid scientific evidence.

[Slide.]

The review criteria consists of nonclinical studies. The studies are usually done in laboratories and they are done to develop analytical information, as well as to validate the assay.

During these studies, characterization of the components of the assay, which includes description of the antigen, the antibodies, the quality control material, as well as standards, all calibrators are conducted.

The limits of detection of the assay must also be evaluated. Cutoff values or setting of the cutoff values

are conducted. Reproducibility studies or precision studies are done, and those include intra- and inter-assay, as well as lot-to-lot reproducibility.

If the device is intended for point of care, then, the reproducibility studies should be conducted at representative sites.

Cross-reactivity studies for malaria antigen tests cross-reactivity studies are conducted to challenge the antigen or the antibody selected.

Retrospective specimens from patients infected with other Plasmodium species and other similar parasitemias are evaluated, and patients infected with microorganisms that affect similar symptoms.

Interference studies are evaluated and they are usually done using samples containing substances, either endogenous or exogenous.

Stability data is also conducted to stress the storage and shipping conditions of the sample and the assay.

[Slide.]

Clinical studies are the most important part of the studies that are done, because during the clinical studies, the manufacturer or sponsor must demonstrate that the assays are safe and effective for the persons for whose use the device is intended. The studies should support the intended use of the assay and the probable benefit to health

should outweigh any injury associated with its use, and the reliability of the device must be demonstrated.

Clinical studies must be conducted following a unified study protocol. Clinical studies done abroad are acceptable. If the studies are not conducted in the United States, they should follow the Declaration of Helsinki.

[Slide.]

The following studies are important evidence of safety and effectiveness:

First, clinical sensitivity. To demonstrate the sensitivity of the assay for detecting Plasmodium falciparum or for differentiating Plasmodium falciparum from the other Plasmodium species, the following type information is provided.

1. There should be clearly defined populations to reflect the intended use of device.

2. A clear description of how disease status was determined, for example, clinical presentations, microscopic examination of thin and thick blood smears. Patient histories, to include the symptoms, diagnosis, and any other laboratory diagnosis. Patient consent forms are also included.

3. A clinical protocol must be submitted. The protocol should clearly define the objectives of the study, exclusion and inclusion criteria, and the study design. All

test methodologies, microscopic procedures, quality control and quality assurance methods must be developed and included.

4. The device should be tested at a minimum of three distinct geographical locations. Sites and investigators should also be identified.

[Slide.]

Clinical studies also should include clinical specificity. To determine the specificity of the assay for detecting Plasmodium falciparum or for differentiating falciparum from the other Plasmodium species.

The population tested should include patients with microscopic evidence of other Plasmodium species, other parasitemias, and other conditions with similar symptoms. A description of the method used to determine disease conditions should also be included.

[Slide.]

Validation of Cutoff Values. The clinical studies should include a validation that the cutoff values determined in the non-clinical studies are appropriate for the target population. The patients tested should also represent the spectrum of disease, that is, early through fulminant stages.

[Slide.]

Statistical analyses, when possible, should

include an analysis of Receiver Operating Curves and consideration for use of equivocal zones to help minimize false positive and false negative results.

[Slide.]

Our Division looks forward to working with sponsors to help get new malarial diagnostics into the market, and we also meet with manufacturers and review clinical protocols before the studies are implemented.

Thank you, and I will take any questions after.

Presentation

Robert A. Gasser, Jr., M.D.

DR. GASSER: I am Colonel Bob Gasser from the Department of Immunology at the Walter Reed Army Institute of Research.

[Slide.]

The Department of Immunology is really the malaria vaccine development group at the Walter Reed Army Institute of Research. As Dr. Syin indicated, for the last two years I have been the principal investigator behind our effort to identify a malaria rapid diagnostic device that we could bring in to the military inventory.

[Slide.]

Malaria is a gigantic problem around the world and it's a major problem for U.S. armed forces that were forced to deploy in endemic areas around the world.

[Slide.]

For the past 100 years, since malaria parasites were first characterized, light microscopy has been the benchmark standard for diagnosis. In expert hands, this technology is extremely sensitive and highly specific, and in addition to identifying the presence of the parasite, a single assay provides a wealth of clinically relevant data including the species of the parasites present and the severity of the parasitemia which correlates directly with the severity of disease.

[Slide.]

Problems with light microscopy, though, have existed really since the outset of its use, and these problems have not been amenable to ready solution over the passage of the years. Notable problems include skills of the microscopist. This is difficult to maintain in nonendemic areas. That is a major problem within the United States and for Western physicians in general, for Western laboratory personnel, and in endemic areas of the world, the financial resources available for health care systems are usually sufficiently limited that maintaining high degrees of skill in the laboratory personnel is also difficult.

In addition, light microscopy has some inherent problems. Infection with Plasmodium falciparum, which is the most serious form of malaria, during its 48-hour asexual

life cycle stage in the blood, the latter half of that 48-hour period is associated with parasite sequestration in the visceral circulation with the potential for false negative blood smears to result when peripheral blood is sampled and stained.

Mixed infections have historically been substantially under-diagnosed by light microscopy. This is becoming increasingly clear as new technologies, such as PCR and antigen capture tests, have allowed a re-evaluation of the presence of multiple species in single clinical specimens.

In situations of low-power parasitemia, light microscopy faces limitations, and in the endemic areas of the world, the cost and the fragility of the equipment required pose major problems, as well.

[Slide.]

For these reasons, alternative assays have been sought. In the military, we identified certain characteristics that alternative diagnostic technology would have to have before it would be useful for us.

First of all, sensitivity and specificity would have to approach those of microscopy in expert hands. This is substantially in excess of the sensitivity and specificity of microscopy as routinely performed in most settings.

Secondly, to the extent that reagents are involved in such assays, the reagents would have to retain stability after a substantial exposure--and we define "substantial" as being on the order of one year--substantial exposure to ambient environmental conditions of heat and humidity.

In addition, such assays would need to be simple to operate. There is no point in replacing the complex skills required by microscopy for a complex set of skills required to operate one of these assays, and for military purposes these assays would need to be physically robust. They are going to get beat up in helicopters, trucks, et cetera, and we need to know that they are going to be able to stand up to the kind of abuse they are likely to take.

[Slide.]

During the 1990s, civilian sector development of rapid malaria diagnostic devices advanced. Most of these devices employ a common technology, which is antigen capture on a membrane, and then additional reagents to produce a visible readout, in other words, immunochromatographic types of assays.

[Slide.]

The next series of slides gives a graphic demonstration of the technology involved. I apologize, I realize that this a sophisticated audience and you may be very familiar with the concepts here, but I would like to

make sure that everybody has a common frame of reference as we talk about this.

This slide illustrates a blood sample, typically in a well, or it could be applied directly, at the base of a wick. The wick is typically nitrocellulose. To the wick is bound a monoclonal antibody stripe targeted against a specific Plasmodium antigen. The blood sample will either have Plasmodium antigen in it if the patient has disease, or not if the patient does not have malaria.

[Slide.]

The blood carrying the malarial antigen with it wicks up the strip.

[Slide.]

As the antigen within the wick blood passes the bound antibody stripe, it binds in turn to that antibody stripe.

[Slide.]

A clearing reagent, typically containing a detector antibody with some sort of labeling on it--and different types of labeling technology are used, either colloidal gold, liposomes with dye, et cetera--is then added to the well or to the bottom of the strip.

[Slide.]

This, in turn, wick is past the bound antibody stripe creating an antibody-antigen-antibody sandwich.

[Slide.]

And the detectors give a visible readout. Typically, these assays require anywhere from 10 to 30 minutes to run.

[Slide.]

By the summer of 1997, we assessed that these types of assay had matured to the point that they were of potential military utility, however, then, as now, there were none of these devices which are FDA approved, nor which are commercially available in the United States.

The Department of Defense today is required to have FDA approval for diagnostic devices, drugs, and vaccines that it uses, so purchasing an off-shore manufactured device that didn't have FDA approval is not an option for us.

[Slide.]

In preparation for attempting to bring one of these assays into our inventory, we sat down and defined what kind of sensitivity and specificity criteria these devices would need to meet. We made several assumptions. First, we gave high priority to the detection of Plasmodium falciparum since this species of malaria-causing parasite is associated with the greatest morbidity and with the risk for mortality. The other species of human malaria very rarely, if ever, cause fatality.

Secondly, we placed priority on detecting parasitemia at higher levels of parasitemia for several reasons. Higher levels of parasitemia are obviously associated with greater imminent morbidity and risk of mortality, and secondly, if a test failed to detect parasitemia at lower levels, but had the potential to salvage a diagnosis as the parasitemia increased, it would still have potentially utility.

Specificity is important, but we felt that the risk of a false positive result was less than the risk of a false negative result, and with those basic criteria in mind, we developed these criteria. Keep in mind that 5,000 parasites per microliter, which is what we defined as high parasitemia, is equivalent to 0.1 percent parasitemia.

Typically, in severe malaria, you have 1 percent parasitemia, so this is two orders of magnitude below what would typically be expected to be severe malaria caused by Plasmodium falciparum.

[Slide.]

We selected after a thorough survey, we selected four assays for testing. Only one of these assays at the time was commercially available overseas. That was the ParaSight F assay. The other three assays we tested were all essentially prototypes.

[Slide.]

The objectives of our studies have been to compare these assays simultaneously under clinical conditions using microscopy as the reference standard, and to define the sensitivity for both P. falciparum and P. vivax at defined levels of parasitemia and to define the specificity of these assays for both of these two species.

[Slide.]

The subjects that we have enrolled have been patients presenting at the local clinics in endemic areas for diagnosis and therapy of possible malaria. Our study sites have been Iquitos, Peru, and Maesot, Thailand. We have also had a study site at the University of Toronto, the Travel and Tropical Medicine Center there, but our subject enrollment there obviously has been much, much lower than it has been in these other endemic area sites.

[Slide.]

Subjects. Inclusion criteria included the presence of one of the following three symptoms: fever or headache or history of fever within 72 hours of enrollment. We enrolled patient 1 year or older in Peru, and due to local human use considerations in Thailand, we were restricted to enrolling patients 15 years of age or older there.

[Slide.]

Exclusion criteria in the study we conducted last

year was that we excluded patients who had received antimalarial drugs, and this would also include antibiotics, such as sulfa drugs, floroquinolones, tetracyclines. It might have been given for bacterial infections, but which would be recognized to have antimalarial effects, or who were taking actual antimalarial therapy at the time of their presentation.

[Slide.]

Subjects were interviewed, their temperature taken. If eligible and willing, they were enrolled, and we drew their blood into EDTA containing tubes.

[Slide.]

Two study slides were prepared and one slide was prepared and given to the local clinic staff. The local clinical staff stained their own slide, read their own slide, and made their treatment decisions based on the slide that they had stained and read themselves. We saved two slides for subsequent staining and interpretation by our study staff.

[Slide.]

Precisely quantitated amounts of blood were delivered to these rapid diagnostic assays during our protocols. We recognize that this is not quite the way that they will be used in the typical clinical environment, but we wanted a high level of precision for our studies.

Each assay was tested by a separate technician who was blinded to the results of the microscopy and was also blinded to the results of the other assays which were being performed.

[Slide.]

Both of the two study microscopy slides were stained by protocol method. We had two microscopists read the first slide. The second slide was saved for later, as I will explain in a moment.

Using identical model microscopes and lenses at all sites, the microscopists were blinded to each other's interpretations. They had to read a minimum of 200 thick film oil-high power fields before calling a slide negative.

[Slide.]

Concordance of both microscopists was required for the presence of parasites, the species of the parasites, and the quantification of the parasites within a factor of 2.

[Slide.]

If the microscopists agreed, their reading was defined to be the true study result for microscopy. If their readings were not concordant, a third microscopist both slides 1 and 2, and using the combined findings on slides 1 and 2, that result was used as the final true microscopy result.

[Slide.]

We enrolled 2,162 patients in Thailand last summer of whom 2,155 were evaluable. The 7 excluded patients essentially were patients in whom it turned out the history forms had been filled out inadequately or in whom there were technical problems with the way the informed consent had been filled out.

[Slide.]

Similarly, in Peru, we had 838 evaluable patients out of 844 enrolled.

[Slide.]

Of our total patient enrollment of almost 3,000 subjects, 18 percent had Plasmodium falciparum, 22 percent had Plasmodium vivax by microscopy, 1 percent were found to have mixed Plasmodium and vivax infections. About 0.1 percent had Plasmodium malariae, and 59 percent were negative.

Six percent of the subjects had nonconcordant microscopy readings. More than half of these nonconcordant results involves discrepancies in the quantification of parasitemia at very low levels of parasitemia where the difference in identifying one or two parasites was sufficient to throw of the quantification by a factor of more than 2. So, we felt that this high level of microscopic concordance, which is really quite in excess of what you will see in most published studies that have looked

at this, testified to the high level of skill of our microscopists.

[Slide.]

For the ParaSight F test, which detects only falciparum, we came up with the following results: the overall sensitivity was 95 percent, sensitivity for parasitemia in excess of 5,000 parasites per microliter was 98 percent. The sensitivity dropped slightly as the parasitemia dropped, but even at very low parasitemias, parasitemias of zero to 500 per microliter, the sensitivity remained at 83 percent, and the specificity was at 86 percent.

As you can see, the sample sizes were large enough that we were able to get tight confidence intervals.

[Slide.]

The ICT malaria test, which is manufactured by an Australian firm, Amrad ICT, also performed very well. You can see the sensitivity was extremely high, 100 percent for parasitemias in excess of 500 parasites per microliter. Specificity for this assay was lower, though, only 67 percent.

[Slide.]

The ICT malaria test also detects vivax. The format of this test is that there is one test stripe that comes up positive for non-falciparum malaria or vivax

malaria only, and there is a second test line which comes up for positive for falciparum only. In some falciparum cases, both test lines may come up, so if you get both test lines up, it is an ambiguous result. It could represent either falciparum alone or it could represent falciparum plus vivax, mixed infection.

You will see two sensitivity results listed in this table, one defined as strict. The strict result was that case in which the vivax line came up and the falciparum line did not come up. The open sensitivity is the result in which both lines came up. Results in which the falciparum line came up are clearly false negatives for vivax.

This device, therefore, had pretty significant limitations in its ability to correctly characterize Plasmodium vivax although in those in those patients who had Plasmodium vivax, it did fairly well at detecting the presence of malaria.

We do not know at this point to what extent this represents a failure of microscopy to correctly identify mixed infections, and we are further examining that issue using PCR.

[Slide.]

The results of our studies from last year indicated that no one of the assays we tested met all of our needs although several came close. We provided feedback to

the manufacturers, conducted a second trial this year with refined products, and the results of which are pending, but which look improved at this point.

[Slide.]

We believe that this technology does hold near term promise for reliable malaria diagnosis and that there are several potential arenas in which it might be useful. Obviously, the first one and the one that we in the military are the most interested in is that it offers the opportunity for rapid and reliable clinical diagnosis in field settings. There is also the potential to use these assays as epidemiological tools potentially in malaria control efforts and potentially for blood product screening.

However, we recognize that for these different uses, modified formats and/or altered calibrations of performance emphasizing sensitivity even more highly over specificity might be required.

Thank you.

DR. HOLLINGER: Thank you.

Presentation

Phuc Nguyen-Dinh, M.D.

DR. NGUYEN-DINH: My name is Phuc Nguyen-Dinh. On behalf of my colleagues at the Division of Parasitic Diseases at CDC, I would like to discuss with you the potential role of testing for antigen and antibodies in the

prevention of transfusion-transmitted malaria.

[Slide.]

First, a reminder about the extent of transfusion malaria in the United States. This is a review by Dr. Mary Munga and her colleagues of the past 35 years. The bars show the number of cases, and the curve shows the incidence of cases. What this figure shows to us in that in the last decade there has been no major variation in incidence of transfusion malaria. The values remain relatively low as mentioned by Dr. Syin earlier at 0.25 cases per million of units collected. That is an estimate, of course.

[Slide.]

During that period, there were 91 cases for an average of 2 to 3 cases per year. There were 10 deaths, which is a case fatality rate of 11 percent.

As mentioned earlier by Dr. Syin, all the four species were involved in the transfusion cases, with an increase during the recent years of the proportion due to Plasmodium falciparum, which is the most virulent of the four species.

[Slide.]

These are the guidelines to prevent transfusion malaria, and we can just review them quickly. The current criteria for deferral are based on history. Potential donors are going to be deferred if they have had malaria, or

if they have been in a malaria endemic area.

For the travelers, the deferral periods vary from one year to three years, depending on whether the donor has been a resident of an endemic country or not.

[Slide.]

This slide shows that these deferral criteria did not completely prevent transfusion malaria. Among the 91 cases, in 58 donors there was enough information to indicate that 62 percent of the donors should have been excluded if the donor deferral guidelines had been correctly applied.

Of greater concern, in 38 percent, the donor would not have been excluded by these criteria. Among these donors, two-thirds had Plasmodium malariae, which is the species that can persist for a lifetime while causing only minimal symptoms in the carrier.

The next question then is whether these donors would have been deferred if, in addition to screening by history, a laboratory test had also been used.

[Slide.]

Laboratory tests can be useful in identifying the implicated donors. Among 65 such donors, in most cases the identification was by a laboratory test. Most often is was a positive malaria serology, showing that the donor had been exposed to the parasite.

Serology was done by immunofluorescence, IFA, and

in most of the cases that was done the CDC lab. In a smaller proportion, parasites were demonstrated by microscopic examination of the donors' blood, which is the proof of active infection. In some cases, a very small proportion, 4 percent, the donors were identified because they were the only donor implicated.

Please note that these laboratory tests were not performed with the leftovers or the segments of the donated blood units. They usually were conducted during epidemiologic investigation by going back to the donor and collecting blood from them. Thus, these test results do not necessarily reflect what would be found in the donated blood itself.

[Slide.]

In the implicated donors in whom blood information was available, one-third has a positive blood smear, and practically all had a positive serology.

Thus, if these donors had had these tests done, practically all would have been deferred on the basis of serology, and a third would have been deferred based on the findings of parasites.

In this case, we are talking about the microscopic examination of a blood smear, which, when performed by a well-trained laboratorian, as mentioned by Dr. Gasser, should detect parasites densities, which are as low as 5

parasites per microliter. That is something like 250 parasites per drop of blood.

[Slide.]

Next to microscopy, as again discussed by the previous speakers, there are other techniques that have been developed. However the techniques for antigen detection do not have a much better sensitivity than microscopy, as was shown earlier.

Here I have some figures also from other studies. In a study in Kenya, the immunochromatographic test for HRP-2 detected with 100 percent sensitivity only the blood specimen that had 100 or more parasites per microliter, a reminder again, a good microscopist would detect 5 parasites per microliter.

In travelers seen at the Hospital for Tropical Disease in London, the test for pLDH, which has also again been mentioned by Dr. Gasser, approached 100 percent sensitivity only in patients with 500 or more parasites per microliter.

One test that shows a much higher sensitivity would be PCR. A paper published from Vietnam describes its use for blood screening and in a dilution study described in that particular paper, PCR was found to detect parasite densities of 0.1 parasites per microliter for Plasmodium falciparum and around 1 parasite per microliter for

Plasmodium vivax.

[Slide.]

This figure is comparing the various techniques for detecting malaria parasites and their products.

The horizontal arrow shows increasing concentrations of parasites in the blood, and the vertical arrows show the sensitivities - the lowest parasite densities that can be detected by the various tests.

As said before, microscopy in the green arrow done by a skilled technician, and again we have to insist on the skilled technician, would detect down to 5 parasites per microliter. The various techniques for antigen detection that have been described by Dr. Gasser and also the previous table will detect at best 10 to 100 parasites per microliter, and also please note that one of the antigen detection systems, the one that is measuring HRP-2, is going to detect only 1 or 2 of the 4 species that infect humans, so it will not detect, for example, Plasmodium malariae, which you remember was a big component in the species infecting the blood transfusion units.

The test that can detect the lowest concentration of parasites is in the orange arrow, that is PCR, and it has a threshold 50 times lower than microscopy. Its threshold is 0.1 parasite per microliter.

The next question then is how would these tests

function to prevent transfusion malaria?

[Slide.]

Not too well, I am afraid.

Classic studies have shown that a human can be infected by inoculating 10 parasites. Specifically, in that particular experiment, it was Plasmodium vivax. If we suppose that those 10 parasites are in a unit of blood, that corresponds to a parasite density of 2.5×10^{-5} per microliter of blood, and that is at a very far away end from the PCR, which is the most sensitive technique that is currently available. It is 4,000 times lower than the threshold by PCR, and of course it is much lower than the threshold by the less sensitive techniques, such as microscopy and antigen detection.

One note of caution, however. We do not know where the parasite densities in the actual blood units that were given to the cases of transfusion malaria, where those parasite densities would be.

We know from the last three cases that were seen at CDC, that the parasite density was low enough not to be detectable by microscopy, but high enough to be detectable by PCR. So, it was somewhere here. But the universe of the 91 cases, we don't know where the parasite densities would be. Maybe they are here, and they could be detected by this technique.

[Slide.]

An alternate approach would be to detect individuals who have antibodies against malaria, and who are thus at risk of still having infection. Antibody detection does not quantify parasite densities. Even very low parasite densities can result in production of antibodies.

That serology might be a good option is suggested by the fact, that I have shown earlier, that it can be more effective in identifying implicated donors, and that the near totality of implicated donors were positive.

In addition, seroconversion occurs during the couple of weeks following infection. Thus, the seronegative window would be during or shortly during the travel or during the malaria episode, a period at which the donor would be deferred at any rate based on the history.

[Slide.]

The main disadvantage of serology is that positivity can persist, as you know, for a long time, years after the infection has been cleared. This will result in the exclusion of individuals who have retained a positive serology from a past infection that is now cured.

If serology were to be adopted for mass screening, there would be technical problems to address. Large amounts of antigen would have to be produced, and that would have to be provided by something else than the crude parasite

extractor currently being used. Probably one would have to resort to other things such as, for example, recombinant protein or peptides.

The test should also be able to react and to detect antibodies to all the four species, not only Plasmodium falciparum.

The test also should be automated, using a format such as ELISA, rather than the more cumbersome IFA. Finally, the issue of whom to test should be addressed. Should it be all the potential donors regardless of the history, or should it be only those donors at risk based on recent travel?

To address these questions, it might be informative to see what the other countries do.

[Slide.]

In France, they use a combination of history and serology as criteria for deferral. Individuals with a history of malaria are deferred permanently. Returning travelers are deferred for four months. From that time on, up to three years, they are screened with serology.

The serology is by immunofluorescence, using an antigen which is only Plasmodium falciparum, and not the other species, using these parasites grown in culture. The test is either made in-house, using an in-house format, or it uses a commercially available kit, which is called

Falciparum Spot. Note that the antigen is only one of the four parasite species, but the French people have told me that it has 85 percent cross reactivity with antibodies to the other species.

Another serology technique, the ELISA, has been used for a couple of years by the French group. That was using a commercially available ELISA kit, but they have stopped doing that due to reported problems with sensitivity.

[Slide.]

We also talked with our colleagues in the United Kingdom, and they have introduced since '98 some new criteria. They are currently using again a combination of history and serology as criteria for deferral.

All the donors having been in an endemic area are deferred for six months--that is compared to the four months of the French--after which they are screened for antibodies, and they are accepted if the serology is negative.

Note that the travelers who live in a non-endemic area do not need to be screened after 12 months, while the residents--this definition here of residents--who are arriving from endemic areas will need this screening beyond one year, and they will be deferred until they are shown to be seronegative. This new classification derives from a case of transfusion malaria that was fatal, that the British

had I think in '97.

[Slide.]

As for the people who have a history of malaria, they are deferred for six months, after which they need antibody testing before they can donate.

The antibody testing in the United Kingdom is by ELISA, using a commercially available kit, and when that test is positive by ELISA, it is confirmed by IFA. Note also that between the various countries of the United Kingdom, the country of Scotland, for example, is not doing serology. This is the only that differs from the three others.

The people who have a positive antibody test are deferred permanently unless they are tested again and the test reverts to seronegative, and if a test cannot be performed, the person is going to be considered as positive.

[Slide.]

In summary, the deferral criteria currently used in the United States, based on history, are not always able to prevent transfusion malaria.

If we search for other potential screening method, we should note that currently available methods for detecting malaria parasites or their products are not sensitive enough to detect the very low parasite densities that theoretically can result in infection.

However, we have no data on the actual densities in the incriminated blood units. Thus, we don't know whether their parasitemias would have been detected by the various techniques now available.

This information could be obtained by systematically using these laboratory screening tests in all future cases of transfusion malaria.

[Slide.]

As an investigative tool, antibody testing has proven more effective in identifying infected donors.

However, if it were to be used as a large-scale screening process, it would result in the deferral of donors who are no longer infected, but are still seropositive due to past infections.

However, we do not know how many such unnecessary exclusions would occur, since we do not have data on seropositivity rates in current U.S. donors.

[Slide.]

In comparison, other countries such as France and the United Kingdom use serology as a screening tool. But this is more to increase their donor pools by looking for acceptable donors among their travelers rather than to increase safety.

Finally, should mass screening using serology be adopted, some important technical issues must be addressed.

Immunofluorescence tests would not be practical, and they would need to be replaced with automated approaches, such as, for example, ELISA, and the antigens used should be amenable to mass production, for example, recombinant proteins or peptides.

Thank you.

DR. HOLLINGER: Thank you.

Committee Discussion and Recommendations

DR. HOLLINGER: Dr. Tabor.

DR. TABOR: I enjoyed that talk. I think you said there were 91 transfusion cases in the United States in a period of, what, 10 years?

DR. NGUYEN-DINH: Thirty-five years.

DR. TABOR: Thirty-five years. So, 91 cases in 35 years is about 3 per year if you really average it out. That would be 1 in 4 million blood donors, which is a pretty low incidence to justify instituting some kind of serologic screening.

The reason I am saying this--and I am not saying that is my position, I am just making an observation--is in the recent MMWR report on three cases of transfusion-transmitted malaria that no doubt you must have been involved in evaluating, if I recall correctly, all three or at least two of the three, but possibly all three were cases that would have been excluded by current questioning, if the

questions had been answered honestly or correctly, and as a result of the questions not being answered correctly, perhaps--again, I don't remember the cases in great enough detail--but perhaps none of the three would have been subjected to serologic screening in France or the U.K. because they would not have fallen into those categories.

So, I just make those observations, so they are taken into consideration in any discussion.

DR. HOLLINGER: Along those same lines, Ed, two-thirds of those would have been excluded if the guidelines had been followed, so that even lowers it even further. I think their comment was that it is really malariae that probably, in terms of the deferral, is the one that would be perhaps potentially more likely to slip through the screening methods.

Somebody said I think like was it two-thirds of the ones that would not have been excluded were probably malariae, something like that?

DR. NGUYEN-DINH: That's correct.

DR. HOLLINGER: Dr. Nelson.

DR. NELSON: While I agree that the major risk seems to be low, one is that it could have been underestimated, and secondly, I think we have to realize that this is a dynamic situation, and with things like conflicts in malarious areas where substantial numbers of

people who are either military or advisers, or whatever, that the situation could really change.

The other issue is that with the history, we are excluding a large number of donors who maybe need not be excluded, and therefore, I think that some sort of a serologic testing, if it could be implemented and shown to be effective, would have great benefit, I think.

DR. HOLLINGER: Many of the committee received a letter from an individual named Swift, Steven Swift, the last time, because of the action last time by the BPAC on whether to defer donors who come into a port, for example, from a malaria area and are there only in the daytime, and the drift of this letter apparently was that a Norwegian shipping, I guess, or cruise, cruise lines comes into Labady, into Haiti, which is a malarious area, and is there for only about six hours, and then leaves during the daytime, and that there is, about twice a week, two boats twice a week, about 2,000 each, and how much that represents, people going into the area.

I did have a chance to talk to him on the phone. It was a very thoughtful letter, I felt, and I think anybody that write a three-page, thoughtful letter deserves a call from somebody. So, I did talk to him and speak to him with the fact, however, that he is not excluded from ever donating, it's a one-year exclusion, and that after a year

he is eligible to donate blood again, at least I hope that was a reasonable thing for him to understand.

Go ahead, Mark.

DR. MITCHELL: I had a question about the PCR testing and whether that would detect the four different strains.

DR. HOLLINGER: The question is whether the PCR will differentiate the four strains or will detect the four strains.

DR. NGUYEN-DINH: Yes, generally, PCR will be able to differentiate between the four species. We are using at CDC the primers described by SuNunu in England, and, in fact, we are now using the PCR to improve on our microscopic diagnosis when we see a parasite which, by microscopy, we cannot quite identify the species, we use PCR to tell us what the finding is.

Does that answer your question?

DR. MITCHELL: Yes, I appreciate that. My comment is that I also agree that it would be very valuable in subpopulations to have such a test available, such as the military and others who may be exposed to this. Also, it is clear that there needs to be more information, that there has to be a test in order to get the kind of screening information.

You know, we talked previously about passive

estimates versus active screening to try to get estimates of the amount of exposure that people have, and I think that a test would be very valuable in getting that information.

DR. HOLLINGER: Dr. Tabor.

DR. TABOR: I guess the point I was trying to make was that with very low incidence at present, admittedly, it could change dramatically, and dishonest or incorrect answers to the current screening questions, applying the test based on answers to the screening test, the way it is done according to your talk in France and England, probably would not effective.

If you had a good screening test, how would you decide who it should be applied to? Presumably, you wouldn't want to use it universally, or would you?

DR. HOLLINGER: Colonel Fitzpatrick.

DR. FITZPATRICK: Speaking from our viewpoint, we would want to use it universally, and we have had instances in the past where we have had to defer large groups of donors because of deployments, and if we had a means of not deferring large groups of donors, but testing them and excluding them based on the result of the tests, we would be expanding our donor pool, which would be very helpful at this time.

DR. HOLLINGER: I am sorry. Would you rephrase it again? I am not I understood what you were saying.

DR. FITZPATRICK: During Desert Storm, we deployed a large number of people including a large number of reservists. Some of those were malaria risk areas. The incidence was very low, but they still had to be deferred for periods of one to three years depending on how long they were there.

We are currently considering and evaluating expanding the area that we defer individuals in Korea for because of increased incidence of malaria in Korea, and those are short tours there, so that will impact the Army more than the other services, just as CJD impacts the Air Force more than the other services.

So, if we had a means of instead of just deferring those donors because they were in that area, but testing them and accepting them based on the results of the tests, we would do that universally and expand our donor pool.

DR. HOLLINGER: And this would be after how long a period of time? If they were in that area, at what point would you be able to screen them and be assured that they are not incubating or having infection?

DR. FITZPATRICK: If it is a PCR test, I assume we would be able to screen them immediately upon their return.

DR. HOLLINGER: This is after they come back, screen them, and therefore, if they are antibody-negative or whatever the test is used, PCR, whatever, then, you might be

able to use them.

DR. FITZPATRICK: Right.

DR. HOLLINGER: I think an interesting aspect, though, I found with this is that it is so different from the PCRs and viruses, and so on, is that usually, if the PCR is negative, infectivity is usually very limited, I mean by and large, and here it is just quite the opposite in that even though the PCR is positive--and that may just have to do with sensitivity or other things--but anyway, that still that blood can be infectious down to a fairly low level, so it is really surprising.

Dr. Nelson.

DR. NELSON: I think that is the issue in that malaria is spreading--Korea as an example--but people are predicting wider spread, and what we are doing now with the history is that it is a problem of the specificity of the history, which is not good at all.

We are excluding a large number of people that may not need to be, and it may come to a point that it really impacts on the donor. If we had a more specific test that was also--and I don't think that the history is all that sensitive either--I mean people slip through.

I would be very interested in what the data are on transfusion-transmitted malaria in France and the U.K. because with the proximity to Africa and probably more

travelers to Africa where malaria is highly endemic, probably the donor population has a higher infection rate, but how many transfusion cases are there, and we have to think about the fact that their geographic exclusion is less rigorous or less than ours. Ours is for a year, theirs is for four months or three months, but they implement also serologic testing, and I think that makes sense.

I would like to see what their results are. Do you have any data?

DR. NGUYEN-DINH: I have some information, but this is by secondhand, and you really want to check it, and we can check it for you if you ask us to do that, you know, from the French and the United Kingdom.

The French tell me that they have had since they started this system in 1983, one case of transfusion malaria, but then immediately after that, they follow by saying look, you know, our system is not ideal, et cetera, et cetera.

The British, I know had that case I mentioned in 1997, where someone died, and that was a major problem there.

Again, if you wish, we can definitely get very precise epidemiological information from those two countries and also the European Union, because they have guidelines that are very interesting to look at, I think.

DR. NELSON: Is there data on how many donors are deferred based on visiting an endemic area? My guess is it is probably much higher than the U.S. Both of those data would be interesting. I mean there were data there that could be useful to the committee. I would suggest that we try to get it.

DR. HOLLINGER: Thank you. Excellent.

Any other comments about this topic? Jay.

DR. EPSTEIN: Just a follow-up comment to Dr. Tabor's with which I agree. The challenge of parasitic diseases is really quite different than for viral diseases. You can have infectivity with very low levels of parasitemia, and for many of these conditions, including Chagas and babesiosis, we have very, very low population prevalence and incidence, and so the challenge of deciding whether we should or should not screen the blood supply is really twofold.

I mean first we would need tests of high sensitivity and high specificity, and then even at that point, we have a challenge in deciding whether it makes any sense to screening when the yields would be so extremely low.

In each of these cases, the concept of selective testing has emerged. I think the concept that we heard today, which was that there could be selective testing to

try to requalify otherwise excluded donors is novel, and the concept of selective testing based on history to decide whether to qualify a donor is not novel, but has been daunting in every other context where it has been attempted. I submit Chagas as the other example where we went that route. These are tough questions.

DR. HOLLINGER: Corey.

MR. DUBIN: It seems pretty clear that we are deferring people we shouldn't be deferring at a time when we are questioning supply quite a bit. It seems pretty straightforward on this one. I agree with the comment at the end of the table, that the data would be interesting to have, but I still think it is staring us in the face pretty clearly that a lot of people are being deferred that shouldn't be deferred if we were doing serologic testing.

DR. HOLLINGER: Dr. Kleinman.

DR. KLEINMAN: The testing back in philosophy that was described in France and the U.K., I think, you know, although you could argue that might have some problems in that we don't know how sensitive the tests are, I think it would be important to follow up on what Ken said, and if you got the data from those countries and found that, in fact, they have very low levels of transmission with that strategy, it would give you a little bit more confidence that a positive test, even though theoretically, there are

units that would be missed because of limited sensitivity, you know, it still might give you confidence that that might be a strategy that could be implemented to get over the problem of unnecessarily deferring donors.

I agree with Jay that there are an incredible number of problems in implementing that kind of novel strategy, but, you know, I mean in a sense it is a reentry strategy. You are going to reenter based on the donor history rather than waiting for one year or three years.

I think the other thing we need to remember is that although, you know, we are deferring that number of people every year, and once they are deferred, when you are deferred for a year or three years, you know, I don't know what the figures are, but it is a low percentage of people who want to donate blood so badly that they come back again. I mean once you have told them they are out temporarily, they will say fine, thanks, and they are out.

DR. HOLLINGER: Jay, just a question that Dr. Fitzpatrick mentioned. What is the issues with being able, in a unique situation, like the military, of allowing that organization to do the thing that he has just suggested, which is to reenter the donors if they come back, and they are using an antibody test or something of that nature instead of going through the whole year or more deferral, is that a real problem otherwise?

DR. EPSTEIN: Well, I think, you know, Steve Kleinman hit the nail on the head, we are talking about a reentry strategy, and if we were to approve it for the military, we would have no reason not to approve it also for the civilian sector.

After all, many of the soldiers don't stay in uniform, you know, they leave the service, and then they are just civilians. So, the question would arise in either context, and I don't see a reason that we wouldn't pursue it. It just becomes a validation issue.

MR. DUBIN: Jay, isn't it also true that if the Joint Chiefs decide to do something, FDA doesn't have a whole lot of authority?

DR. EPSTEIN: The world is not that simple, Corey. They are armed, and we are not, but --

MR. DUBIN: I don't mean it in an armed sense, Jay. I don't mean it in that sense. Don't they make medical decisions independent of the structure?

DR. EPSTEIN: They do, but there are political forces that obligate the military voluntarily to comply with the FDA standards.

MR. DUBIN: Right.

DR. EPSTEIN: It is from a purely legal point of view, military compliance is voluntary.

MR. DUBIN: Right, that is what I meant.

DR. EPSTEIN: However, there is a long-standing recognition of the need for voluntary compliance because the military is faced with the challenge of needing to assure that the soldier gets the same protections as the civilian, and that has always been the goal wherever it is achievable.

There are situations where it is not achievable, but it is always the goal, and that leads to a philosophy of voluntary compliance. I think we end up with a harmonized system in the end.

MR. DUBIN: Right. I mean it was impressive how fast the Pentagon undertook lookback from our perspective. You guys were way down the field pretty quick, and it is obvious why. You have got soldiers in the field, you need to know.

DR. FITZPATRICK: There have been cases where we have implemented stricter guidelines than the FDA voluntarily, but there have not been cases where we have not done what the FDA asked.

MR. DUBIN: Right. I was going the other way, Jay. I wasn't suggesting they would not comply. I was going in the other direction.

DR. EPSTEIN: No, no, but the reentry scheme in the absence of an FDA standard for it would be more permissive, not less permissive than the FDA standard, so that would not be an example where, at least historically,

the military would implement without FDA approval.

It is true also at a state level and a local level. It is possible to be more stringent than the FDA standard, and it is true of a lot of AABB standards, for instance, which are universally followed. But to go the other direction is perceived as highly problematic by the military, and it is just not generally the practice.

MR. DUBIN: I understand. I wasn't suggesting that.

DR. HOLLINGER: I think we will call a recess for today. There is one topic tomorrow. It is on medical device panel reclassification of HIV drug sensitivity. Before you leave, I want the committee to stay for just a few minutes, but the session will start tomorrow at 8:00 a.m.

[Whereupon, at 4:55 p.m., the proceedings were recessed, to be resumed at 8:00 a.m., Friday, September 17, 1999.]

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CERTIFICATE

I, **ALICE TOIGO,** the Official Court Reporter for Miller Reporting Company, Inc., hereby certify that I recorded the foregoing proceedings; that the proceedings have been reduced to typewriting by me, or under my direction and that the foregoing transcript is a correct and accurate record of the proceedings to the best of my knowledge, ability and belief.

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