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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

WORKSHOP:

SAFETY AND EFFICACY OF METHODS FOR REDUCING PATHOGENS IN CELLULAR BLOOD PRODUCTS USED IN TRANSFUSION

VOLUME I

Wednesday, August 7, 2002

8:02 a.m.

MILLER REPORTING COMPANY, INC.
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P R O C E E D I N G S

DR. VOSTAL: Good morning. My name is Jaro Vostal, and I'd like to welcome you to Washington, D.C., NIH, and to the workshop on pathogen reduction in cellular blood components. We have a very exciting day today, packed with lots of information and discussion, so we have to get started.

We will also try to stay on time because it's important for us to cover all the topics from morning until afternoon.

There's one order of business I'd like to bring up, and that is, people have approached me about whether we've been collecting information on conflict of interest for the speakers. Since this is a public workshop, we don't have a policy to do that, only for advisory committees. But if you're a speaker and you have some potential conflicts, we welcome you to reveal those on a volunteer basis at the beginning of your talk.

So, to get started, we have Dr. Jay Epstein, who's the Director of Office of Blood Research and Review, and he will give the introduction this morning.

DR. EPSTEIN: Well, thank you very much, Jaro. It's my pleasant task to set the stage, but I think it's obvious that the real thanks go to Jaro and his group for

developing and organizing the program, and special thanks also to Joe Wilczek for handling our logistics.

As Jaro said, our goal is to review the emerging technologies that are applicable to pathogen reduction for cellular blood products. And how do I get the first slide on?

Just with some acknowledgment to the cartoonist, I think it's obvious to everyone in the room that bacterial, viral, and protozoa pathogens have been identified in blood products, that mortality and morbidity are associated with transfusions that are contaminated by these pathogens. FDA and industry as a whole are committed to reducing the incidence of pathogen contamination in blood products. And as you can see from the organization of the program, we are mindful that there are a number of different ways to approach the challenge of decreasing contamination.

Broadly speaking, these methods include efforts to primary prevent contamination of the collection. This includes the skin preparation method as well as the possible effectiveness of diverting an initial volume of the collected blood away from the final storage container.

We then move to efforts to detect pathogens in the collection as a way to avoid use of contaminated units. And we have, again, a set of technologies directed at

different pathogens: nucleic acid testing, bacterial culture, use of bacterial fluorescent probes, and some novel biochemical tests for pathogen proliferation.

Failing that, there's then the new technology challenge of methodologies to decontaminate a contaminated unit, and we have targets that are directed toward nucleic acid, and these include both methods that are chemically spontaneously reactive as well as those that require activation by UV light.

So what we hope to do at this workshop is to evaluate these several different approaches to pathogen reduction. From the FDA's standpoint, each individual approach will need to be evaluated both for safety and efficacy within its context of intended use. Of these three basic methods, the use of decontamination is both the most novel and the most complicated in terms of the safety and efficacy assessment and, therefore, will merit a considerable amount of our time.

Decontamination methods involve the addition of mutagenic and potentially carcinogenic chemicals. We are aware that residual amounts of these chemicals can remain within the transfusion product and then be transfused. And we also recognize that in some cases these chemicals may interact with the product itself, potentially changing its character.

The focus of the workshop, again, as noted earlier, is on the cellular product, and the cellular products, as we know, are unique because they are, for the most part, not frozen. And storage temperatures above freezing do allow particularly for bacterial proliferation.

Additionally, these cells are susceptible to damage both from chemical exposure and UV light treatment, and the evaluation of toxicity or damage to the cells is itself a complex task.

The intent, then, at the workshop is to promote the discussion of the scientific aspects for evaluating pathogen reduction in cellular products. We hope to hear public opinion on the appropriateness of the approach that the FDA is taking toward evaluating these methods, and we hope to encourage the development of novelty contamination methods by outlining the necessary steps toward validating clinical use.

We then will review the different approaches to evaluating pathogen decontamination methods. We hope to establish the appropriate methodology for testing efficacy, and we hope to obtain some level of a scientific consensus on the minimum level of efficacy that will be required.

Concomitantly, we hope to get a framework in place to evaluate toxicity of these methods. We will then review the current methods for evaluating the efficacy and

the safety of cellular products. And we will specifically focus on the question of FDA's approach toward assessing toxicity when there's a question of mutagenicity or carcinogenicity.

So, as you can see, we've set ourselves a rather ambitious task, but we know that we have assembled in the room some of the leading experts in these areas, and we look forward very much to the insights that we may gain from this dialogue toward establishing the framework of FDA decisionmaking that will permit product development to go forward.

So I thank you all for coming to the meeting and look forward to your contributions throughout the sessions. Thank you. I give the podium back to Dr. Vostal.

[Applause.]

DR. VOSTAL: Okay. Thank you, Dr. Epstein.

So now we'll move into the first session of the workshop, and this will deal with an overview of the different types of pathogens that can be transmitted by blood transfusion, and our first speaker is Dr. Roslyn Yomtovian, who is the Director of Blood Bank, Transfusion Medicine Service and the Acting Director of Clinical Pathology at the University Hospital of Cleveland, Ohio. She will cover the bacterial contaminants.

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DR. YOMTOVIAN: Well, thank you so much, Dr. Vostal, and others, for inviting me back again. I feel like I'm in the movie "Same Time, Next Year," for those of you who are familiar with that movie. But I'm certainly happy on behalf of our group in Cleveland to talk to you about this very important topic and give you an overview on what I feel are the key issues in bacterial contamination.

To start on a little light note here, there have been two other workshops on a related topic in the past, so the question is how many acts will we finally have. I hope that this is indeed the last act before we finally do something about what I consider a very important issue in blood transfusion safety, as I hope to remind you or convince you of before I'm done with my talk.

And so, really, I'm going to cover with you very, very briefly two key issues, and they are to recognize the clinical significance especially of platelet bacterial contamination, and then very briefly pick up where Dr. Epstein left off, just going through what are some of the strategy or strategies to prevent or interdict the problem.

Now, I will focus on platelets, and in trying to convince you that that's appropriate, I paraphrase Willie Sutton, who actually never said that he robs banks because that's where the money is. He said--"I rob banks because that's where the money is" is not really what he said.

It's modified from the irony of using a bank robber's maxim as an instrument for teaching medicine is compounded, I will now confess, by the fact that I never, never said it. Why did I rob banks? To me the money was the chips, that's all. So, to me, platelets are the chips in terms of bacterial contamination, that's all.

Actually, Willie Sutton wrote two books, believe it or not, so he did more than just rob banks.

Well, to begin with, a little bit of an overview on the subject, and I don't think I have to convince anyone here of what's written on this slide: In an era in which the risk of transmission of recognized transfusion-transmitted viruses, particularly HIV, has been virtually eliminated, it is paradoxical and somewhat ironic that the earliest recognized infectious transfusion complication, bacterial contamination, is now the most frequent and indeed is the most daunting and proving to be the most difficult to eradicate.

And we fell into this not because, I must say, I was born with a genetic inclination to study this, but because about ten years ago we had a cluster of four episodes of bacterial contamination at our facility, which was thoroughly investigated by the CDC and FDA and was reported in MMWR and later expanded into a fuller report in Infection Control and Hospital Epidemiology. And, in

essence, what we discovered, what everyone discovered was we weren't doing anything wrong. That was our great fear in the beginning. What was unique about our facility? Why did we have all these contaminations? We were doing nothing wrong. Our technique was proper, et cetera.

Since then, of course, we discovered that this is a problem, for whatever reason--and I don't have time to go into the details--that does seem to cluster. It's a very interesting aspect of this problem, and perhaps we can talk about it at the panel.

But I want to now just take a couple of minutes and give a very truncated, historical overview, really starting with the platelet story. Of course, contamination of blood goes way back to the early part of the century, if one looks at red cells. But I want to look at platelets, and, therefore, I'll go back to 1969 in a very important paper by Murphy and Gardner in which they discovered that you could increase the shelf life of platelets greatly by storing them at room temperature. And, of course, development in plastic bags made that possible.

But one of the things they worried about was if there would be a risk from inadvertent bacterial contamination. Now, they felt there was not a risk, and that was based on a very small study--very small. I think "n" was, you know, no more than about 100 in their study,

and, therefore, it was not surprising that only a couple of years later Buchholz's group reported on a case of transfusion-induced enterobacter sepsis, and they admonished that, although there is utility to room temperature storage of platelets because you could keep them longer, the platelets are functional longer, there may be a serious risk to those receiving such products. Platelets stored at room temperature should be used with caution, especially in high-risk populations.

And shortly thereafter, they used that incident as, of course, we've used our experience in this area to do a larger study, and they found that up to 1.6 percent of platelet units are contaminated, and they noted that to be storage-time-related, as many others have noted since. And they further said that the risk of bacterial proliferation may warrant a review of current methods of platelet collection and of ambient temperature platelet storage. And, again, they cautioned use of these products, especially in recipients of impaired host defense mechanisms. And, in retrospect, it fell largely on--it fell silent. There was really not an audience for what Buchholz and others were saying.

Another group--this is not the same Dr. Jacobs in our group--at about the same time, again, worried that platelet concentrate stored for four days at room

temperature would facilitate bacterial proliferation, and in a very prophetic recommendation, which I don't know if they ever followed up on, but it certainly rings true for today, they proposed use of a direct film made from a sealed segment of the tubing incubated at 37 degrees overnight when the parent bag is stored at 22 degrees, and they said that should provide a reliable indication of bacterial contamination at the time the platelet concentrate is being distributed.

That's really the essence of what's going on now with the various culture schemes where you have a holding period at a higher temperature to encourage or augment the bacterial growth. Very prophetic.

Well, the saga went on. New bags were developed to store platelets, and instead of being worried about bacterial contamination, the storage time of platelets was actually extended with the new generation of bags from five to seven days, and it was only then in 1986, in response to an increase in number of reports of platelet transfusion-associated sepsis, that the Blood Product Advisory Committee to the FDA recommended going back from seven days to five days. And, actually, if you read that report carefully, you could make a case that they should have gone back to four days. But I think this was a compromise at

the time of trying to keep the supply up and reduce the problem.

So what is the risk of contamination in platelets? If one looks at the FDA's own data derived from the mandatory reporting of transfusion-associated deaths, one notes that in the two time frames, an earlier and a more recent one, although the total number of deaths is about the same, the percentage due to contamination has gone up. And that's likely due to the increased use of platelets in this time period, which is greatly increased, and perhaps some better recognition.

Oops, sorry. If one--I am going, I think, the wrong way. No. Sorry.

Okay. I'm not going to belabor the BaCon study because you'll hear about that later, but that is the second way that the risk has been evaluated of bacterial contamination. And, of course, that study did use rigorously defined criteria to capture cases. And I'll get back to that in a little bit because, by being so rigorous, obviously the total number of cases would be limited.

And, in fact, in the BaCon study published results--and, again, I'm sure you'll hear an update of this later--here are the results. There were five cases in red cells and there were 11 cases in pooled random units and 18 cases in single-donor units with fatalities in all of these

cases, a much higher rate in the platelets. Not to say the problem doesn't exist in red cells, but it's certainly far greater in platelets.

Now, both the FDA reporting mechanism and the BaCon study are dealing with the tip of the iceberg, and I'll explain that shortly. The work we've done through the years is trying to look at the rest of the iceberg, which I will try to convince you there are many clinically significant cases in here. And even cases that are unlikely to be clinically significant may be important in an epidemiologic sense or to warn us that something may be percolating. So these cases, I believe, are also important to recognize.

And so what I have done is tried to summarize on one slide--and this is probably the most important slide that I will show today--that if you normalize our experience to 100,000 platelet transfusions in the denominator, I'm comparing the risk of transfusion-transmitted bacterial disease per 100,000 transfusions and an estimate of transfusion-transmitted deaths from this problem in the BaCon study and in our experience. And the differences are, you know, orders of magnitude different. So BaCon would have one per 100,000 of bacterial transmission. Our number suggests it's something like 200 per 100,000. And deaths, BaCon reported 0.2, if you look

at their publications, in transfusion per 100,000; in our experience, it's approximately 10 cases per 100,000. Very different numbers and a very significant problem.

And work that we've done also showed that unit per unit, the risk of contamination in a single-donor platelet apheresis unit versus a random unit is statistically the same. Now, obviously, since random units are pooled, the risk is much greater, approximately--you multiply the risk by the number of units in the pool. But unit for unit, the risk appeared to be the same.

I wanted to share with you just a few very concise clinical vignettes from our studies through the years on bacterial contamination of platelets as a way of illustrating why the data from a study like BaCon, which is a very useful study, but it's very restricted in the numbers of cases that were finally reported because of the rigorous criteria that it used, and also it was voluntary and it certainly wasn't a prospectively designed study, as were our studies for many years. So let me share with you a few cases.

A 45-year-old patient was receiving multiple antibiotics, developed shock beginning 30 minutes after a transfusion of an apheresis platelet, Streptococcus bovis was isolated from the platelet bag, but her blood cultures were negative. So it wasn't accepted as a BaCon case

because it required that the blood cultures be positive. But many patients that receive platelets are protected in the sense--protected from the growth of the organism, certainly not from the endotoxin, because they're on antibiotics. This patient did not die from this event but died shortly thereafter from an unrelated cause.

Another case is a 63-year-old patient with AML who developed rigors 15 minutes after a transfusion. No other signs or symptoms. Was given a pool of five random platelets. A very astute nurse, however, obtained two blood cultures right about that time, and the platelet pool bag was part of our surveillance, prospective surveillance program, was positive for coagulase-negative staph, as were the two blood cultures. She was treated with vancomycin, recovered. This was accepted as a BaCon case because the organisms were identical by RFLP. The blood cultures were positive. The pool bag was positive and so forth. But I venture to say very few hospitals would do blood cultures on someone only having rigors after a platelet transfusion with no other signs or symptoms.

Two more very interesting cases are a 27-year-old with ALL who received a pool of platelets, and I will say uneventfully, absolutely uneventfully. Twenty hours later he felt chilly, and 22 hours later after he got the transfusion, he spiked a very high fever. We were doing

surveillance cultures on our bags. That was positive by then for Staph aureus. A blood culture was drawn. It was also positive for Staph aureus. He was treated with vancomycin and also required granulocytes. This was also not accepted as a BaCon case because of the delay in the symptoms, significant delay. And we've seen that more than once.

A 72-year-old with aplastic anemia received five random platelets in a pool, again, I emphasize uneventfully. We were doing surveillance cultures of our platelets. It was positive after the fact for coagulase-negative staph. Two blood cultures were obtained at day four. One of the two cultures was positive for what seemed to be an identical organism, at least with antibiotic susceptibilities. A corresponding red cell unit from that donor was also positive. She was treated with vancomycin, and, again, that was not accepted as a BaCon case because there were no clinical symptoms.

So the point of this is simply that the problem is far greater than what has been reported to the FDA and what has come out from the BaCon study. And this just summarizes what I've already mentioned.

In the remaining time, I want to just very briefly and quickly review the different strategy or strategies to interdict bacterial contamination because

it's possible that, unlike the paradigm in blood bank for many years in which you wanted, you know, one strategy to deal with the problem, we may need multiple strategies to deal with this problem, although I'm sure that will be a topic for discussion later.

So I've just put these into what I consider four paradigms: the bacterial contamination avoidance methods, inhibition methods, detection, and elimination methods. And, of course, this meeting is going to focus primarily on the elimination methods.

But starting at the beginning, an avoidance method would certainly be the ideal approach since it would avoid the need downstream for bacterial detection, growth inhibition, or elimination. And avoidance strategies depend on the bacterial source, obviously.

There are two ways, two main ways that blood products can become contaminated. One would be donor bacteremia, and certainly with platelets, the most common way is donor phlebotomy. Of course, to deal with donor bacteremia, the ideal way, if possible, would be donor screening. Obviously that won't be 100 percent. And for phlebotomy, an ideal way would be to have the best arm preparation possible.

But even with the best arm preparation possible, you're not going to eliminate this problem because it's

been shown fairly convincingly that bacteria don't reside only on the superficial skin surface, but bacteria are harbored in the deeper layers of the skin and skin appendages. And, for example, the American Society for Microbiology even allows a blood culture contamination rate of up to 3 percent, and in part, that reflects the fact that you cannot totally decontaminate the skin.

In a table that was published by Dr. Ernst in one of--a throwaway journal but a very useful table, nonetheless, on the percentage of organisms as contaminants, it's interesting that certainly two of the ones that we see commonly in platelet contamination, bacillus and coag-negative staph, are also the ones that are found most commonly as false positives, quote, false positives, in blood cultures. So certainly some of these are likely related to the phlebotomy process.

Now, there is likely a correlation between the type of skin prep and the rate of culture positivity. Many papers through the years have noted that iodine tincture is a superior microbicide compared to povidone, iodine, and other methods. In fact, you in some papers get a 50-percent reduction in spurious contamination, and you even get a reduction in the quantitative level of bacterial growth.

However, a very recent paper by Calfee, et al., in the Journal of Clinical Microbiology, actually refutes that and doesn't show a statistically significant difference in these methods. So I'm convinced there likely is a difference in methods, but I'm not expert enough to tell you what the best method is. It's certainly something that needs ongoing evaluation.

Likely of much more importance is the training of the phlebotomists, and in three studies--two by Weinbaum and one by Schifman, et al.--they compared the rate of contamination of trained phlebotomists with non-phlebotomists, and this is very germane because in an era where hospitals are trying to cut costs every which way and practically take people off the street to do certain tasks, really this is food for thought, that people that don't know what they're doing will have a much higher rate of contamination than trained people. So this is a very important point.

So, in summary, based on what has previously been published, it appears that iodine tincture disinfection is preferable; use of trained personnel is important. Obviously, the phlebotomy site must be selected with care. Scarred areas and sites near indwelling lines harbor more bacteria, so those need to be avoided. The phlebotomy site needs to be prepared with care. It's been shown that use

of friction when prepping the site will disinfect it better, and, of course, one must allow the disinfectant to dry and not touch the site. And certainly if one uses single-donor platelets, you statistically reduce the risk of contamination because it's one venipuncture versus multiple venipuncture, so the rate will be lower.

Now, switching to a very brief overview of bacterial growth inhibitory methods--and I'm really just touching on some highlights here. I've already mentioned that there's--and Dr. Epstein has mentioned, bacterial platelet contamination is linked to room temperature storage and the time of storage. If you reduce the storage time, you'll reduce the problem. It's not that the bacteria aren't there. They just need time to grow up to clinically significant numbers.

So if you could refrigerate or freeze platelets, you would greatly reduce the problem. The problem is that we've already learned from Murphy's work et al. that cold temperature irreversibly damages platelets, so that's a problem. You'd need a cryoprotectant to protect the platelets at colder temperatures. And there has been a lot of work on this ongoing to figure out a method to preserve platelet function when they're stored either frozen or at 4 degrees. And the work that's most successful and is ongoing in this is work from Life Cell Corporation in which

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they have now some studies that have already been published and some studies that are in progress showing that when you use their proprietary agent called Thrombosol with a lower concentration of DMSO, and you use that to freeze platelets, it's a much easier method to employ. You don't need mechanical freezing, and you could thaw the platelets quickly. And, furthermore, you don't need to wash out the agent before transfusion, and you get equivalent or improved post-thaw platelet function. So although this is really not ready for clinical use at this point, there may be something here to keep following.

So switching gears to bacterial detection methods, the challenge here is: What is the level of clinically significant or clinically tolerable bacteria that you want to detect? Will you allow a level of 10^1 or 10^2 and say I don't care about it, I'll only detect 10^3 or 10^4? What is that level?

Well, based on our experience, I believe the level is around 10^{2}-^{3} CFU per mL, because from our experience through the years, we've shown that a level of even that range may be clinically significant. The ideal method would be rapid, inexpensive, sensitive, specific, practical, and simple. That's why I say there may not be one method. Testing should be as close to transfusion as possible to enhance detectability. If you test too early,

the bacteria will not have grown up enough to detect them. So you need to test close to transfusion and to remind people you should never do the sampling from a segment or a link that's made at the time the original product is made. Statistically there will not be enough bacteria to be present in these small volumes that are in these to be positive.

So this next slide summarizes--and I've adapted this from a nice review article that Dr. Brecher's group wrote a few years ago--the different approaches to bacterial detection. And the one group of approaches that's being used most successfully are under the category that I called "cell growth," so culture methods which are very sensitive and a type of surrogate culture method, percent oxygen in air, which is a method that's being developed by Pall Corporation. There are certainly other methods that I've put under cell marker methods that are based on antibiotic probes or antibody probes or a very new method that's coming on the scene based on epifluorescence microscopy, which may be quite sensitive, although maybe slightly less than cell growth.

For some reason, the molecular biologic approaches haven't been as successful, but that may be a technological issue rather than an inherent problem with these methods.

And this does not include all detection methods, but it's just designed to give an idea of order of magnitude of sensitivity of different methods. A Gram stain, which we did for many years, is extremely insensitive. You need about 10^6 CFU per mL to detect bacteria; whereas, culture methods are right down around 10^1, 10^2 CFU per mL and right now really are the most successful in preventing this problem in institutions that are using this method.

This is a summary of our cases of contamination over not quite ten years, clinical outcome, number of isolates, the specific bacteria, and CFU per mL because we were doing quantitative cultures on the bag in all the implicated cases, and, again, these are largely based on prospective studies.

The point is the red numbers here indicate positive blood cultures. So even numbers as low as 10^3, in one case as low as even 10^2, were linked to some sort of clinical symptom, even if they were only very mild symptoms. So I think the issue of what level you'd like with detection is, as I've indicated, ideally around 10^2-10^3. Whether that can be achieved by anything other than culture would remain to be seen.

This just gives a little bit of our experience with Gram stain. We stopped doing Gram stain in 1999. We

only ever interdicted--we did interdict some cases, so I'm not going to knock Gram stain completely. But, obviously, many cases we did not interdict using a Gram stain because it's very insensitive, and really, you're only going to get rid of the absolute--you know, the blood products that are literally cultures at that time.

So, shifting gears to bacterial contamination prevention methods, filtration, phlebotomy diversion, and, finally, you'll hear a lot more about the photochemical decontamination have been examples of prevention methods. Early on filtration was shown to be reasonably effective for Yersinia enterocolitica. But, of course, that's a problem largely of red cells, and, of course, now that universal leukoreduction is sort of the trend of the future, this problem is likely to become less and less, and it certainly is not going to solve the problem with platelets. But it has a role, a limited role, especially with select organisms, and, for example, Yersinia.

Regarding the diversion technology that Dr. Epstein mentioned, there have been several articles published on this. There have been two clinical type articles from Europe. This is an older article that was published in Vox Sanguinis that showed that in about 3,500 whole blood units that were collected over four months, two bags, two 15-mL bags were attached in series--maybe not in

series, but in sequence. Blood was diverted first to P1 and then to P2. And what they showed was in the P1 bag, the blood that was diverted, the initial diversion was much more frequently contaminated than the P2 bag, suggesting that diversion may be effective in reducing the level of contamination.

A very recent study appearing in this month's issue of Vox Sanguinis took 7,000 whole blood units which were collected with something they call the camposampler attached to the blood collection system to divert the first 10 mL, and then they tested the sampling bag by the BacT/ALERT following collection, and they had about 18,000 control whole blood cultures.

Now, their results are provocative and interesting. They did a one-sided test, statistical correlation test to look at the reduction or the incidence of bacteria in the study versus the control group, and doing the one-sided test, there was kind of marginal statistical significance for overall reduction in bacteria with the diversion group versus the control group. But, very interestingly, using just standard, regular statistics, two-sided, they found that there was a very significant reduction in coagulase-negative staph in the study group versus the control group. So it may be that the diversion method may be particularly apropos and good

for prevention of coagulase-negative staph. So I refer you to that study.

I'm really not going to spend time talking about this because this is really what this meeting is about. But to date, there have been publications on two broad methods of photochemical decontamination. The psoralen method of decontamination, which forms an adduct with nucleic acid and has been shown to be quite effective, although not absolutely effective--but you'll heard about that, I'm sure, later--in eliminating bacteria. There is a little bit of inconsistency, and the second different method is based on a riboflavin B2, which is an oxidative process, and it also has been shown to be somewhat effective in reducing bacteria, and I'm sure we will hear that data at length so I'm not going to dwell on that.

So, in summary, bacterial contamination is an ongoing, recurrent complication of primarily platelet transfusion therapy. There is no systematic approach in the U.S. to reduce or eliminate this problem at this time. We haven't even defined an approach, let alone implemented an approach. So I hope what comes out of this meeting will be some approach.

A single ideal preventative strategy--safe, rapid, simple, inexpensive, sensitive, specific, and practical--has not been developed. In fact, it's unlikely

that there will ever be one single such strategy. So we may be best to look at combined strategies, because there are numerous strategies alone, but better together, that not only will reduce--certainly alone they'll reduce, but perhaps together they may eliminate the occurrence of this problem.

And, finally, and very importantly, prevention strategies for once may prove cost-enhancing if linked with a seven-day platelet storage product. And I know we'll be hearing more about that at this meeting, and you can't ignore costs in this day and age where everyone is under the gun always to save, to save money. And I just thank the members of our group who worked on this through the years because I'm only just one of the people that happens to come and speak at meetings. But Dr. Jacobs and a crew of very talented microbiologists, Dr. Sepatnekar now at the Cleveland Red Cross, Dr. Palavecino, our current blood bank fellow, and Sara Lee and Ann, who are very talented research microbiology techs, have certainly done much of the work through the years.

I thank you for your attention.

[Applause.]

DR. VOSTAL: Thank you, Dr. Yomtovian.

We have time for one burning question, but hopefully it will be short. Anybody have a question?

[No response.]

DR. VOSTAL: Okay. Thank you.

We will now move into viruses, and this presentation will be given by Dr. Michael Busch, who's the Vice President of Research and Scientific Affairs at the Blood Centers of the Pacific, and also a professor of laboratory medicine at UCSF. Dr. Busch?

DR. BUSCH: Thank you, Dr. Vostal.

First, in terms of conflict, I think I've worked with every company that both tests and inactivates, but none of what I say, I think, will have any relevance to conflict.

What I've been asked to do is to update on current risks of virus infections from transfusions, and then particularly discuss the issues of the levels of viremia in these various infections during the progressive stages of infection, and then at the end I will address the issue of the levels of viremia necessary to transmit. So, again, current risk update.

In terms of looking at the patterns of viremia, I've divided up my presentation into sort of three categories of viruses.

The first, the major transfusion-transmitted virus is for which routine screening is currently in place,

and not only serologic screening but the advances with implementation of nucleic acid testing.

Second is pathogenic viruses that we do not routinely screen for, and this includes the Herpes viruses as well as consideration of Parvo B19 in hepatitis A.

And then a third category, which is viruses that actually are fairly prevalent and have been discovered over the last few years, but that have yet to have an established pathological relationship. But I think that as we'll look at these viruses, I'll suggest that these may be a good model for the next emerging agent, and they may be a tool to assess the efficacy of pathogen reduction methods in routine clinical practice because these viruses are being transmitted daily and, as you'll see, can exist in fairly high titers in donor units.

Finally, at the end I'll briefly address the issue of the relationship of viremia levels to infectivity.

This slide just summarizes the enormous progress over the last several decades through implementation of both enhanced donor selection criteria, but most important, really each of these dots represents advances attributable to improved screening. And the strategy of adding enhanced tests, as we've discovered viruses and built better tests, has clearly been successful, but I think one of the promises of pathogen reduction is that it might avoid the

need to add a new test for each specific agent and allow us to feel more comfortable not adding new tests as new pathogens are discovered or as issues of residual transmission are documented.

But I think as we'll come back to this at the very end and in the discussion, in my opinion I do not expect that pathogen reduction methods will allow us to discontinue any of these assays that are currently in place.

In terms of the current risk, the classic way that we've been estimating risk over the last 10, 15 years has been using the so-called incidence window period model, where we quantify the rate of new infections in the donor pool. In the repeat donors, we assess the rate of seroconversions. We then estimate incidence overall and by factoring an increased rate of seroconversion for first-time donors. And then by knowing the durations of the window periods, either the serologic window period or with the introduction of NAT, the window periods that precede detection of RNA by either mini-pool or single-donation NAT, we can calculate out these risks of donations being given during the early window period.

And as you can see here, we've, again, made enormous progress as we've moved from serologic testing to the introduction of mini-pool NAT, and could make slightly

better--the blood slightly safer by moving to individual-donation NAT. But, really, we're at sort of the asymptotic level of these risk curves, with risks now in the range--with mini-pool NAT, in the range of 1 in 2 million for HIV and HCV. At present, we're not performing mini-pool NAT for HBV, in great part because if the very small incremental reduction that could be achieved over surface antigen test by mini-pool NAT. We could increase safety moderately for HBV and really minimally for HIV and HCV by moving to individual-donation NAT. And we'll talk through the window period data that really explains this in a few minutes.

In addition, sort of a newer approach beyond the classic incidence window period model for estimating risk actually involves using the yield of mini-pool NAT as a tool to estimate risks associated with mini-pool NAT-negative units. And this is simply a strategy that uses the rate of detecting mini-pool NAT donations on a total-donation rate. This is actually Red Cross data, so we're picking up mini-pool NAT-positive units at 1 in 270,000 for HCV and about 1 in 5 million for HIV. And by knowing the relative durations of the window periods, the mini-pool NAT-positive pre-seroconversion window period, versus the total pre-mini-pool NAT window period or the individual-donation NAT window period, we can, in essence, adjust the

rate of mini-pool NAT yield to estimate the risk of blood or the yield of individual-donation NAT.

And I'm not going to go through this in any detail, but suffice it to say that these numbers now based on mini-pool NAT yield are virtually identical to the rates from the incidence window period model. So very reassuring, I think, that our risk is in the range of 1 in 2 million for each of these viruses, and that the yield of individual-donation NAT will be extremely low, probably about 1 in 5 million, for each virus.

Now, as Roslyn emphasized, what we've done is to drive the risk of the viruses down to ranges of 1 in a million, and what we're left with is other problems from transfusions, such as bacteria that are, I think, much more important today. We also have a number of non-infectious complications, and so this is a slide from Sunny Dzik that I think puts into broader context where our problems lie. And I think today in transfusion medicine really infectious risks, with the exception of bacteria, are a very small contributor to the overall transfusion problem.

Moving on now to the issue of viremia, for each of the agents we're sort of working towards understanding the sequential stages of infection and the levels of viremia that exist. And as we look at the current viruses and some of the new emerging agents, we'll be talking about

these kinds of stages--in eclipse phase, which is the period following an infectious exposure, but before one can detect virus by testing the blood, even with highly sensitive nucleic acid methods.

Then during that period of eclipse, we often see intermittent blips of viremia, and this has been termed recently "pre-ramp-up viremia." I'll illustrate that. Then there's this exponential explosion of viremia in the blood, sometimes associated with symptoms, as in primary HIV, but often asymptomatic, as in hepatitis C. We term this "ramp-up viremia."

For some viruses, with, for example, HCV, there may be a prolonged plateau phase during which high-titer viremia exists for months before seroconversion. Other viruses, like HIV and HBV, tend to have a peak of viremia associated with seroconversion and then a clearance of that high-titer viremia.

Often around the time of seroconversion, we'll see dramatic fluctuating viral load, often to negative values, only to be followed by a persistent viremia. And we've termed this "peri-seroconversion viremia." And then with seroconversion, people tend to either become persistent carriers, typical of hepatitis C or HIV, and they'll establish a viral load set point in the context of a seropositive state, and understanding that viral load is

obviously important for targeting pathogen reduction methods.

Some people will resolve the viremia, fairly common in hepatitis B and about 20 percent of HCV-infected people.

Now, other kinds of odd events have been documented, what are termed "immuno-silent carriers," people who may have long-term persistent viremia in the absence of seroconversion. And there's also been documented examples where people can become transiently viremic, clear the viremia, and never seroconvert--in essence, an abortive infection or a successfully resolved infection perhaps by cell-mediate immune responses but without antibody conversion.

This slide summarizes the story for HIV, and then I'll show a few examples. This is kind of the classic pattern. In a subset of people, probably 10 or 15 percent of the panels that we've studied, we can detect a blip of viremia, transiently detected--I'll illustrate that--about the time of exposure or within days of probable exposure. Then we have this explosive ramp-up phase, and we can quantify, as I'll illustrate, when individual-donation or mini-pool NAT or T24 antigen would detect viremia during ramp-up. We understand the dynamics of that ramp-up viremia quantitatively.

Peak viremia for HIV typically is in the million to 100 million range, so very high viral loads exist transiently during this acute dissemination phase. And then usually the viremia stabilizes down to set points in the range of 10^2 to 10^5 genome equivalent in the setting of a chronic asymptomatic HIV carrier.

This is just one example that illustrates a few points of a seroconversion panel that we've studied. This is a panel identified by Alpha Therapeutics and Bioclinical Partners. This person was actually not found to be seropositive until out here at day 16, with day zero defined as the first quantifiable RNA load sample. But these samples existed in the freezer to allow testing back and really careful study.

What you see here is about two to three weeks prior to the early ramp-up viremia, this phenomenon of intermittent or erratic detection of a blip viremic event. In this case, you know, one bleed had one out of ten replicate high-sensitivity PCRs positive, and then the next seven out of eight. And then we continue into the eclipse phase when there's no detectable viremia for two or three weeks, and then very early phase ramp-up, with, again, three out of eight reps positive. This is a very sensitive assay. And then you enter this exponential ramp-up phase,

this person peaking at about 3 million before they stabilize down. So a fairly typical case of early viremia.

This is just to illustrate how we can quantify the ramp-up phase. This is, I think, something like 45 plasma donor panels where we can quantify the exponential increase in viral load, and you see many of these people will have peak viremias in the range of 10 million or greater.

We can use that model, that regression line from the ramp-up phase, to estimate the viral loads at which different assays can detect the viremia. And this is really to emphasize here that in the setting of now mini-pool NAT, we can fairly confidently say that units should not be getting through the system that are mini-pool NAT-negative that have greater than about a thousand genome equivalents or viral copies per mL. And, clearly, if we moved individual-donation NAT, that viral load would be reduced to less than a hundred.

So in the setting of contemporary screening, the residual viremia that should be present and associated with transmission should consistently be less than a thousand or, potentially with individual donation, at a hundred copies per mL. So, in a sense, the challenge for residual clean-up by pathogen reduction.

This just illustrates that with progressive seroconversion, the viremia in a population analysis, again, tends to stabilize with the average viral load in the range of 10^4 to 10^5 in people who are chronically seropositive carriers.

Moving on to hepatitis C, similar early in that there is a period of eclipse during which we see this intermittent viremia, pre-ramp-up blip viremia, followed by the explosive ramp-up phase. Again, we can precisely estimate when different markers would detect that early ramp-up viremia. But very different from HIV, with HCV there's a prolonged plateau phase. It lasts about two months, and during this period people are asymptomatic. Most of this phase, the ALT levels are normal. Only in the latter stages does the immune response, cellular response kick in and you get liver damage.

The viral load during this plateau phase is enormous. It's in the range of 10^5 to 10^8 genomes, I believe, infectious copies per mL. So, really, a high viral load that, were we not to be doing RNA screening, would be, I think an enormous challenge for pathogen reduction to reproducibly eliminate.

With seroconversion, most people remain chronic carriers, about 80 percent of people, with highly variable viral loads ranging from 100 to 10^7 in people who are

persistently seropositive. Again, most of these people asymptomatic. Historically, of course, most HCV transmissions were occurring from donors who were seropositive chronic carriers.

Just a few specific data slides. This is one of these plasma panels for HCV. This donor was detected by mini-pool NAT screening at NGI, and out at these time points, so this is a month or more before eventual seroconversion. And, again, by testing the stored plasma units, we could quantify the viral load during the early phase of plateau, this person in 10^7 to 10^8 copies per mL. These two points were contributing to the ramp-up phase analysis I'll show in a moment. But this slide is mostly to illustrate this phenomenon of the pre-ramp-up viremia. In this somewhat extreme example, for about two months prior to ramp-up we detected erratic virus only in two or three of the four replicate TMA--this is full input gen probe TMA assays. So it's this kind of low-level viremia that is probably accounting for most of the rare residual transmissions. Even with mini-pool NAT and even with individual-donation NAT, I think we could only erratically detect these low-level viremic very early phenomenon.

For HCV, again, similar ramp-up phase. This is from about 40 or so plasma donor panels that had values during that very brisk early viremia. Again, many of these

people achieving viral loads up as high as 10^8 per mL. And from this kind of analysis, again, we can model the detection of mini-pool or individual-donation NAT.

In this example, we have the brisk ramp-up, and then just to illustrate one example of the plateau phase lasting in this case 46 days between the initial peak viremia and antibody conversion.

This is a summary from Sue Stramer from the Red Cross, just to show the time from detection of donors as mini-pool NAT-positive to seroconversion. The yellow is the period during which these people are viremic. These are whole-blood donors picked up by mini-pool NAT at the Red Cross. Red is when they seroconvert, and you can see that most of these people seroconvert in the range of 50 to 60 days after initial detection.

But this slide also illustrates two examples at the bottom of people who were immuno-silent carriers, picked up by mini-pool NAT, who remained mini-pool NAT-positive and antibody-negative for well over a year and well over two to three years. So these are examples of chronic carriers who never seroconvert.

Again, just to illustrate, this is data from Dave Thomas. This is injection drug users who acquired HCV while in follow-up. Some of these people resolved infection and completely RNA-negative over prolonged

periods. But this is just to illustrate that after seroconversion, most people, 80 percent of people, stabilize at fairly high viral loads. The average here is about 10^6 copies. So most seropositive HCV-infected patients have really pretty remarkable chronic viremia.

One example for hepatitis B, this person is, again, a plasma donor panel who became infected with HBV. This is showing DNA load and surface antigen levels prior to seroconversion and then how this is resolved down associated with anti-surface and anti-core. The emphasis here is that, again, viral loads during this early peak viremia can be up in the range of a million per mL or greater. So quite a challenge for inactivation.

Data again from Sue just showing in a population level the viral load over time as people develop surface antigen positivity, mostly here to emphasize again that in the later stages of primary viremia, viral loads in the range of a million or greater per mL are not unusual.

As we've enhanced the sensitivity of antigen assays, the residual units that would be missed by antigen tests have lower-level viremia, down in the range of 10,000. And as we go into the phase of NAT testing for HBV, we further reduce that. This is illustrated in a slide from a study that should be submitted shortly from a collaboration of FDA and REDS looking at different antigen

assays and the viral loads, and then as we get to mini-pool or individual-donation NAT, the viral loads that are still allowed to be, you know, released should be reduced well below 10,000 with contemporary antigen assays. And if we do implement NAT, we should be able to reduce the viral loads in the residual infected units down below 100 to even 10.

Changing gears a little bit, we've talked about the three major viruses, talking about viruses that we do not routinely screen for, and starting with CMV, with CMV the frequency of transmission by CMV seropositive units is in the range of less than 1 percent. We just completed a study in collaboration with John Roback that looked at 1,000 donor samples to assess the frequency of detected viremia. These were tested by several serologic tests and by two DNA assays that had been validated through a previous multi-laboratory study of performance of assays.

Each of these CMV, DNA, PCR assays had sensitivities of about 10 genome equivalent per 100,000 PBMCs. So these are very sensitive. In this example, we're targeting CMV-infected cells.

In this study, the seroprevalence was about 42, 44 percent. When we completed the study with coded panels and retest panels, only two out of 416 seropositive donors,

or 0.2 percent, were found to be viremic, and none of the seronegative donors were viremic.

Importantly, in these seropositive donors who were infected, the viral loads were very, very low, less than 100 genome equivalent per 250,000 PBMCs. So this is in the setting of a kind of cross-sectional seropositive study.

In a separate study that we've been doing looking at seroconverting donors, and specifically looking for plasma viremia, we completed a study that's been submitted that looked at a series of both serial samples from seropositive donors but, most importantly, focusing on seroconverting donors for CMV. And in this study, the only setting where we could find plasma viremia was in seroconverters. About 1 percent of these paired bleeds from seroconverters had detectable viremia. And in collaboration with Harvey Alter, we looked at serial samples from infected transfusion recipients who developed CMV, and we could quantify this period of primary infection where we could detect CMV DNA in the plasma often for two or three weeks, peaking at fairly low viral loads, at about a thousand per mL, during primary infection.

Now, we don't have a lot--this is plasma viremia, because there are not a lot of cell samples from seroconverters for CMV stored in any repositories. So I

contacted John Roback, who recently has done studies in the murine CMV model looking at acute infection, infecting these animals and then monitoring the blood, and this is showing copies of CMV DNA in the plasma following acute infection, and very similar to what we see in humans, there's an early period of plasma viremia, but quite low viral loads, in the range of 100 to 1,000 copies per mL.

In stark contrast, though, they were able to study the frequency of CMV-infected cells, and they're finding viral loads as high as 10^5 to 10^7 per mL during acute infection. And this is probably the case in humans based on some limited human data, that in primary infection there may be quite high viral loads of infected cells.

Another virus that's gained some attention recently is Herpes 8, which is the Kaposi's sarcoma virus. However, the studies that we've done, and also CDC has recently completed some large studies in Africa, suggested this virus, although there are a fair number of donors who are seropositive, the donors are consistently DNA-negative and that there is not transmission of this virus by transfusion. So this is a study that the REDS group has just completed with Phil Pellet from CDC which involved evaluating I think seven different laboratory assays for the prevalence of CMV antibody, and these tests detected CMV antibody ranging from 0.5 percent to 5 percent of the

donors were said to be seropositive, and a latent class model analysis supported an overall estimated seroprevalence rate of about 3 percent in the REDS donor pool. However, all of these samples were PCR-negative, and, again, studies from CDC recently have shown in a large study in Africa that there does not appear to be any transmission of HHV-8 by blood transfusions.

Parvo B19 is an important agent of concern to blood banks these days, a lot of considerations about the need to add screening for this by NAT methods. This is a non-enveloped virus that seems to be relatively refractory to inactivation.

This virus tends to--it infects erythroid progenitors and causes anemia. It can also cause a problem in pregnant women and newborn infants. This has epidemic nature.

Now, in terms of the viremia pattern, DNA is detected typically quite rapidly following infection, within four to eight days, and transiently for about seven days. IgM antibody is usually associated with resolution of the viremia. However, there have been examples of low-level persistence of viremia even in the setting of seroconversion.

Now, very importantly, the viremia in acute infection is enormous, ranging as high as 10^{14} per mL. This

is an example that Sue Stramer shared with me that shows one case of a plasma donor who was detected as viremic, and, again, in this example the viremia peaked at 10^{12} per mL, and this person remained actually PCR-positive at very low levels for months following that initial peak viremia. So, really, an enormous challenge for inactivation methods in terms of the viral load.

Hepatitis A, classically foodborne hepatitis, but there are rare transfusion cases. Again, a virus of focus these days in terms of possibility NAT screening. Classic dogma suggests that hepatitis A is cleared very rapidly as seroconversion occurs, typically to undetectable levels within four to six weeks. There's classically no chronic carrier state, and people who have converted are thought to be immune for the rest of their lives.

However, recent work from CDC has suggested that viremia may be prolonged and may persist in some people, so they had 13 individuals who acquired HAV while under HBV vaccine studies, and these people had viremia that preceded ALT by up to over a month. So before one would have any evidence of hepatitis, they were viremic. And in some cases, it lasted about three months. And the viremia levels, again, can be quite high, 10^5 to 10^7 per mL during the peak, and then staying up in the 1,000 or more for periods of months following conversion.

The last group of viruses I want to briefly discuss are really viruses that over the last, you know, five to ten years have been, you know, sort of the focus of a lot of attention and then sort of put on the back burner because of the absence of confirmed evidence of clinical significance.

However, I think that these viruses do present us with a potential model of efficacy, to study the efficacy of pathogen reduction, so I want to briefly summarize their characteristics.

The first of these is hepatitis G virus, or GBV, discovered back about early 1990s and, again, thought to be a potential cause of residual non-A/non-B hepatitis, but subsequently shown to not be definitively hepatotropic or associated with any known disease. But, importantly, the rate of viremia in the donor pool is fairly high. In the range of 1 to 2 percent of U.S. volunteer donors are viremic, and much higher levels, 17 percent, in commercial plasma donors, clearly transmitted by blood transfusion. This is a slide from Harvey Alter looking at some transfusion-acquired HGV infections, and the point here is, again, very high viral loads. This person had a peak viremia of over 10^7 per mL. In this example, the person resolved the viremia associated with seroconversion, but there are also a number of people who remain persistent

carriers, often with viral loads well in excess of a million per mL. These people are giving blood every day, and this virus is transmitted at close to 100 percent frequency.

The TTV family of viruses, these are also non-enveloped viruses, so a problem in terms of potential other methods for inactivation. These are typically transmitted both by parenteral and fecal-oral routes. They do seem to be present in the liver but, again, there's not definitive proof that these cause any liver pathology or other diseases.

Over the past five years or so, it's been shown that this is actually a very diverse sort of family of related viruses that include the more recently discovered SENV variant.

In terms of prevalence in the donor pool--this is data from Harvey Alter--7.5 percent of the blood donations in the NIH, I guess, over the last, you know, ten or so years were positive for TTV DNA. In other studies from Japan, 50, 60 percent of donors are viremic for TTV. So a very prevalent virus.

The viral loads in acute infection tend to be a little lower than with HGV, around 10^4 per mL, peak viremia, and although this slide shows some associations between

that peak viremia and ALT elevation, larger studies have failed to prove that there's a causal relationship.

Finally, SENV, again, it's a related virus to the TTV family. However, this is a virus that there is some suggestion may have hepatitis associations. In transfusion recipients we see, you know, 30 percent; in drug users, 60 percent. All of these viruses I've just spoken about, readily transmitted blood transfusion.

In the U.S. donor setting, the prevalence of viremia is around 3 percent, but in other populations like in Japan, up to 30 percent of blood donors are viremic which SENV.

This virus, again, does have an association with hepatitis. In Harvey Alter's group, 11 of 12 residual non-A/non-B hepatitis cases were positive for SENV viremia. However, Harvey's current conclusion is that this is not definitive evidence that there's a causal relationship. There's no question that this virus is readily transmitted by transfusion. And, again, one example of a recipient from Harvey's studies that had an early acute viremia, up over 1 million per mL, associated with some ALT elevations, and people can become persistent carriers with this virus as well.

I want to just briefly touch on infectivity versus viremia to make the critical points that the level

of virus detected in most of these studies by nucleic acid methods is probably a good reflection of the infectious titer of virus that needs to be eliminated by a pathogen reduction method. And the best data for this is really coming from our recent experience with breakthrough infections by NAT screening.

For example, in this case reported in JAMA a couple years ago, a donor seroconverted to HIV in Singapore, and prior donations on lookback were demonstrated to transmit the virus. And when we went back and compared the ability to detect that infectious donation, which was in storage, using the newer NAT methodologies, what we found is that these methods were able to detect the infectious unit with full input assays. But as one diluted the samples out, mirroring the mini-pool screening context, these donations began to score negative. So this suggested--this was prior to widespread implementation of mini-pool NAT. This suggested that mini-pool NAT would miss some infectious units and that infectivity was present when very low viral loads existed.

This is data from the San Antonio transmission case this last year where we similarly had plasma available from the implicated transmitting donation, and we were able to do the two licensed--the two commercialized NAT methods and show again that undiluted these methods could detect

them. But as we diluted the sample out to the mini-pool sizes, erratic detection of the infection. So, again, clearly showing that HIV is transmissible when very, very low viral load is present.

This has also been shown with hepatitis C. This was a case reported in Lancet a few years ago from Germany where a donation given eight weeks prior to C seroconversion was associated with transmission of HCV by the platelet but not the red cell concentrate, sort of illustrating that the volume of plasma in the unit is another variable that determines the total input of virus and, therefore, the infectivity.

In this study, they were unable to detect the viremia present in that clearly implicated donation, and their conclusion was that they had transmission from a NAT-negative unit, emphasizing the point that even single-unit NAT may not prevent all transmissions.

We actually acquired that sample and tested it and were able to show that we could detect very low level viremia, but only erratically. So this is very similar to the kind of blip viremia that we talked about earlier.

So these studies, I think, show that window phase, antibody-negative units can transmit even when very low virus is present.

Now, what about people who seroconverted? A lot of people have argued that once people seroconvert, there's neutralizing antibody, there's defective virus, so transmission should be, you know, suppressed by these phenomenon. But, actually, if you look at the data on real human transfusion transmission relative to viral load, there's strong evidence that very low level viremia transmits even in the setting of seropositive individuals. This was a study we did from the TSS cohort where we looked at transmission from seropositive donations and correlated the virus load in the unit and the duration of 4-degree storage of the unit with transmission.

You probably can't see it very well, but there's really only a small percentage--over 90 percent of HIV seropositive units transmit. The only units that did not transmit are out here where they were stored for prolonged periods. If the sample wasn't in the fridge for more than a couple weeks, there was essentially a 100-percent transmission, irrespective of viral loads. And we're seeing transmissions even with units that had less than 100 genomes per mL in the setting of the seropositive person.

And we have similar data for hepatitis C from the TTVS where we're seeing transmissions from seropositive donations, even if the viremia can only be detected by full input TMA analysis, 100-percent transmission, even with

extremely very low viral load. So, essentially, if viremia is detectable, transmission is occurring at close to 100-percent rate.

So, finally, just a summary slide to point out that, as we focus on these agents and we try to understand the viremia that is the challenge for pathogen reduction, for the major viruses where we're doing mini-pool NAT, the viral loads should be quite low, the challenge is low. But were we to not be doing mini-pool NAT, considering dropping NAT, were we to go to pathogen reduction, the viral challenge would be quite high. The window periods have viruses in concentrations of 10^6 to 10^8 per milliliter. In chronic infection, there's still very high viremia, so consideration of dropping antibody testing or NAT testing I think would result in a very high challenge viremia for the pathogen reduction methods.

For the cell-associated virus, again, window period viremia, infected cells can be quite high, typically very low level viremia in the setting of antibody conversion; and, of course, leukoreduction for these viruses should reduce the viremia to low levels, although there's clear evidence that residual infectivity exists even in leukoreduced products.

For these two viruses, hep A/B19, the viremia is enormous, 10^8 to 10^{12} during the window period, and there is

increasing evidence that some people can become chronic carriers after seroconversion with low-level viremia.

And then, finally, these sort of sentinel agents that I think serve as opportunity to further evaluate the efficacy of pathogen reduction. They're being transfused routinely today. These established chronic infections with viremia in the 10^4 to 10^6 range. So I think these viruses are an opportunity to really evaluate in clinical context the effectiveness of pathogen reduction.

Thank you.

[Applause.]

DR. VOSTAL: Thank you, Dr. Busch.

In the interest of time, we'll move on to the next talk, and you will have a chance to ask Dr. Busch when he heads up the viral inactivation discussion panel later on this morning.

The next speaker is Dr. Leiby, and Dr. Leiby's a senior investigator at the Holland Lab at the American Red Cross, and he will cover the parasites and other pathogens that can be found in blood and transmitted by blood transfusion.

DR. LEIBY: I'd like to thank the organizers for having me come here and talk to you about something slightly different, and that is, parasites and other pathogens that are also transmissible by blood transfusion.

Now, we've already heard from Ros and Mike about the bacteria and the viruses, and we're going to venture into an area that's a little more gray, an area in which there's not as much information; and as far as pathogen inactivation and reduction, there's been relatively few studies, and those are the parasites and other pathogens.

What I would like to do is give you an overview of those agents which we know are transmitted by blood transfusion, the relative levels of transmission cases which we do see, and then some of the issues that, as one begins to develop techniques for pathogen reduction, we have to take into consideration with these agents because they are in many ways different than what we see with viruses and bacteria.

Broadly, the agents I'm going to talk about are the protozoa, the Rickettsia--which, in fact, are bacteria, but I think they fall better into a parasite classification--and, lastly, an agent of something that we might see in the future, I have included TSEs, now because we know they're transmitted by blood transfusion but because pathogen inactivation as a whole has a promise of being able to address agents for which we don't know about yet or which we might see in the future.

I'd like to propose as we get into these agents, these parasites and others, that we, in fact, are raising

the bar. In this sense, it's becoming more difficult as we address these agents to actually reduce them in blood and blood products.

First of all, they are phylogenetically much more complex. They are carryouts as opposed to the agents we've been talking about so far this morning. In essence, these cells are more complex. Their outer cell surfaces in some cases are more complex, so they may in some instances be more difficult to kill or reduce.

They also in many cases have very privileged intracellular niches. This is not to say that we don't find bacteria and viruses intracellularly, but some of these niches are rather unique, inside phagosomes, inside phagolysosomes. And so these may present problems which we don't see with the other agents.

Many of these agents can fall under the classification of being emergent, so we know very little about them. We don't have tests available to tell us how often they are transmitted. We don't have models, animal models or other culture models, that allows us to measure how well the inactivation techniques are working. So as a group, then, these become a little bit more difficult to work with.

Lastly, and perhaps most importantly, is the consideration of experimental models, because as we measure

inactivation, not only do we need the agents to put into the blood products to test the inactivation strategies or reduction strategies, but we also have to have ways of measuring how well these strategies actually work. In the case of these, we don't have the simple culture methods we have for bacteria or viruses. We may have to go to actual animal models. We may have to go to unique culture systems in order to get the things that we need.

In fact, as a whole, we need specific life-cycle stages, and as these organisms' parasitic life cycles sometimes are very complex, we need specific stages that may only be obtained from one source. As I said, the reduction and viability measurements are not so straightforward. We can't simply play it out on a culture plate to see if the bacteria grow, and we have to find a system that allows us to tell not only are the organisms there but, more importantly, are they still viable.

And, lastly, we get into cellular survival and integrity, and this is a question that I'm sure will come up later with any of these techniques, what kind of damage do we see with the cells that are left after the reduction techniques.

T2A The agents I'd like to focus on today are the ones that I believe are the most important. The first one I'll talk about is Trypanosoma cruzi, which is the

etiologic agent of Chagas' disease. Next I'll talk about the broader group of Plasmodium, which causes malaria. We'll talk a little bit about Babesia microti, which is closely related to Plasmodium, which causes babesiosis. Then we'll surely venture into the Rickettsia, which includes Ehrlichia and the recently renamed Anaplasma, which causes ehrlichiosis. Then lastly, as I said, not an agent that is known to transmit by blood transfusion, but we'll talk about new variant CJD.

The first one I'll talk about is Trypanosoma cruzi, and I'll do the same thing virtually for all these agents. I'll provide a little bit of background, then some information on the seroprevalence or transfusion transmission and some of the problems which we may be facing with pathogen reduction.

This is actually the agent of Chagas' disease, and as you can see here, it's a flagellated protozoan which is found extracellular. Unlike Ros and Mike, I can show you nice pretty pictures of these agents.

It causes, most importantly, a chronic, asymptomatic, and untreatable infection, and it's actually those first two things that make it most important as an agent transmitted by blood transfusion. The donors are, in fact, asymptomatic, have no knowledge of the infection. They're also chronically infected. They're probably

infected as children when they lived in an endemic country of Latin America. And for the rest of their lives, they are then capable of transmitting the infection during blood donation. As I said, it's endemic to portions of Mexico, Central America, and South America.

Now, there's four primary routes of transmission. First of all is vectorial, which is actually the insect or the natural way in which it's transmitted. It's transmitted when the feces of the bug, which contains the infective stage, is rubbed into a bite wound or some other mucosal surface.

It can also be transmitted congenitally, which is from mother to the unborn infant. Most recently, it's been reported--and this has been known in other places, but there is a case reported in MMWR in March of this year of transmission of Chagas' disease by organ transplant, in which a single organ donor transmitted the infection to three organ recipients, at least one of which died from Chagas' disease.

Lastly, of course, the topic we're concerned about is blood transfusion.

Now, as far as T. cruzi transmission by transfusion, if one looks at seroprevalence, in some at-risk populations the level is as high as 1 in 5,400 donors. In fact, Ira Shulman had a study in Los Angeles in which he

put that level at about 1 in 550 donors. So it depends on which population you want to look at.

Nationwide, it's probably about 1 in 25,000 donors in the U.S. are, in fact, infected with T. cruzi. And this number will vary depending on the number of at-risk donors in a given area, but I'd like to stress that there are no areas in this country where you would not find at-risk donors. The numbers just may vary.

In a study we've done at the Red Cross, we found that of these seropositive donors, 63 percent of them are, in fact, parasitemic, so they actually have parasites in their blood, and we know that we are transfusing these parasites to donors.

We've only seen those six transfusion cases as a whole in the U.S. and Canada. Certainly blood transfusion cases occur throughout Latin America, so this is nothing novel. The numbers seem low here. In these cases, they all involved immunocompromised recipients who got fulminant Chagas' disease. So what I always like to propose is that these six positive individuals in these six transfusion cases certainly served as sentinels, and they were the most obvious cases; whereas, most of them are either misdiagnosed, in many cases there have been underestimates of true transfusion transmission.

Now, if one wants to look at pathogen reduction for this agent, what are the issues which one needs to consider? Well, first of all, unlike what Mike just talked about, the high levels of viremia--and Mike proposed that as an issue when you're trying to deal with inactivation. I'm going to propose just the opposite. With T. cruzi there is intermitted to low level infections. Sometimes you'll be able to measure parasitemia in the blood. Sometimes there are no parasites present. And, in fact, when parasites are present, they're present in extremely low numbers, maybe less than ten in a unit of blood. So when you're designing a technique that's designed to eliminate such a low number of organisms, it gets to be difficult in your experimental models to not only measure that but also to reduce those parasites that may be there.

One advantage with this agent as opposed to the rest of the ones I'll show you, it's actually extracellular, so it's pretty easy to get at. It's found both in platelets and red cells. They're both capable of transmitting the infection. But when one gets into model systems of T. cruzi, things get a little bit more complicated.

First of all, it requires metacyclic trypomastigotes--those are the infective stage of trypomastigotes--in order to really mimic what goes on in

the blood bag. Those type of trypomastigotes can only be obtained through animal models or actually through sophisticated culture techniques.

And then in the back end, the measurements of inactivation require two things, either culture or animal models, and there we must distinguish between what, in fact, are parasites that are present--one could easily measure by PCR, but that does not give you any measure of true viability. So one must consider the viability or the infected--whether or not these organisms that you see are, in fact, infective.

Shifting to a slightly different agent, we'll talk about Babesia microti, which is the agent of human babesiosis, at least in the United States. It's an intracellular pathogen of red cells, and you can see some of the parasites in these red cells. It's a tick-borne zoonosis, transmitted by Ixodid ticks, more commonly called the black-legged or deer ticks. These are the same ticks that transmit not only Lyme disease but also vialichia (?), which I'll show you in a minute.

As far as babesiosis in this country, there is local and regional distributions, primarily in New England, the upper Midwest, and then there's some agents that are similar in nature, which I'll show you, from the West Coast.

When we get into Babesia and the rest of these agents, they all have what one describes as flu- or malaria-like symptoms, so they're very nondescript and difficult to identify individuals who are infected.

Infections generally as asymptomatic and self-limiting, so unlike Chagas' disease, which is chronic and untreatable, in fact, babesiosis is quite readily treatable with antibiotics. The problem, though, is that in people who are either elderly, immunocompromised, or asplenic, the disease can be rather severe or even, in fact, fatal.

Now, when one talks about transfusion transmission of this agent, the seroprevalence in studies that are published to date are somewhere about between 1 percent to 4.3 percent in donors. So it's not uncommon. Most seropositive donors are, in fact, also parasitemic, so we can measure the parasite in the red blood cells. And this parasitemia varies anywhere from a rather short period of a couple of months to greater than one year. And this suggests the possibility that, in fact, we may see chronic carriers or donors who have this infection for long periods of time and appear asymptomatic.

Now, unlike Chagas' disease, there's been quite a few transfusion cases, 40 to 50 transfusion cases in the U.S. within the last 10 years. And we hear reports of transfusion cases almost monthly, so this is one that is

becoming more and more prominent. In fact, this would certainly be an underestimate because most transfusion cases, again, are not recognized, particularly when you consider you have flu- and malaria-like symptoms. And they do cause some fatalities, so this is not a benign illness.

Well, what about the issues with Babesia? How are they different? Well, first of all, it's an intracellular agent, so we have to get the--whatever reduction method we use has to be able to get inside the cells. The other one is that there are similar emergent agents, so-called WA-1, CA-1, and MO-1. These are Babesia-like agents which are found primarily on the West Coast, transmitted by a different tick. There's been at least two transfusion-transmitted cases of WA-1, but genetically these agents are somewhat different than Babesia microti. So the methodology used to reduce these agents may be slightly different and will have to be considered.

These agents can be found, as you know, obviously in red cells, but due to red cell contamination, platelets have also been implicated in transfusion studies that have transmitted the agents.

Now, when one speaks of model systems, we need, first of all, required--it requires infected human red cells. One could use animal models, in which we used

hamster infected cells, but in a model system those would not be the priority, I think.

The measurements of inactivation get a little more complicated because there, in fact, are no culture systems available for this parasite. And it's long been thought kind of Holy Grail. People have looked for culture systems, and at least for the human Babesia, these do not exist. So the bottom line, the measurement for inactivation would have to be in some type of animal model to see if these organisms are, in fact, really reduced or inactivated.

Now, the close cousin of Babesia microti are the Plasmodium, the agents that cause malaria. These are also intracellular pathogens of red cells but also liver cells. But since we don't transfuse liver cells, we won't worry about those.

It's mosquito-borne, primarily by Anopheline mosquitoes, and primarily limited to the tropics, so it's not something we face actively here in the United States. It causes flu-like symptoms, which often have a periodicity, meaning that the symptoms will come and change every two or three days or four days, depending on which agent it is. So it varies by infecting species.

These are the two problems which we encounter in transfusion transmission in malaria. Although our current

strategies of using questions about travel and where people have come from work quite well, it's when we get into the asymptomatic carriers or more likely the people who are semi-immune and actually have low levels of infection that we actually see transmission cases.

The other complexity with the malaria is one designs techniques or pathogen reduction, and this is actually the list of the four human malarias: P. falciparum, P. vivax, P. malariae, and P. ovale. It's the rather complex life-cycle stages they have which might require different techniques or different constituents to remove them or reduce them, anywhere from these small ring stages to tropozooites to gametocytes. And all these organisms go through these similar complex life cycles. And as you can see, they're all intracellular as well.

Well, what about transfusion transmission? In the U.S., seroprevalence is unknown because it really is not actively transmitted, so it would be very difficult to get any measure of what the actual seroprevalence is. Generally, in the U.S., there's about one or two transfusion cases per year, very low levels, and primarily, as I said before, these involves asymptomatic or semi-immune donors.

Now, as far as issues that surround the development of tests for Plasmodium, these agents, again,

are intracellular in red blood cells, as shown here. There's a variety of species and stages which would have to be considered. The model systems, again, would require infected human red cells, and then, again, the methods of inactivation, the measurements, would have to apply probably in culture systems or perhaps even animal models.

Now, a group of agents perhaps you know less about than the others ones I've just mentioned are the Ehrlichia or the Anaplasma. These are the agents of human ehrlichiosis causing human monocytic ehrlichiosis and human granulocytic ehrlichiosis. There's actually even a newer form, Ehrlichia areini (?), that also is found among human leukocytes.

By and large, these are all newly emergent agents, appeared in the 1990s. You can see some pictures of them here inside, some granulocytes, and actually the arrows are pointing at the parasites themselves, the small--actually, which they are--they form what are things called morulae, which is a Latin term referring to the raspberry/grape-like structure.

Again, these are tick-borne Rickettsia transmitted by the same one that transmits Babesia and Lyme disease. It's intracellular, as I said, in leukocytes. And once again, we have the same nondescript flu-, malaria-like symptoms. And among patients who are infected, 10 to

20 percent have more serious complications. In fact, certain numbers of them also die as well.

When one talks about transfusion transmission and when one talks about seroprevalence, there are very limited studies, virtually none published, but we think somewhere--in the recent one we have in press, the levels are anywhere from about 0.5 to 3.5 percent of blood donors in Connecticut and Wisconsin. There is one presumptive case of HGE transmission reported at AABB a few years ago, and in this case, the red cell units were implicated, thereby once again suggesting that it contained leukocytes that were infected.

The problem with this agent, primarily because it's so new and emergent, is that it's underrecognized and also misdiagnosed.

Well, what about the issues, again, as far as developing techniques for reduction? Well, first of all, the first issue, again, is that it's intracellular, but in this case, there may be some advantage, in fact, that universal leukoreduction may, in fact, remove most of these infected cells.

As I said, it's newly emergent. In this case, it virtually lacks any test. We have no way of knowing how many donors are infected, how many are positive, and so forth. So there's a lot of issues that need to be worked

on in order to get a better handle about how big a threat this agent actually is.

There are several model systems. There are very good systems that allow ones to infect human leukocytes in culture, which works quite well, and that, as I get into measurements of inactivation, allows one to do it in cell culture. The point was that you need infected human leukocytes in order to do the experiments.

The last one I'll include--and this falls into, I guess, the category of the other as far as my talk of parasites and others, and I throw this out not as an agent. I said before that actually we know to be transmitted by transfusion, but one which needs to be thought about, as well as other agents that are newly emerged, and also the promise itself of pathogen reduction to address those agents that we don't know about yet or we will see in the future, and this includes, as I said, transmissible spongiform encephalopathies, which we know includes new variant Creutzfeldt-Jakob disease. It's a prion protein, so I can't show you any neat pictures. It's an abnormal isoform of a cellular glycoprotein, thought to be transmitted at least initially through contaminated beef. That's how it got into humans, or at least we think. It invariably causes a fatal brain disease, the incubation period in years, and untreatable. There, again, anytime

you get any of these agents that are incubated for years, we get into problems with our model systems for measuring inactivation or reduction.

Well, a lot of these things are easy to answer this time. If one talks about new variant CJD and we talk about seroprevalence, well, we really don't know, and as far as blood transfusion, we have no evidence that it's transmitted by transfusion.

But the issues that one would have to address if one wanted to look at this agent are complex. As I said, it's newly emerged, and the need for model systems, while there's a lack of tests and, as I already mentioned, very long incubation periods, it makes it difficult to look at these agents.

So just to summarize parasites and other pathogens in this topic, they are indeed more complex organisms, and they require more complex model systems. And the fact that they're newly emerged makes the ability to find these systems or develop these systems slightly more difficult.

Well, what about pathogen reduction itself and also the toxicity one may encounter? First of all, when you have an agent like some of these in which they have very low levels infection, it may be more difficult to eliminate them because we may have to increase the amount

of whatever reduction technique or inactivation agent we use in order to actually see that they've been removed. So I propose that perhaps in some of these agents that only have one or two per milliliter--or a hundred milliliters, rather, it may be more difficult to measure their inactivation as opposed to the viruses which might refer to that have hundreds of thousands or millions.

What about the cell viability? As we get rid of these agents which are found intracellularly, we may, in fact, also harm the cells. So those, I'm sure, are topics we'll hear more about today.

And, lastly, what about diminishing returns? I raise this issue that in some of these more rare event cases in which we see maybe only a couple of cases of malaria per year or several cases of babesia per year, perhaps pathogen inactivation may be an overkill as a technique to limiting these agents where some of the more tried and true systems we already have may be the easier route and, in fact, the more cost-effective route. So I suggested that we may be treating many just to reduce a few cases.

Thank you.

[Applause.]

DR. VOSTAL: Thank you, Dr. Leiby.

Miraculously, we've managed to get back on time. Are there any questions for Dr. Leiby?

[No response.]

DR. VOSTAL: If not, we'll move on to the last talk in this session, and this will be presented by Dr. Richard Diamond. Dr. Diamond has just recently joined the Center for Biologics, and he's now the Assistant to the Deputy Director for Medicine, Dr. Jesse Goodman. Fortunately for us, Dr. Diamond has a 30-year history in academic medicine as an infectious disease specialist, most recently coming from the University of Boston School of Medicine.

Dr. Diamond?

DR. DIAMOND: It's a pleasure to have the opportunity to speak to you. I was asked to focus on bacterial infections, perhaps because there's a broad range of topics to address, and there's a need to keep things simple in terms of time. And there are critical areas to address in relation to bacterial infections that haven't been yet. And what I'd like to focus on in particular are what happens and what's the significance of organisms that are transfused and what about bacterial products; and as the prototype bacterial product, we'll try to address endotoxin in particular as the most clearly studied

bacterial product which is known to have significant consequences in the human circulation.

You've already heard that there have been a variety of studies of what happens when you add bacteria to various blood components. With red cells that are stored at 0 degrees--and a variety of different groups have added anywhere from one per mL to 100 per mL bacteria and seen how long it takes for inocula to grow, and really minimal inocula in that range of Yersinia enterocolitica, Enterobacter agglomerans, or Pseudomonas species, reach log phase growth and high levels of endotoxin, lipopolysaccharide by two to three weeks, with a generation time, even though it's slowed down, of anywhere from 15 to 22 hours.

Other organisms that we would consider less pathogenic can also grow at these temperatures, even though we don't usually call them--consider them to be major risks for growing in solutions at low temperatures, but they certainly can. And, presumably, within this time period patients would receive significant inocula if they were transfused. Platelets, of course, being stored at room temperature, minimal inocula very quickly give you log phase growth, in less than one day in most of the studies that have been looked at, with stationary phase growth with numbers above five times 10^8 per mL, often about 10^{10} per mL

in units that are four to five days--after four to five days of storage.

Less pathogenic bacteria that we don't associate with human infections as much but certainly have caused them in recipients of contaminated blood, like Propionibacterium acnes, which is one of those organisms that can enter as a skin contaminant, may take a little bit longer to grow and have a slower doubling time when one artificially inoculates them into platelet samples, but certainly can reach very high numbers by four to five days and have caused significant infections.

So how often does contaminated blood cause bacteremia? Well, if you look at the data--and this is from a really broad series of collected numbers in the literature--it's hard to come out with defined conclusion, but you can make some sense out of this. And I think the important message is that if one looks at the estimates of clinical bacteremia that occur from either red blood cell contamination or platelet contamination, it is only a small fraction of the--the rates of clinical bacteremia are really only a small fraction of the numbers of contaminated units that are transfused.

In other words, even though these are high-risk patients, particularly those who are receiving platelets, they don't get infected most of the time when they receive

contaminated blood. Some of them are receiving antibiotics, but not all of them are in that category at the time of transfusion.

Even in high-risk patients, infusion of contaminated blood then usually has apparent effects. It's estimated, for example, after--the infection rate after receiving contaminated peripheral blood stem cell infusions is about 13.7 percent. Now, most of those patients are receiving antibiotics. Those numbers are colored, obviously, but the bottom-line message is that infusion of contaminated blood doesn't necessarily cause clinically apparent infection.

If one looks at various rates of transmission of transfusion-related bacteremia and fatalities, it's hard to look at the multiple different series individually. This is from one series from CDC which I selected, not because it's necessarily better than any of the other ones in the literature, but because it's from the most recent time period, and one can at least say that these patients presumably receive state-of-the-art treatment or closer to it than some of the older studies. And one can see that the rates in that series of transmission of single-donor platelets, pool platelets, and red blood cell units, and the fatality rate in terms of percentages.

So, again, it depends on the series, and it depends on who is and how the counting is done, but certainly not all contaminated transfusions lead to fatalities. So what variables affect clinical outcome? Obviously, the infecting organisms makes a difference.

There's an enormous difference in strain and species virulence. However, common skin contaminants such as Bacillus species, Propionibacterium acnes, and so on can cause significant infections, and not only in those who we would think would be ultra-susceptible to infection, in other words, pancytopenia patients. The concentration and the rate of the bacterial infusion obviously makes a difference, and a variety of host factors, not all of which are very well defined, clearly neutropenia and immune status, but also a variety of other factors that have to do with cellular immune status, pre-existing antibodies to organisms or organism products in question, and receptors.

It's known from mouse studies that there is significant genetic variation in receptors for Gram-negative organisms. The endotoxin receptor, the CD14 family of receptors, in terms of responses, is linked to toll-like receptors which seem to be responsible for transducing the signals to endotoxin exposure. And as this is being studies, it's very clear that at least different mouse strains have definable genetic variations in these

receptors which can definitely determine outcome of infection, related receptors in the toll 2 family have been shown to be linked with survivability of staphylococcal sepsis. It's certainly highly likely that analogous genetic variation in humans might well be linked to some of the explanations for why seemingly normal people have very different outcomes to bacteremias, something that we who struggled with patients in clinical settings have been pondering over for a very long time.

Now, also there's the issue of general clinical stability of patients, and that's where it makes it very difficult to interpret the very different series and different listings of fatality rates from the small series of recipients of contaminated blood that have been published. These can have major factors in both responses to a load of bacteria as well as to a load of bacterial products like endotoxin because the pre-existing status of the patients, the ongoing interactive factors are of major significance, and then therapy obviously makes a difference. During the transfusion, antibiotics can have a multiplicity of effects. First of all, antibiotics can raise the level of endotoxin liberated from bacteria 3- to 30-fold, depending on which series that one looks at. And this can be demonstrated in vitro and in vivo model systems. And, in addition, certain antibiotics bind

endotoxin as well as binding and increasing clearance of organisms. Polymyxin has been used in vitro and in vivo to demonstrate for clearing endotoxin, and antibiotics that are in common use, some of the aminoglycosides like gentamycin, although they're not as potent binders of endotoxin as polymyxins, certainly are capable of binding endotoxin. And a variety of anti-inflammatories can have major effects on the cytokine responses that determine the effects and the outcome of bacterial and endotoxin exposure. And then how timely is recognition and specific treatment?

From an analogous lesson of following immunocompromised patients for years, the old saw was, well, you better start treating these patients immediately when they get a fever and cover them with broad spectrum anti-Gram-negative coverage because these infections are rapidly fatal in neutropenic patients. And Gram-positive infections cause significant morbidity and some fatalities, but not as rapidly.

If you take that as a lesson, the lesson here is there are major differences in host susceptibility, but also the lesson in recent years is if we look at that group of patients, they all are treated so promptly with broad spectrum Gram-negative coverage that it's hard to find a series where the survival of Gram-negative bacteremia is

less than 90 percent in those patients, so it's hard to do meaningful comparisons of agents or regimens.

So host factors do play a major role, and, in fact, if one looks at all these series, what comes out is, if you get contaminated red cells, your major risk of post-transfusion bacteremia correlates with pancytopenia and immunosuppressive therapies. Surprise, surprise, that doesn't tell you anything you didn't know or anything that's terribly helpful.

There is a presumed relationship to impairment of bacterial clearance mechanisms which is responsible for this difference. And these patients are--one commonly sees that clinically significant infections can be initiated by less virulent bacteria and lower bacterial inocula. These have been shown in experimental models and to a lesser extent and more indirectly but with some compelling evidence in clinical and epidemiologic studies.

In transplant recipients, for instance, symptomatic bacteria occurred in one out of 1,700 pooled random donor platelet unit infusions that were transfused, and that's one out of 350 transfusions in these patients. When one looks at it in that context, this is happening pretty often in susceptible groups of patients.

But, really, is there a definable threshold infectious dose? The short answer is, well, you can't

really tell in an individual patient. Infectious doses and attack rates are hard to determine in clinical and epidemiological studies. You don't exactly know what went in. There aren't good measures of inocula. We don't tend to measure them in advance.

Most bacteremia-associated contaminated platelet infusions probably contain between 10^6 and 10^{10} bacterial per mL. Of course, if somebody has an immediate reaction, which they might very well to a massively contaminated unit, they don't get the whole unit. And most of the reports don't characterize exactly what they got, so it's very difficult to tell even if you knew what was in the bag at the time of the infusion, and there's precious little information that characterizes things that much.

You don't know what the real dose was that was given to the patient, and, clearly, many patients get infected by units that are less contaminated than that. And, in fact, as you've already heard this morning and was intimated, it's much harder to tell when that happens, because the paradigm has been that contaminated blood and measurement of transfusion reactions are limited to immediate reactions. And we know from human studies and from animal models that inoculation intravenously with contaminated solutions of any kind, not just blood, the effects may be delayed and delayed significantly in animal

models, the lower the inoculum one gives, and depending on the host factors that are present.

So bacteremia after continued IV infusion we know can occur with 100 to 500 milliliter infusions with 10^5--between 10^5 and 10^7 bacteria per mL in maybe 10 to 30 percent of non-immunosuppressed patients based on series of not blood contaminated infusions but contaminated intravenous solutions where it's been possible to at least come up with a little bit of an idea of the range of contamination within the solution.

But even then the information that you'd want to know in detail what the true inocula were and what the exposures were and what the rate of infusions were are not available clinically and epidemiologically for you to make hard and fast conclusions about that.

But what does come out of this is that the mortality rates, considering the amount of bacteria infused, are not all that high. Now, they're higher than anybody would like to see, but considering the human experimentation of infusion of intravenous bacteria, these patients--most of these patients survive.

Lower numbers of bacteria certainly can cause bacteremia in immunosuppressed patients, and probably--and, again, very difficult to judge and extrapolate from the epidemiological data that are available, but from some

anecdotal series, 10 to 100 mL infusions intravenously probably containing anywhere from 10^2 to 10^5 bacteria per mL have initiated bacteremia, but not invariably.

If one looks at then something that we would hope would be more definable, what are the reactions to endotoxin, lipopolysaccharide? This has been well defined in non-human primates, and there are some very nice models there. If one looks at septic shock, E. coli septic shock models in Rhesus monkeys, you can see the levels of infusion, 375 to 500 microgram per kilogram per hour for eight hours, or in baboons, one and a half milligrams per kilogram over ten minutes. The minimum lethal dose seems to be on the order of three to six times 10^6 endotoxin units intravenously, and that will kill about 15 to 30 percent.

E. coli sepsis, non-fatal sepsis with 5 to 40 times 10^8 colony-forming units over two hours, four times 10^{10} is lethal to baboons uniformly. But humans are maybe 100 times more sensitive to lipopolysaccharide than any of these primate models, so they're of limited value in judging what will happen when we infuse endotoxin or bacteria.

So what are the responses to endotoxin in humans? The minimum dose, in the range of 0.1 to 0.5 nanograms per kilogram, that will cause fever has been defined. That's 0.1 to 0.5 endotoxin units.

If one infuses 2 to 4 nanograms per kilogram IV, which were the standard doses for normal volunteer studies in humans--and these are healthy, normal volunteers--one gets levels within 15 minutes to an hour that peak between 16 to 240 picograms per mL. These are all healthy volunteers, and they actually precede the peak effects. Signs and symptoms that develop are fever, chills, nausea, myalgia, headache, leukocytosis, and hypotension. They're somewhat delayed in onset after infusion, and that's presumably because they are related to the physiological responses that have to do first with primary release of TNF alpha and then with a whole slew of other cytokines and mediators, and there's also activation of the clotting and fibrinolysis system, although in these normal volunteers, this does not proceed to clinically evident BIC. So there are ways of controlling these processes.

And then when one gets up to 20 nanograms per mL or 100 endotoxin units, you get into a range of serious toxicity. This is sort of a guess from--again, from epidemiologic data because this isn't the kind of study that is approved for general human studies use.

So what about endotoxemia? First of all, it's not always detectable in septic shock. About 30 to 50 percent of septic shock patients where endotoxin levels are measured have detectable endotoxin, and usually it's on the

order of 400 picograms per mL or above if present. But continuous exposure differs from a bolus dose, and the bolus doses that we see in volunteers aren't the same kind of exposure--it may be similar to what happens if you kill off all the bacteria in a blood unit, and then infuse it with the endotoxin that's left behind. But it's not going to be analogous to a situation of ongoing sepsis where the complex mechanisms involving endotoxin clearance balanced with ongoing effects on the immune and inflammatory responses are going to change the output of cytokines and the cytokine profile and inflammatory mediator profiles drastically, not to mention the complexities of inter-current factors in unstable patients where endotoxin may have very unpredictable interactions with other ongoing processes that affect the inflammatory and immune mechanisms.

And high levels can occur without bacteremia, for instance, in ARDS patients, pancreatitis, cirrhosis, maintenance hemodialysis, and, interestingly enough, in one study ultra-marathon running. What's interesting there is they measured--some runners had over a thousand picograms per mL after running 81 kilometers. I'm not sure--I was dumb enough to run about ten marathons years ago, but I couldn't even conceive of doing that. But all they felt was some nausea and vomiting, which I think you have a

right to feel after 81K, associated with this level of endotoxin. So depending on the circumstances, it's very hard to predict what effect a given level of endotoxin is going to have in an individual patient by itself, and that other factors presumably have major effects on modulating what responses you're going to see. It just makes it hard to predict what a given measurement or level means.

So levels correlate very inconsistently with outcome, and complex dynamic factors determine what the levels are. So if you look at one case, for example, of an outbreak of 11 cases that were reviewed by CDC of Yersinia enterocolitica sepsis after contaminated red cell transfusions, the mortality was 45 percent. Endotoxin levels were tested in 5 out of the 11 patients. The median was 11,645 nanograms per mL. That's a few logs higher than the last slide we were talking about in the levels for what's commonly described in sepsis with a range of 3,500 to over 17,000 nanograms per mL and most were receiving antibiotics, which, as you recall, can increase 3 to 20 times or more the endotoxin levels in the circulation. What effect that has is unclear.

And at least some patients, in this case the majority, 6 out of 11, can survive with enormous circulating endotoxin levels. That's about the only thing I can make out of this.

So we've got examples of bacteremia--even though most cases of contaminated blood products that lead to bacteremia seem to be relatable to skin contamination, some have been documented as related to episodes of occult bacteremia in donors. And, in fact, I remember as a clinical associate making rounds in the clinical center in the early 1970s an outbreak on the childhood leukemia service of four cases of Salmonella choleraesuis bacteremia. The only common feature these people had, these kids had, was they had received platelet units from the same donor, and traced back to the donor, and the donor had an occult Staph--excuse me, Salmonella osteomyelitis, which presumably led to intermittent bacteremia in very low levels that one would have to go to some extremes in those ancient days 30 years ago to detect.

So platelets are particularly vulnerable because of the storage temperatures, but also because so many organisms are adherent to platelets and can adhere very readily. In fact, I spent the last few years of my laboratory research career looking at some of the antimicrobial effects of platelets and some of the interaction of platelets with a variety of different organisms. It's striking how adherent platelets are and how readily platelets could actually concentrate organisms as one concentrated platelets.

And many organisms can cause prolonged bacteremia without concomitant symptoms. We've heard about some of them. I might mention Bartonella, Borrelia, Brucella, we've heard about Ehrlichia, Treponema pallidum, Rickettsia. There's currently a low prevalence of these organisms in donors, we think, but our lessons about emerging infections in recent years are that things can change. And what that tells us, I think, is that we need to set up epidemiologic screening methods and be ready to change whatever paradigms we have for risk management when we are trying to eliminate pathogens from blood. It's not going to be possible ever to eliminate all risk of everything, and the relative risks for any given group of pathogens is likely to change over time, and we'll need epidemiologic data to be able to respond promptly to how this is taking place.

Again, many organisms adhere to platelets and red cells, and some are invasive. These are red cells that have been invaded by Bartonella. You can see--actually, the slide doesn't come across that well, but there's some stainable material inside the red cells that represent Bartonella. This is a chronic infection that has been recently recognized as being more common than we had thought in the past and where people can have asymptomatic bacteremias that go on for months.

We haven't had a case of transfusion-transmitted syphilis since 1969. Syphilis is very low prevalence in the population, and there are screening tests for people for this infection.

On the other hand, there are other agents that are more common and may become more common yet, and we've heard of a number of examples this morning where there could be rising areas of concern and where there's important needs for epidemiologic data so that risks can be accurately estimated.

Even with infections due to agents that invade and reside in leukocytes, depletion of leukocytes from blood doesn't preclude transmission by transfusion. This is human granulocytic Ehrlichiosis infecting a leukocyte. It's been shown with Ehrlichia and a number of other organisms that reside intracellularly in leukocytes that one can, even after depletion of leukocytes, demonstrate organisms in supernate, and that these organisms might well be transmitted by transfusions that were leukocyte-depleted. So that by itself might not be sufficient.

So when one considers the pathogenic mechanisms that allow cellular adherence, that allow for cellular penetration, and the various localizations and the tremendous diversity of pathogens, it is an enormous

challenge to begin to pick and choose how to decontaminate transfused blood components.

It is also critical to keep in mind that endotoxin has some important lessons and that it is not the only bacterial product that has clinically consequential responses and provokes clinically consequential responses which may be potentially life-threatening in susceptible patients.

What this means is that any mechanisms that are used for decontamination presumably will release bacterial products. It is essential to know what clinical effects these released bacterial products will have, and it is naive to think that mere elimination of detectable live agents, no matter how effective, will necessarily make transfusion of blood products safe. The consideration of the release of products of the organisms and the effects that they'll have clinically is a critical component that has to be addressed.

So what can we say? Is there a safe level of bacterial challenge? It depends. Bacterial species and strain virulence obviously make a difference. The host immune status and clinical stability makes a difference. Host genetic factors, treatment. The short answer is for most patients probably, but we can't predict for sure which

ones or how much any given individual will be able to clear without any adverse consequences.

Is there a safe dose of endotoxin? In a defined bolus dose in volunteers, yes, there is. That's normal, healthy people who get a bolus of endotoxin. During ongoing sepsis, with ongoing release and unpredictable clearance of endotoxin, expect the unpredictable. Outcomes probably depend on the duration of endotoxin exposure and the nature of interaction with other factors because endotoxin can alter a host of physiological and pathological processes in cells and tissues as well as responses to drugs, mediators, cytokines, and other stimuli. And even safe doses for healthy volunteers may harm unstable immunocompromised patients.

So since I haven't offered you anything helpful, I'd be glad to answer any questions.

[Applause.]

PARTICIPANT: I have a comment and a question. The comment is that the lipopolysaccharides from different Gram-negative organisms have different structures and may have different effects, and we and others have done studies to show that in human, if you use LPS from Brucella abortus and compare it to LPS from E. coli, for example, the LPS from Brucella abortus has a two- to three-log lower potency

than the LPS from E. coli. So I think all Gram-negative organisms and all the LPSs are not equal.

The question I have--well, I have a question to follow that. The question I have is related to your comment, which I think is very important, that Gram-positive organisms and Gram-negative organisms, when you inactivate and you may still have cell wall components that may be very active, and there's been a lot of recent literature about how even Gram-positive cell wall components can activate toll-like receptors and stimulate TNF release.

DR. DIAMOND: Absolutely, and there's a limited amount of time to discuss all these things, but certainly there are--just as there are strain differences between bacteria of the same species, the variations in endotoxin structure actually that have relation to biological effects have been reasonably well characterized on a molecular basis. And certainly there are enormous differences in endotoxins between different species, and in peptidoglycans and other bacterial products between different species.

The point is not about any specific one, but I think more in a generic sense that all of these compounds are going to be there. There are too many that might be of consequence, and what we desperately need are some sort of biological correlates that we can begin to use as an index

for what we may be doing when we're making any changes in organisms, and then infusing what we have left.

DR. VOSTAL: Thank you, Dr. Diamond, for tackling that difficult subject, and also thank you for pointing out another reason why not to sign up for those ultra-marathons.

We're now up against a break, so if we could take a short break and come back at 10:45, we'll get started on the other sessions.

[Recess.]

DR. VOSTAL: Okay. Now we're going to move into our next session, and this session will cover the methods of decontamination or of pathogen reduction. Our first speaker will be Dr. Steve Wagner, who's the director of cell therapy and blood cell therapy development at the American Red Cross, Holland Labs. And Dr. Wagner has actually been helping us in planning and carrying out this workshop, so we really appreciate his input. So please welcome Dr. Wagner.

DR. WAGNER: Thank you very much. I'm very grateful to be able to help plan this workshop and also to participate in it, and my task today is to give a general overview on methodologies for pathogen reduction. In addition, what I'd like to do is talk about a few points that I think might be important in trying to analyze some

of the potential benefits and risks that are involved in these methods.

We've heard from different speakers some of the rationales for inactivation. They are to eliminate or to reduce the residual infectivity in blood from pathogens. We all know what the danger of pooled products are where infections might be disseminated to many individuals, and certainly inactivation techniques might help in that regard. Inactivation techniques also might provide an additional layer of safety on top of testing and other things that we might do.

There are some agents, as Dr. Busch and David Leiby have indicated, that we currently have no test for, and so pathogen inactivation might provide some additional safety in those situations. We haven't talked very much about--and I certainly won't talk much about the fact that some agents mutate and there are different varieties, and certainly this has been the case with HIV. And there have been some--there are differences in terms of the serological characteristics of different strains of HCV. And so variant agents which may not be amenable to current tests might be another category of agents that might be--where safety might be improved with inactivation techniques. And people have talked about new agents, and,

of course, there's public and political expectations of a zero-risk blood supply.

With all that said, pathogen inactivation is not necessarily a simple problem to solve. There are inherent challenges. Pathogens may appear in different compartments. Many of the pathogens have different structures. Dr. Leiby talked about that. And, in particular, in the virus families, there are some non-enveloped viruses whose capsid proteins are so tightly interdigitated that it's very hard for small molecules to permeate the capsid, and, in particular, the picornaviruses, of which hepatitis A is in that family, the capsid proteins are so tightly interdigitated that even a cesium molecule is unable to get inside the capsid. And so you might expect that these types of agents would be very difficult to inactivate.

The processing that occurs with pathogen inactivation may reduce cellular yields. If you have to move blood from one container to another that might have different removal devices or other things, there are going to be some cells left behind. And so we shouldn't expect to have 100 percent of the cells that we started with after the inactivation technique.

In addition, most of these agents work by targeting nucleic acids, but like most things in life,

nothing is perfect, and there are unwanted side reactions from the treatments. And these side reactions may lead to loss of cellular function or loss of recovery or survival. And so there's going to be some price to pay in terms of both cellular yields and the effects of these side reactions on the product, and that's going to have to be weighed against what increases in safety you might expect from pathogen inactivation.

In addition, there are some other types of things that really need to be considered that cannot be dealt with in traditional clinical trials because they're low-frequency events. Any pathogen reduction technique really will probably require the measurement of low-frequency adverse events, and the reason for that is that the current safety of the blood supply is pretty safe in terms of fatalities from infectious disease transmission. And you obviously want to have a process for pathogen inactivation that would not introduce more adverse events than the events that you're trying to prevent. And so as a result, without implementation and long-term study, it may be difficult to predict the risk to blood bank workers, for example, to genotoxic agents or to recipients by accidental exposure or by residual drug. Without implementation and surveillance, it may be difficult to assess the risk of allergic or hypersensitivity reactions or, indeed,

anaphylactic reactions in susceptible recipients caused by alkylations to proteins or by drug metabolites. And some of the pathogen inactivation agents are alkylating agents, and some alkylating agents have been used obviously in chemotherapy, and I just draw your attention to this article that has to do with the hypersensitivity reactions that people have seen to cancer chemotherapeutic agents. Obviously, you don't want these reactions to be greater than the infectious disease risks that we're trying to prevent.

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

And so what's the risk of fatalities from infectious disease transmission as we know it now? Probably the most frequent fatality, as defined by the BaCon study, is about 1 in 450,000 units of blood. And so if you're going to do pathogen inactivation, you don't want someone to have an anaphylactic reaction more than 1 in 450,000 times. And so that's obviously going to require quite a lot of surveillance in order to assure that we're not actually making the problem worse.

There's a number of approaches that have been taken to inactivation I'm sure you've all heard. For platelets, there are the psoralens, S-59 and riboflavin. For red cells, there's a FRALE compound, S-303, and Inactine, which is a PEN110. And in plasma, people have looked at solvent detergent, methylene blue, and S-59. I won't be talking about plasma in my talk today.

This is the structure of S-59. It's a plainer compound that's capable of interpolation in between the bases of nucleic acid. It's got a positively charge amine group that gives the molecule quite a lot of solubility. And its mechanism of inactivation is by intercalation, as I said, between the bases of double-stranded regions of RNA or DNA, and upon absorption of UVA light, psoralens can make mono- or diadducts with pyrimidine bases in nucleic acids. The diadducts can form a cross-link between nucleic acid strands, and it prevents subsequent nucleic acid replication from the pathogen.

The steps for S-59 pathogen reduction involve collecting an apheresis unit that's suspended in a platelet additive solution, addition of the S-59 to platelets, transfer of the platelets into a UVA-permeable plastic container, then illumination with red light and transfer of S-59 phototreated platelets to a container that has an absorbing resin to remove S-59, free S-59 in some

photoproducts, and transfer of the phototreated platelets with the reduced free S-59 levels to another storage container. So there's a lot of transfers and a lot of opportunities for leaving cells behind in different containers. So one shouldn't expect to start out--to end up with 100 percent of the platelets that you started out with.

S-59 and UVA has been demonstrated to work and inactivate a wide range of enveloped extracellular viruses. It inactivates intracellular viruses. There are some non-enveloped viruses that have been shown to be inactivated. In general, these are non-enveloped viruses that have capsids that allow the permeation of the drug to the target DNA. As far as I know, there's been no demonstration against hepatitis A or parvovirus.

Some parasites have been shown to be inactivated, and both Gram-positive and Gram-negative bacteria have been shown to be inactivated. But the technique is probably not effective against spores. It's acknowledged that spores will probably germinate in a rich media. However, it's not clear that every spore that's in a bag will germinate before the inactivation process occurs. So they might be carried forward. In addition, probably the technique is not effective against endotoxin.

But nucleic acid is not necessarily the only target of psoralens. There's quite a lot of literature indicating that psoralens can produce reactive oxygen species. These include singlet oxygen and superoxide. These oxidative species can diffuse and tend to oxidize membranes and that's been observed after psoralen phototreatment. And psoralens, of course, are used for the treatment of psoriasis, and there's been observations of skin erythema caused by psoralen treatment, and this erythema from the skin photosensitization can be inhibited by reactive oxygen scavengers. And so psoralens not only react with nucleic acid. They also act as photosensitizers and can produce reactive oxygen species. Reactive oxygen species, many times the target are cellular membranes.

Here's some data that's been published in Vox Sang where the investigators were looking at S-59 treatment and were using electron microscopy to measure platelet lysis. And by day five, there's a significant increase of lysis from a control value of 5.5 percent to 14.4 percent lysis that are measured in platelets that were treated with S-59 and UVA light and where the S-59 had been removed by a compound absorption device.

This result was obtained even though the platelet count as measured was totally unchanged, and so there is

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some effect on platelets, and it may be due to some of these oxidative processes that I talked about.

In addition, a well-known target for psoralens includes unsaturated lipids as well. Much of this work has been done with eight (?) , but UVA light and psoralens can form adducts with unsaturated fatty acids at the side of the double bond. In addition, not only fatty acids this reaction occurs but also true lipids. So a removal device to remove the psoralens will not necessarily remove the material that's bound to lipid, and that's going to be transfused to a recipient.

In addition, although more rarely, there has been evidence that psoralen can react and produce alkylations to proteins. There's a receptor that's been identified on HeLa cells that has been shown to be alkylated with psoralen. And that receptor reacts at a tyrosine residue to produce a photoconjugate. And so in addition to lipids, proteins may be alkylated. There is some concern in protein alkylations that you might make a hapten situation with a potential immunological response to the hapten. And so the biological--and sometimes those responses are not seen very often. It may be a very rare event. And so you really have to have very good surveillance if you're going to implement processes where proteins might be modified to make sure that the safety is such that the intervention

that you're doing is not worse than the problem you're trying to prevent.

We'll switch over to riboflavin now, which is, again, a tricyclic system, a plainer system with a sugar moiety. It's a natural protein, and it can be a photosensitizer under certain circumstances. Riboflavin is known to bind to DNA by intercalation. It can produce guanine oxidation. It produces 8-hydroxy-guanosine. It also has been associated with light, with the formation of single-strand breaks in nucleic acid and the formation, interestingly, of covalent adducts. And riboflavin can be activated by not only UVA light, but light in the blue region as well. A couple references indicated there.

Extracellular enveloped viruses can be inactivated by riboflavin as well as intracellular viruses. There's some non-enveloped viruses that have been shown to be inactivated but, again, because of the size of the molecule, one might not expect some of the picornaviruses or perhaps even parvoviruses to be inactivated.

Riboflavin and light treatment is probably not effective against bacterial spores, again, because of permeability issues, and it's probably not effective against endotoxin as well.

Reactive oxygen species, as unfortunately I know so well, can cause membrane damage to cells. Most of this

has been seen in red cell systems. And so there's a couple observations in the literature where riboflavin and light causes red cell damage, typically measured as hemolysis. And so the issue is what's the potential for damage to the component that we're interested in looking at, in this case platelets, and that we await more information.

Riboflavin also can cause some protein alkylations. Riboflavin can react, photoreact with tryptophane residue in human albumins. In addition, the investigators were able to take these albumins with adducts and use them as a hapten to raise antibodies, and these antibodies were found to react against eye lens proteins. And, interestingly enough, the eye lens proteins of older animals reacted more strongly to these antibodies than younger animals, and the investigators also looked at the reactivity of people who had lenses from cataracts, and they found the strongest staining of the antibodies to lens proteins from cataracts. And so there may be unintended or unknown immunological consequences of transfusing cells or proteins that have alkylations of small molecules.

The molecule S-303 is being considered for inactivation processes in red cells. It's structurally very similar to a well-known and well-characterized molecule quinacrine mustard. Both are tricyclic compounds made of acridine moiety, so three-ring fused structure.

Connected to the structure is a alkyl chain, which is a linker region in this compound which they call FRALE, which stands for frangible anchor linker effector. So this is the anchor here. It brings it to the nucleic acid for intercalation. This is the linker here which connects the anchor with an effector, and in this case, the effector is a nitrogen mustard.

The molecule quinacrine mustard is well characterized; it's genotoxic; it's a clastogen. And S-303 has a structure that has many similarities, with the exception of this ester bond in the middle of the molecule, and we'll talk about that in the next slide.

The mechanism of inactivation by the FRALE compounds is that the anchor or the acridine moiety of FRALE compounds intercalates between the bases of double-stranded regions of DNA and RNA. And the nitrogen mustard moiety or the effector of the FRALE makes adducts with nucleic acid bases. Diotics can form, and they form a cross-link between nucleic acid strands that prevent subsequent nucleic acid replication of the pathogens, and that the ester moiety, which I showed in the previous slide, in the anchor--in the frangible linker region, it hydrolyzes and forms a negatively charged acridine compound that should not further interact with nucleic acid. And,

in addition, the negatively charged acridine compound can be removed by a resin as well.

S-303 can inactivate enveloped extracellular viruses and intracellular viruses. It can inactivate some non-enveloped viruses, but, again, it's unlikely that it will be able to deal with picornaviruses or perhaps parvoviruses. It can inactivate a wide range of bacteria, but, again, it's probably not effective against spores and probably not effective against endotoxin.

Acridine nitrogen mustards can alkylate proteins. There's a large body of literature that shows that nitrogen--that quinacrine mustard can form an alkylation with the acetylcholine receptor and that it affects the activity of the acetylcholine receptor. In addition, it's possible to make antibodies against conjugates of quinacrine mustard and the acetylcholine receptor or against a conjugate with serum albumin.

This is data that I took from a patent from one of the companies, and the main thing that I want to show is that a FRALE compound, in this case it's called PIC-1, can form--can react with proteins on the red cell surface, and that those proteins can be visualized by doing flow cytometry using antibodies that are fluorescent against acridine. And so here's a control where there's hardly any fluorescence, and you can see the increase in fluorescence.

And what the investigators found is that if they include the compound glutathione with the reaction, it could prevent the reaction of the protein with the red cell surface. And so in the presence, for example, of two millimolar glutathione, there's a great reduction in the fluorescence of detection of this reaction to the red cell surface.

So it's possible to perhaps distinguish between nucleic acid and alkylation of surface membrane proteins by the addition of glutathione. But what if you do add glutathione? What does that mean?

Well, if you're adding glutathione, if exogenally added glutathione reacts with FRALE compounds and acts as an extracellular quencher, and if FRALE compounds can permeate cells and inactivate intracellular virus, can FRALE compounds permeate red cells and deplete their intracellular glutathione pool? And if indeed that's true, would those red cells be susceptible to subsequent oxidative damage in a patient who might be given an oxidative drug?

This is a cartoon structure of the compound that some investigators called Inactine. It comprises a covalent modifying group connected to a cationic tail. The cationic tail is supposed to confer DNA binding to nucleic acid. It supposedly stabilizes the molecule, and this

whole molecule is very small in comparison to some of the other ones that I've shown you.

If you go to the patent literature, you can actually find some examples of these Inactines. Here's one, PEN102. This is not the one that's being used for red cell decontamination efforts. They're using PEN110. But basically there's a zero DNA ring that is--and there's a tail with two sites of positively charged species separated by a couple carbon atoms.

Ethylenimine is well known from toxicological literature. It's genotoxic, and it's considered a clastogenic agent.

Inactine forms adducts with guanosine at the N7 position, and this causes a stop signal for replication of polymerases for the pathogens. And so that's the mechanism of inactivation.

In addition, there are repair enzymes that can recognize this alkylation, can cause a loss of a base, causing an abasic site which can result in strand breakage.

This is a DNA sequencing reaction where this is a control situation of undamaged template. And here's a situation where the template has been treated with Inactine, and you can see that in the primer extension that there are stops that correspond primarily with C residues, and those C residues, of course, in the primer correspond

to the template at the G residue showing the stoppage of the polymerase when it tries to run past the G.

The methodology for Inactine or PEN110 is to start with a conventional red cell concentrate and add Inactine, followed by an incubation to allow the reaction to occur to inactivate pathogens and followed by removal, and in this case removal is accomplished by extensive washing.

Inactine can inactivate enveloped viruses and intracellular viruses. This compound, because of its small molecular weight, inactivates a wider range of non-enveloped viruses. For example, they've demonstrated inactivation of an animal parvovirus, which is difficult to inactivate. I believe--I haven't seen data on inactivation of agents from the picornavirus family. I haven't seen data showing inactivation of HAV. There's some evidence of inactivation of some parasites and bacteria, and I'm not sure about the activity against spores. And, clearly, if there's going to be extensive washing, that may help with respect to endotoxin levels where endotoxin might be in the supernatant but there may also be endotoxin that's stuck on cells that's transfused.

Ethylenimine can also alkylate proteins. There's evidence in the literature that ethylenimine can form covalent bonds in lysozyme. There's also an interesting

study where some investigators were looking at inactivation of a minute virus of mice, which is a parvovirus, and they found that ethylenimine had slower kinetics of inactivation for the minute virus of mice than another virus, which they were looking at (?) forest fires. And as it turns out, in looking at the mechanism of this slowly inactivated virus by ethylenimine, they noticed that the proteins--the viral proteins, when run on a gel, had altered, slower mobility. In addition, they had different isoelectric focusing points. And, in addition, when they did studies with the ethylenimine, the virus was not able to enter cells, all of which seem to suggest that proteins rather than nucleic acid might be affected, at least in the case of this one virus that was less sensitive and took more treatment to inactivate.

In addition, there's evidence of ethylenimine reacting with cysteinyl residues in reduced proteins. It's used actually as an assay for analysis. And so the question is if cysteine and reduced proteins can react with ethylenimine, is it possible that some of the other agents that are used for pathogen reduction might react at glutathione, which has cysteines?

So, in conclusion, it's kind of look all roads lead to Rome. All methods target nucleic acids. Methods can reduce the infectious titer of extracellular and

intracellular enveloped viruses, and with the exception of one compound, the activities against non-enveloped viruses are less well characterized. Methods can reduce the count of a number of bacteria and some parasites. Implementation and surveillance may be required to assess low-frequency risks. Low-frequency risk assessment is essential for establishing that fatalities from the pathogen reduction process are less than the current fatalities from infectious disease transmission, and non-nucleic acid, what I call side reactions, may be important to understanding some recipient reactions as well as to explain loss of cellular function, recovery, or survival.

Thank you very much.

[Applause.]

DR. VOSTAL: Thank you, Dr. Wagner.

Any brief questions?

[No response.]

DR. VOSTAL: Okay. Thank you.

I have a brief message from the management. NIH has a strict policy of not allowing food or drink in this auditorium, and I hope since we're just guests over here, you'll be able to comply with that request.

We will now move into an area that is of particular interest to the FDA, and that is, how do we evaluate the efficacy of these pathogen reduction methods?

And we're going to have a couple talks to survey the current methods of efficacy evaluations that we have at CBER and also what is done in Europe.

The first one will be with Dr. Mahmood Farshid, who is a viral inactivation specialist in the Division of Hematology at CBER.

DR. FARSHID: Thank you. My talk basically will focus on how we evaluate the viral validation studies because the previous talk more or less covered most of the methodologies that I was going to talk about.

Biologics, or some prefer to call them biopharmaceuticals, which require viral validation studies basically fall loosely in three different categories. These are cell line-derived products which include monoclonal antibodies and recombinant products produced in cell culture. These products basically are produced from well-established and extensively tested starting material, and they have an excellent viral safety record.

The animal-derived products, these include, for example, anti-lymphocyte produced in rabbits or anti-venom, such as anti-snake or anti-scorpion, and also include some of the products produced in transgenic animals such as transgenic sheep and transgenic goats. The starting materials for these products are variable. There are some strategies for herd selection and also some limited testing

is being done. And viral validation studies are indicated as a requirement for these products.

The third categories are blood and blood product and other human-derived product, but the talk was to concentrate here on blood and blood products. These are, as we know, also the starting material are very variable and constantly changing, and they have been implicated in the past in transmission of the viruses and the potential residual virus infections still exist. And there are, as we heard this morning, there are various complementary approaches for reducing the viral risk in these products. These include donor selection and donor screening. These are basically--they were covered by Dr. Busch, and I don't think I need to go in any further detail.

And pharmacovigilance, and finally, in the case of plasma-derived products, which is basically the topic of this talk, is inclusion of viral validation of the manufacturing process is also a requirement. And that is that validating the manufacturing process for removal or inactivation of the viruses.

Therefore, the aim of viral validation studies to provide evidence that the production process will effectively inactivate or remove viruses which could potentially be transmitted by a product. Basically here we talk about the human pathogen and mainly HIV, HBV, and HCV.

In addition to that, the validation to provide indirect evidence that the production process has the capacity to inactivate or remove novel or undetermined viruses. These are essentially viruses that we heard this morning from Dr. Busch which they are not screened for and also some of the emergent agents could be included in this group.

These are basically not complete--incomplete list of the viral clearance methods. Essentially, viral clearance refers to viral inactivation and viral removal, and inactivation could be a chemical inactivation, which most common one, solvent detergent, or physical, such as heat inactivation, and there are some combined methods as we saw by--Dr. Wagner covered that, such as photochemical inactivation which essentially using dye and chemical and exposure to light. And in the case of removal, it could be precipitation of chromatography or membrane filtration.

Basically, in case of plasma-derived product or manufactured product, there are--the steps which are taken for doing viral validation include scaling-down process because the viral validation is not being performed in actual manufacturing setting, and that is contrary to GMP, and also is not practical to do such study in large scale. Therefore, a model, laboratory model of actual

manufacturing process for the steps which they are intended to be validated will be scaled down.

Then the step which is to be validated, the spike with appropriate virus, with high titer, and this virus could be a relevant virus or it could be a model virus.

Then in subsequent steps, determining the virus reduction factor for that step or for different steps.

And, finally, summing up, the reduction factors to give a total log reduction, provided that the steps being validated, they work in orthogonal manner, they basically inactivate or remove by independent mechanism.

In looking at this scheme, there are a number of validation steps or components in the whole validation studies which one need to be evaluated. These include test viruses, the choice of viruses that they have been used in the studies, and also the design of the validation studies, which that include the ability to scale down the process, basically to establish the relevance of the laboratory model to actual manufacturing process, and also providing the kinetics of inactivation, the inactivation is time-dependent, and this needs to be shown by providing the kinetics data.

And robustness of the process as a whole also needs to be demonstrated by introducing deliberate change

in the process and showing that the process of reproducible and effective after introduction of these changes.

And, finally, the assays which are used to quantify the level of viruses need to be validated.

The final would be determining the log reduction for each particular step and also total log reduction.

I will through some of these components very briefly basically to demonstrate what we look for when we evaluate these studies.

In case of virus selection, the viruses that can potentially be transmitted by a product, which we refer to as the relevant viruses, and if relevant viruses cannot be used because there is no in vitro culture for it, for example, like HBV or HCV, then model viruses, which these basically resemble the relevant viruses. And, in addition to that, viruses with wide range of chemical properties also will be included to evaluate the robustness of the process, and basically these will cover, as I indicated, the viruses which they are nondescript or they are yet undetermined.

The selection of viruses basically is dependent on the starting material, if it's cell-derived or human-derived or animal-derived, and there are also some practical consideration, and that is availability of the suitable culture system and availability of high-titer

stocks, and availability of reliable methods for quantification.

This shows the panel of the viruses which can be used to validate the human drug product, in this case plasma-derived product. HIV is being used as a relevant model for HIV-1 and 2 and also HTLV. Hepatitis B, there is no culture for hepatitis B. There are some animal models like chimp could be used, but these are a problem with availability and also the cost, and it's not a requirement, especially if you are revalidating a previously established inactivation of removal method.

There are some animal hepatitis B viruses which could be included in the study, like woodchuck hepatitis B or duck hepatitis B virus.

For hepatitis C, there are a number of specific model viruses, and among them, BVDV, which taxonomically is very similar to hepatitis C in terms of its size and physical chemistry, and so particularly if removal is to be validated, BVDV is a good specific model because of its size.

And there are other model viruses for hepatitis C, namely, CMV is one of the more commonly used, is slightly larger than HCV, but more resistant to inactivation and probably will be better model if one wants to validate the inactivation process. And we encourage the

manufacturer to include both BVDV and CMV because most of these validation studies include both removal and inactivation.

Hepatitis C, there is a culture (?) hepatitis A virus which can be used as a relevant virus, and also because of the potential transmission of some of the Herpes viruses, one of them needs to be included in a panel and PRV is more commonly used. And this also will be useful as having enveloped DNA virus to cover for HBV as well.

B19, as we heard this morning from Dr. Busch, it can be very high titer, and it is basically increasingly becoming a concern. And inclusion of the specific model virus to remove or inactivate this virus is essential, and PPV is included in this panel. And also B19 is not only a relevant virus, but also will be a good test to show the overall capacity of the process in removing and inactivating viruses because this is a very highly resistant virus.

I won't go through this for the interest of time, but basically just to underscore the point that selection of viruses should be dependent on the starting material, and here we're talking about cell line-derived product and the panel which is used is different. And if one uses the animal-derived product, the choice of viruses should be relevant to the starting material.

And virus selection basically should include DNA and RNA genome, viruses with DNA and RNA genome both single- and double-stranded, lipid enveloped and non-enveloped viruses, large, intermediate, and small size, and in terms of resistance from highly resistant to easily inactivated viruses. And that will cover basically the whole range.

The other validation component is the scale-down model. This is very important if one is to establish the relevancy of the data to the actual manufacturing process.

And this is essentially same thing as I said in previous slide, and all the critical parameters, both functional and product specification need to be maintained and shown that the scale-down process actually mimic an equivalent to the actual manufacturing process.

There are some validation components that we look in terms of what are chemicals. This is more or less a last-minute slide I included there since we talked about photochemical, and that is concentration of the chemical changes in donor plasma need to be validated. And the effect of impurities in the donor units, for example, presence of lipids and hemoglobin in case of hemolysis, and maybe bilirubin and their effect on overall inactivation should be determined.

And if removal of impurities is needed, the degree of impurity removal prior to the treatment should be also validated. And obviously the total quantity of light as well as its intensity and wavelength need to be determined.

There are a number of variables which may affect the overall efficacy of the treatment because of the light absorbencies, including plastic bag transparency and sample depth. And also mixing efficiency needs to be validated.

And, finally, residual level of chemical and its breakdown needs to be determined and the assays that are used to determine this breakdown of the initial chemical should be validated.

Regardless of what inactivation or removal steps are being used, there are certain criteria which are indicative of effective clearance steps, and that includes that significant viral clearance should be provided by the method and should be reproducible and controlled at the process scale and modelable at (?) to the scale. This particularly is relevant to the plasma derivatives because the validation is being done at the small scale. And it should have minimal impact on the product, and we heard this from Dr. Wagner, and basically it should inactivate the virus and not kill the product, more or less. And it

should not generate new antigen or leave toxic residue. It should not be carcinogenic or mutagenic.

There are other concentrations which basically is mostly relevant to the plasma-derived product, and manufacturing process for blood-derived product must contain two effective steps for removal and inactivation. And effective is defined also--this is more or less open question, but all things considered, all the validation in place, effective is defined as a step which can produce more than four-log clearance of the virus.

Because of increasing concern over non-enveloped viruses, at least one step should be effective against non-enveloped viruses. And at least one stage in the production process must inactivate rather than remove viruses, so total reliance on removal would not be acceptable because of the problem with the robustness of removal process and also difficulty in scaling down and validating such processes. Therefore, one inactivation needs to be included in the process.

There are some limitations in viral validation studies which I'll quickly go through, and that is that (?) strain may behave differently than native strain, and that there may exist in any virus population a fraction that is resistant to inactivation.

And a scale-down may be different from the full scale, and there are inherently some differences between the scale-down and large scale which may affect the overall viral clearance. And the presence of neutralizing antibody in the starting material or in some of the product may cause overestimation of the viral kill or viral removal.

This is basically, again, total virus reduction may be overestimated because of repeated or similar process. The processes that are validated, as I indicated, should function by independent mechanisms. They should be orthogonal. But sometimes there are some overlaps, and that may cause the overestimation in determining the total log reduction. And (?) step to remove virus after repeated use may vary, and this is particularly true in the case of chromatography where if you validate the fresh resin after repeated use, it may not have the same level of removal capacity as it has when it's not. Therefore, revalidation may be needed.

That concludes my talk. Thank you.

[Applause.]

DR. VOSTAL: Thank you, Dr. Farshid.

Any brief questions?

[No response.]

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DR. VOSTAL: Dr. Farshid will be a member of the discussion panel following this session, so you'll have a chance to ask him questions later on.

The next talk will be given by Dr. Hannelore Willkommen, and she is the head of the viral safety section of the Paul-Ehrlich-Institut, and she will tell us about the European approach to evaluating these methods.

x DR. WILLKOMMEN: Thank you very much. Before I start my talk, I want to make two remarks.

First, I want to--I can't speak so much about the experience we have. I want to give some regulatory considerations with regard to blood components which are inactivated.

The second remark is we have no European experience because the components or blood components in general are regulated on a national basis. But this is the issue of the next talk of my colleague, Margarethe Heiden.

I want to focus on some virological aspects which should be considered if we are speaking about blood component inactivation.

He referred already, and I think very in detail and very interesting, about the mechanism of and about the new methods which are used at the moment and which are introduced at the moment for inactivation of blood components. These are photochemical methods--psoralen,

referred about intercept platelet system, Baxter has received recently its certificate. The photodynamic methods using methylene blue, dimethylmethylene blue, thioneine, riboflavin is another component which is used, which is also the photodynamic method, and chemical methods, the very well established S/D treatment or treatment, and the other nucleic acid targeting substances, Inactine and the FRALE substances, you have already about and I don't want to go in details.

For this method of radiation, the application of radiation is necessary. And if you look under radiation, then you can have these three opportunities: first, that the target, the DNA, the protein, the infectious agents, are directly radiated, and inactivation occurs directly via inactivation, where the energy which is brought into the target via irradiation, this is the case for gamma radiation or for UV treatment.

Then if we look here under photochemical methods, then sensitizers are added to the components, and they are treated and irradiated and bind then or form compounds or give a linkage to the target itself so that equivalent linkage to this that the target is inactivated.

And in the case of the photodynamic processes, the reaction occurs really--the sensitizer bound to the target, maybe DNA and RNA, but the inactivation or the

formation of covalent binding occurs with a radical, free radicals or reactive oxygen species, and this reaction can be quenched by Type I or Type II quenchers. And so we have different mechanisms for these reactions, and, therefore, also different possibilities to influence and also different things which have to be controlled, I think.

These substances which are used for these processes, the sensitizers should have specific properties. They should be soluble in water and stable in the solution. They should be non-toxic, non-mutagenic, non-carcinogenic. This is really complicated because most of the components are reactable, have an affinity, special affinity to the genome.

The inactivation process should have a high effectiveness for the range of pathogens. The process should maintain the biological activity of cells on all labile blood products. And I think it is also an important aspect, the photochemical inactivation should be effective at low energy level that formation of radicals or reactive oxygen species can be maintained at a low level. This is, I think, also an important point because the formation of radicals can give side effects which we don't want to have. They can induce alkylation of proteins which are not the target of this treatment. Type I and Type II quenchers can be used to preserve the functional activity of cells and

plasma proteins. So these are (?) , and I want to go to some examples, and I want to go then into some aspects which would be considered.

So first I want to make some remarks on psoralen. As we have heard already from this, this was a publication of Lin, et al., in 1997, and they reported some effectiveness for inactivation of viruses and of bacteria, and they report here, it is reported that the functionality of the cells can be maintained.

Recently I received from Steve Binion of Baxter these intercept platelet product characteristics, and they have a list of viruses which can be inactivated. I mentioned it here on this slide. But I wanted to take this as an example to discuss some considerations with regard to virus inactivation for these products.

First, which viruses should be tested? I think the selection of viruses is not so bad here. HIV is tested, and, I mean, HIV is mandatory in each case. It is not a target virus, of course, but, nevertheless, it has such a meaning, such a political importance, too, that in each case I think it is and should be tested.

CMV is a Herpes virus. Herpes viruses are important for their components, I mean, and Herpes viruses should be considered. But I'm not sure that it is efficient to look on CMV only. Maybe it is also necessary

to look on other Herpes virus. We have heard that the Herpes virus Type 8 is important also, and maybe the behavior of different virus strains is different. And I mean, a very important question from the regulatory point of view, at least, is what is the mechanism of inactivation? What is the most important step which is the key step for the inactivation of viruses? Is it the penetration of--or are these properties of the virus envelope or the virus core that the sensitizers can come to the target molecule of the genome? Is this penetration of the virus core, the virus membrane, this is a very important step? But this would be clarified, and I think it can be clarified only if several viruses and several type of viruses have been investigated. And so I would suggest in this case to extend the Herpes viruses or the number of the Herpes viruses which are investigated. And we have seen in other methods, of course, that viruses of one family can react very differently.

HBV can be tested. That's good. I think that HBV is one of the target viruses, because we have heard this morning from Michael Busch that the highest risk is associated--if we are speaking about the risk of HIV, HBV, HCV, the most important blood-borne viruses, then HBV has the highest risk if you can speak about that.

And the next is that we don't know normally so much about HBV and the properties of HBV in inactivation methods because it is very often not investigated in, let me say, classical inactivation methods which are used for the inactivation of blood products, of blood derivatives.

So it is interesting that the duck hepatitis B virus as here is effective--or the method is effective for inactivation of the duck hepatitis B virus as well as for HBV. But, on the other side, it would be very interesting, and I think that it is not sufficient to demonstrate here the log reduction values. It would be very informative to see the kinetic of inactivation because it is clear that you can see differences in the effectiveness of the method against specific viruses you can see it from the kinetic, especially from the kinetic. And so maybe that this should be investigated, too.

So HCV can be tested here because it is an inactivation method which reacts with the genome. This is also good that we have this information here, and maybe it can be tested by PCR. And we can see here the relation to BVDV which is used normally or very often as a model virus for HCV.

So I'm not sure that we can conclude from this that this method is in general effective against all enveloped viruses. I know that the company doesn't claim

this, but I want to mention only--or I want to say only that we have to be very careful with such consideration or such conclusions, and sometimes I hear that it would be the wish to have a method in place which is effective against unknown viruses. And I think we should be careful with this conclusion. But, nevertheless, the enveloped viruses are highly--according to these numbers, it is to assume, it is to expect that this method would have high effectiveness for inactivation of enveloped viruses.

It is different with regard to non-enveloped viruses. Bluetongue virus is a virus which can contaminate bovine serum. It can come into biological products. It's not the case for blood components, I know, but in general it is an important virus. It is a reovirus and completely different than from the structure from these other viruses. But you see that in the case of the small non-enveloped viruses, this method is not effective, and I think--or I would assume that this may be a little bit a fact of the virus core which may be too--the density may be too high that the sensitizer can penetrate through this. But it would be worth, I think, to know more about all this.

So some remarks for Inactine. I think this there are also clinical trials performed--that have been--they have performed already clinical trials with this substance. I don't want to go through this. You have heard it

already. And I saw a paper describing here this process, and they have changed a little bit. And the first publications reported they remove Inactine after the treatment. In this case, they quench it with thiosulfate. And they report here the same two-phase kinetic with BVDV and PPV. In this case, they provide the inactivation kinetic, and they postulate a two-phase reaction.

It would be interesting to know is the reason for the two-phase--what is the reason for the two-phase reaction? If I assume that the compound react directly with the genome, maybe I would expect that it is more in one-phase reaction. That means that you have real linearity in the time of reaction with--the inactivation would be a linear function from the time of treatment. This is here not the case, and I'm a little bit surprised that these both should be--really should have the same kinetic. These are more or less parallel curves which come up. The titer was here a little bit lower--no, it was lower here, and, nevertheless, it is not so clarified for me.

Inactivation in plasma. Let me make some remarks with S/D treatment. I don't want to go in detail, and you are very familiar with this. It is not really an inactivation method for blood components. It's used for blood components because S/D plasma is in plasma for

transfusion. But because it has a pooling stage, it is some processing by chromatography, it is handled from the regulatory standpoint as a medicinal product.

Nevertheless, we see also in this case, in the case of this well-established inactivation method, some limitations which would be considered, and I see the parallelism to the new methods which are coming into our products. So we know that this method is very effective for HIV, HCV. HBV, I made it blue because we don't know exactly what the inactivation kinetic of this is. We know that it's effective, no question. Otherwise, we would have seen it during the use of this product, and there are also some early investigations with chimpanzees.

So no effectiveness against non-enveloped viruses, that is clear. We have this risk of contamination with non-enveloped viruses, and we see that this treatment also has some negative effect. The activities of inhibitor C and protein S--sorry, it's a mistake here--and alpha 2-antiplasmin are diminished under 50 percent. They are sometimes very, very much diminished. And we see also some limitations with regard to virus inactivation. It is a very nice publication, I think, from Peter Robert, and he published it in Biologicals, and he looked at the inactivation of Factor VIII and Factor IX and demonstrated that vaccinia virus is not inactivated completely (?) is

used as in detergent. And so we see also limitations in well-established inactivation methods, and I think that the conclusion for me is--or the message which I want to give to you is that we have to look very carefully on limitations, side effects of such new inactivation methods when they are introduced into the production of cellular components of medicinal products which are used for treatment of humans.

So very short remarks to methylene blue. Methylene blue is used in several countries routinely already, and we have really some experience with this, but, nevertheless, this product is at the moment not on the market in Germany. This has other reasons.

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We know that in general enveloped viruses are (?)-tive against treatment. Non-enveloped viruses are mostly not effective, and we see also where this method in reduction in the activities of fibrinogen and other coagulation factors, but, in general, this method works quite well. The specific reactivity or the specific effectiveness of this method against different viruses was really rather specific, and it has to be looked on very careful on this.

The process has been improved with the change, cells removed and this new process by membrane filters. A sodium lamp is used for illumination, and methylene blue is

removed. It is obviously an improvement of the situation because, in principle, these compounds can be genotoxic because it reacts with the genome. The kinetic of inactivation, of course, has to be investigated.

So if you look on the new inactivation methods which are introduced and which we are discussing here, then we have to ask some questions from the regulatory point of view, at least. Is the mechanism of pathogen inactivation clarified? This is a very important question for me.

Next, can the effectiveness against several pathogens be predicted on this basis? This is more or less--this question is more or less related to the question which pathogens have to be used for the evaluation of such methods.

Is it a robust process which needs no control during routine use? I think that it is also an important question. If you look on cellular components, we have individual products which may be different. Maybe the lipid content and the plasma may vary. If the plasma is replaced to some extent to 40 percent I think by platelet additive solution, but what is the influence of the composition of the plasma to this? It is only one of--one example which could possibly be important.

What are the variables? This should be really considered. Is the variability of the process of non-

importance? This is also a question. Are virus safety a target? Is it possible to say don't matter, we don't want to know, it's all exactly? It is efficient if the procedure is effective maybe against bacteria or, in general, we have to give an answer on the question what are the target pathogens for the inactivation of cellular components and plasma for transfusion.

If we summarize and see all of this in a more general view, then we can summarize that methods for inactivation of pathogens have been developed. They are promising with regard to their effectiveness for inactivation of viruses and bacteria. And some of them have been tested already in clinical trials.

However, we have to give an answer on the following questions, too: What is the clinical relevance of reduction of cell or protein quality which may occur during treatment? I think it is important. And I have heard here in the talk of Steve Wagner that alkylation is also an important point. We have to give an answer what the clinical importance of such changes are.

What is the long-term clinical importance of agents/additives/photoproducts which remain in the product?

What pathogens are most important? I spoke already about this. Which pathogens should be used to demonstrate effectiveness of treatment? What data are

required to demonstrated effectiveness? Is it necessary to demonstrate effectiveness of such inactivation methods on the same basis as we require this for plasma derivatives? Is it necessary to make extensive studies to demonstrate also factors which can influence us to demonstrate the reliability of the processes? And I think it is necessary to find here a compromise and to create really good data which can demonstrate the reliability of the procedure, but to keep it also in the time frame which can be managed.

How to control routinely the reproducibility and reliability of the inactivation methods? Which parameters are important? Level of lipemic plasma, red cell content, plasma protein contents, others? Is it necessary to control the process under routine conditions?

I think all these questions have been raised, and I think that we have to find an answer on it if we are in the agencies, at least, and have to have an opinion about the reliability, the efficacy, the safety of such products.

Thank you for your attention.

[Applause.]

DR. VOSTAL: Thank you, Dr. Willkommen.

Are there any brief questions?

[No response.]

DR. VOSTAL: Okay. We will move on to the last talk in this session, and this will be given by Dr.

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Margarethe Heiden, who is a colleague of Dr. Willkommen, and she's the head of the blood component of the Paul-Ehrlich-Institut. She will talk about the European regulation of blood components' quality and safety.

x DR. HEIDEN: Thank you very much for your invitation.

As Hannelore already mentioned, in contrast to plasma derivatives, blood components in Europe are excluded from the European harmonization of medicinal products, and, therefore, the regulation of the whole blood banking system in Europe follows only national peculiarities. That means, of course, all the recommendations, international and European recommendations will be taken into account, for example, for donor selection, for preparation, use, quality assurance.

In Germany, blood components are considered as medicinal products and, therefore, are regulated by our drug law and national guidelines. And the authority responsible for marketing authorization, for pharmacovigilance of the blood components, and also together with regional authorities for blood bank licensing and inspections is the Paul-Ehrlich-Institut or agency. And, of course, assessment of these blood components follows the requirements and follows the European standards for plasma derivative, for instance, with respect to source

and manufacture and with respect to the virus validation studies. A lot of it we heard already this morning.

There's a special--there's a peculiarity that in Europe the components, the substances for pathogen inactivation are regarded as medical devices like the bags, and, therefore, they have to fulfill the requirements of the European Medical Device Directive, and this includes not only the production, processing, and so on. This includes all of the whole risk management, risk assessment based on the pre-clinical and clinical studies made by the company.

Quality, safety, and efficacy of blood components are determined by a lot of features, and this pathogen inactivation will change the processing of the blood components, and this change of the processing of the blood components, of course, will lead to changes in quality and safety as well, and hopefully safety will be changed, will be increased with respect to microbial contamination, viral contamination. But the quality with respect to--that means biological activity of the active component, thrombogenicity, side effects, tolerability and so on may be impaired. And that's why if we want to assess the changes in the quality of the component, the blood component, we have to compare it with the components which are already on the market, our market. And we have the

possibility to compare the biological activity of any new blood component with the basic quality data for the biological activity of the, in Germany, licensed blood components.

With respect to the tolerability of any blood component, we have the possibility to compare adverse reactions reported to our institutions for the blood components, and, of course, this will also be an aspect which later on will be compared with the pharmacovigilance data of the new compound. This certainly does not include virus transmissions, and this certainly does not include errors of transfusion.

Unfortunately, especially with respect--the bacteria--transmissions of bacteria contaminations are strongly underreported. It may be, of course, because the reporting of these events is mandatory only since 1999, and the time frame is longer when we compare it, for example, with the French hemovigilance data which the amount of blood components transfused per year is in the same magnitude, the same order of magnitude, but, for example, for 1996, we have a 100-fold higher number of reportings in France compared to Germany.

If we look for the process of inactivation and also on the capacity of the process, we have to look for the very large difference between viruses and bacteria.

Virus have a constant titer of contamination, but in bacteria, the contamination will increase during storage and may have an impact on the design and also on the capacity of the inactivation procedure.

This is an example why it is so important to look for the storage of the blood component. This was a strain of Klebsiella pneumonia which killed a young father two years ago, and after an artificial contamination from the bacteriology department in our institute, we could see that this trend goes up within 24 hours from 1 CFU to 10^9 CFU. And those experiences stimulated us to formulate some requirements which bacteria strains should have which are used for validation studies. They should cover a broad range of several characteristics: blood-borne, environmental, pathogenic and apathogenic. They should be Gram-positive, Gram-negative, spore formers and so on.

A very, very important point is these strains used for validation studies must be able to grow up in the special blood component or at least to survive in this blood component. And we should take into consideration that reference strains are not automatically suitable for these validation studies.

Another very important point is that all these bacteria must be 100 percent healthy if you really will have valuable results from your inactivation process.

These cells have to be intact cell walls, intact membrane, intact cytoplasm, including all the organelles, and they must be free of undefined products of bacterial lysis.

In this bacteriological department, some of the standards are prepared, most of them isolated from blood components directly. They are very well characterized. They all are harvested in the geometric phase of growth, and stable nowadays up to two years, cryopreserved and are ready to use with a defined number of living cells. And they also offered for all people, for corporation, for bacterial validation studies.

Of course, the assessor has to look for other possibilities to improve bacteria safety, and the assessor has to remember any time the pathogen inactivation method is one of the methods one can use. And, for example, pre-donation sampling nowadays is also recommended. In Germany, it will be introduced within the next one, two years.

If you compare the nowaday state of virus safety, of the components we have on the market, when we want to compare with a new pathogen inactivation method--inactivated compounds, we have to state that after introduction of fresh frozen plasma, after introduction of the (?) in '95, we did not have any longer transmissions of HIV and HBV, and following the introduction of HCV NAT,

we do not observe any longer any transmission of these three viruses by (?) fresh frozen plasma. And in this time period, we had more than 5 million units transfused.

If you look for the cellular components, they have a similar picture. Following introduction of HCV NAT in 1999, we do not have any longer transmissions of HCV reported. But we had yet three cases of HIV transmission and one case of HBV transmission. And I hope this number will decrease starting with next year when the HIV NAT will be ordered by our institute for Germany.

That means, taking together all the points, the final assessment in our institute of any assessor has to consider the needs of the patients, that means supply of the medicines in sufficient amount, with good quality, with reliable efficacy, and also with an acceptable safety, not with the total safety.

And the final assessment, moreover, will balance the winnings in safety by the pathogen inactivation compared to the safety level of blood components already on the market, and will balance these against the side effects of inactivating substances--we heard a lot about it--of the impairment, the possible impairment, or already seen impairment of biological activity. That means last not least, the clinical efficacy, and we'll also take into

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account alternatives for improvement of safety, like testing, pre-donation sampling, and so on.

Thank you very much.

[Applause.]

DR. VOSTAL: Any questions for Dr. Heiden?

[No response.]

x DR. VOSTAL: Now we're going to change the format of our presentations a little bit. We wanted to get the opinion of some experts and also give the audience a chance to ask questions of the speakers. So we were going to have a panel discussion format, and we would invite the speakers of the last session and two additional experts. One is Harvey Alter, who is the head of the Infection Disease Section here at the Transfusion Department, and Dr. Roger Dodd, who is with the American Red Cross. And we would like to have them come up here, and we'll have Mike Busch run the discussion. And we've prepared some questions that have been--we'd like to have help and input from these experts on how to deal with these issues.

[Technical problem, microphones off.]

DR. WILLKOMMEN: -- which have been transmitted already. So I think that--on the other side, I mean that this should be controlled. We should know what we have in our hands, and, therefore, I mean, these viruses should be--number of the viruses which would be investigated.

If we want to have an overview what the method can do with regard to viruses, is it to assume that if a method has a broader effectiveness for inactivation of a wide range of viruses, I think we have to test it. We have to find out the mechanism of inactivation, and maybe then we can make conclusions from that. If not, we can claim some effectiveness only from the viruses which we have tested. And so I think it is a very complex question. It is not so easy to answer that. And the most important point is what is the aim of inactivation. Do we want to be sure that pathogens like parvovirus B19, hepatitis A virus, are really reliably inactivated by these methods? Then we have to test that. And we cannot replace maybe HAV with polio virus. We have to look really on HAV that you can make this conclusion. So I think it is really a very big question.

DR. : If I may add, I think if--there should be a distinction. If you are establishing the effectiveness of a new inactivating or removal method, a broader array of viruses should be included in these studies. These should be different than the risk you're re-evaluating or re-validating what is previously established method. Therefore, I think the bar should be higher for a new inactivation or any new clearance method, and larger number of the viruses, both specific model

viruses and non-specific model viruses should be included in a validation study basically to determine the overall robustness of the process. And it cannot be relied only on the viruses which is already being done for very, very established methods.

And I would think that inclusion of some animal studies to establish the effectiveness of new methods may also be required. And I would like to say that the opinion that expressed here by me may not be the opinion of the NSV (?). It's my own.

DR. ALTER: My opinion was on that my own.

[Laughter.]

DR. ALTER: This is a very, very difficult area, and just to raise one problem we've had in trying to use real viruses, like HBV and HCV, and see how much you can inactivate, traditionally we've used these and used the chimp models. So you're starting out with a model that's extremely difficult, extremely expensive, and very unavailable. And then you're using agents which are only available in certain titers. So if you want to look at how much can you inactivate, you really can't look at it because we don't have stocks of viruses with high enough titers that you see in patients. Everybody's using the same materials from our Hutchison strain and the Willowbrook MS2 strain. And they're only available up to

10^4, 10^5 maximum. So you can only talk about--we can inactivate at least $10^{4.5}$.

Clinically it seems to prevent infection if you kill that much, but you really don't know how much--what the total kill could be. So that's one thing.

The other thing is that it would really be nice if the FDA could somehow--and maybe that's what will come out of this meeting--set up this panel so that everybody is using the same agents, just like you would do for an assay, a test assay, and you'd see if a new assay could meet these criteria. It would be nice to have a set panel of a range of viruses that was actually distributed by the FDA and you came in with a new methodology and you had to kill these at that particular level.

DR. WAGNER: Well, actually, it's very hard to add anything to these comments that have already been made. I do agree with them very strongly.

I would also, though, add some caution to the use of some of the model systems, even though they seem to be appropriate. I think that one issue, for example, is the use of porcine parvovirus instead of B19, which is now properly known as an erythrovirus and is quite different in terms of its genetic makeup. And we've already seen data in other environments that show a clear dissociation, for

example, between the inactivation properties of PPV and of B19.

We've also seen data today that show at least two different systems have very different outputs when used to test against porcine parvovirus.

So I would endorse the concept that inasmuch as possible, recognizing the rarity of chimpanzee models and so on, we should look for what we're trying to eliminate and recognize that not every model is good for every process.

DR. BUSCH: A question in the audience.

DR. MARCUS-SEKURA: Yes, I'm Carol Marcus-Sekura from BASI Consulting. I wanted to raise the issue not just of what types and which viruses are chosen, but how these studies are done. And if you take a virus and you spike it into a solution and then you add the compound that you're testing, that's not the same as looking at an intracellular virus and trying to see whether the compound will inactivate a virus in an infected cell.

So I'm concerned about compartmentalization of the virus, the forms the virus may take in the cell. For example, enveloped viruses may not be enveloped until they bud out of the cell.

So I think there are additional issues here that need to be addressed.

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DR. BUSCH: I think we're seeing at least from many of the companies' experiments that do use infected cells, where appropriate, HIV-infected cells or CMV. But I'm not so sure whether these studies are actually being done where these infected cells or viruses are actually spiked into components, red cells, platelets, and the inactive--I mean, I had not heard before today the, I think, very appropriate comments from FDA about concerns over the effects of lipids or hemolysis, et cetera, on the efficacy of these inactivation steps.

Dr. Horowitz?

DR. HOROWITZ: Bernard Horowitz. Mike, I would attempt to answer your question and reiterate some of the things which have been said before. I think experience from the plasma world indicates to us that, in order to convert something which is unsafe to safe--after all, the objective--and given the load of virus which we see present exemplified by your talk earlier today, we're going to need inactivation systems that inactivate at least 10^6 and probably preferably 10^8 infectious doses.

And that may not hold for all viruses, but that would be my personal target if I was writing down the specification for a new method of virus inactivation.

And I say 10^6 and 10^8 in part because I know the limitations of the models that we use, coupled together

with the titers that are present early on in an infection. And if we have a method which is only 10^4, I would think it would fail to achieve the desired purpose of converting something to non-infectious.

With respect to the different models that we have available to us, which models we should use, I think there are two elements here. There are many models which we all use in the non-cellular world. I'd like to echo the fact that the cellular world really is quite different. It's different in two regards, one of which is you have intracellular virus, and the other is that you have virus adherent to the outside of the cell. And we really don't know what the sensitivity of those are, in part because we don't have good models to study it.

That's not to say that we should reject methods because we don't have good models, but we should at least recognize the limitation of the methodologies we use.

Thirdly, I'd like to echo the hepatitis B. I know from our experience with solvent detergent that hepatitis B is easily the most resistant of the viruses that I've personally used with solvent detergent, and I know this, despite the fact that we kill all of the hepatitis B in the chimp model, but I've used the duck model to study kinetics. And the kinetics really do teach us--if we're killing everything that we throw at it, they

tell us how much margin we have, how much spare killing capacity we have, even if it isn't linear. But you feel confident if you can kill everything in one-tenth of the exposure as compared to, say, 100 percent of the exposure.

And so I highly advocate the use of kinetics, and for hepatitis B in particular, hopefully we have enough of those samples around and enough chimpanzees to do studies if we really want to know what's happening with respect to hepatitis B.

Lastly, as we think about killing in bags, which is really what we're talking about, a totally different model than what we're used to in the plasma world where we have highly controlled tanks, bags have dead spots in them, and mixing efficiency was mentioned previously. But it takes on a new element once you're in a blood bag, and you need to be able to figure out ways of knowing that the virus that's spread throughout the bag is killed, not just in sub-samples from the bag. And that almost demands full bag testing when you do your models rather than taking one or two or three milliliters from the bag and asking the question whether or not it's safe.

So that begins to answer some--or at least my personal opinion on some of the questions which are here.

DR. ALTER: I agree with what you're saying, but just one comment. The kill level might be looked at

differently for agents for which we have nucleic acid tests. In other words, the nucleic acid test is going to bring it down already to 10^3 or less, so then you only have to kill the residual. So I think perhaps for HBV, HIV, HCV, 10^4 would be enough. But for other agents where we don't screen the donors with nucleic acid testing, it might not be--it won't be.

DR. HOROWITZ: Which brings up the whole question of whether we should be talking about the amount of virus in a milliliter or amount of virus in the bag. And when you talk about 10^3, I'm assuming you're talking about milliliter. But the amount of virus in the bag, once you multiply that by a hundred, goes back up to 10^5.

So I'd like to hear the panel's opinion on whether or not the nomenclature we're using is appropriate and whether we should be talking about the total dose or the dose per milliliter.

DR. BUSCH: I think we've transitioned to Question 2, which is, you know, the sort of level of kill that we want to try to document. And this question of concentration versus absolute number of particles is a complicated one in terms of, you know, what is the infectious dose, in essence. And I tried to kind of get at that through the human data. We typically quantify in terms of per mL, and then we infuse a larger volume,

obviously, 20, 30 mLs of plasma typically. Whether infection is dependent on a minimum concentration of particles in the infused material or some minimum absolute number is--there's not a lot of data to really address that, in my opinion, in looking at the literature.

I think certainly some hepatology people would suggest that, you know, if there is some fairly small number, perhaps ten or fewer infectious particles in the infused volume, then that's enough to--as it enters the blood and enters the liver, to potentially initiate an infection. And certainly we're seeing from the NAT breakthrough cases that very low level viremia, you know, at or below the limited detection of even individual NAT, may transmit. So I think it does raise that question.

The way the data is being presented from the pathogen inactivation companies, I agree, is often confusing. Instead of talking about kill at a log per mL, it's somehow being translated into a log reduction where they multiply the observed kill by the theoretical volume in a blood component and sort of amplify up their projected kill by a couple orders of magnitude, which I don't think is appropriate, but maybe others could comment on this nomenclature issue.

DR. WILLKOMMEN: I would like to make a comment to this. I think that it is really complicated to say we

have so many viruses in the bag and we need the capacity of so many logs. I think this is--we made estimates. We don't--can't calculate it exactly. We don't know exactly what the variability in such a process is. And so normally we say if we want to have a safe product, we need a method which is really effective, as maybe is it inactivation, where it's inactive or it's a virus that has a fast inactivation kinetic, so that we can be sure that during the whole process the virus which is in is really inactivated.

In the case that you have in inactivation and which is slowly--which occurs slowly and you have virus at the end of it, then I know that--or from my experience with validation studies, this slope of inactivation can vary a little bit, and so you can have more or less.

On the other side, we need blood products, of course, and we have to make a decision finally. And we have to say this is sufficient or this is not sufficient.

I think in the case of blood derivatives we have the opportunity to say to the manufacturer, please add an effective virus inactivation step. In the case of blood components, we don't have that. We can say, good, we see your inactivation, please describe--make so good experiments that you can describe the effectiveness of your process very well, that you really can demonstrate what the

effectiveness is, and then we have to make the decision. The risk/benefit, is it allows a conclusion for the product or against the product? I think this is a completely different situation in the case of blood components than in the case of plasma derivatives.

On the other side, we have to consider also that we bring into--we are discussing now about really new substances. We never use such substances for inactivation of blood derivatives. These are substances which are reactive or react preferentially with the genome, and these are substances which have also some other properties, may (?)-culate products and so on, but we are a little bit familiar already with this. But we should be very careful with--and we should investigate as much as possible that we know what has happened during such inactivation.

Thank you.

DR. BUSCH: Dr. Klein?

DR. KLEIN: Harvey Klein, Bethesda. A comment to the last point is I would just like to know, when the slides go up there and they say log reduction, what you are talking about. So maybe we could define that when each slide goes up so we'll know that we're comparing apples and apples.

I'd like to get back to a point that Dr. Horowitz made, and that is, it's absolutely true that we're not

going to eliminate any of the current tests because of the high titers of virus. But many of us are thinking about the next agent, and we're not going to have a screening test for the next agent, whatever that might be, until it's already here. So we would like to know that at least with our current agents and our model viruses that the kill is pretty high.

And what I would like to have seen, Mike, on your last slide was also some data on the clinical effects on the donor so that we would know that you might pick a donor who was asymptomatic at a time when the acute viral titer was very high, because certainly those who are ill at the time that the viral titer is very high are probably of little relevance to us. Certainly in the chronic case of a viral infection, if you have a high-titer virus and a chronic infection where someone isn't ill, then that's a real problem for us.

DR. BUSCH: Most of the numbers I showed I think are coming from asymptomatic--even obviously acute HCV is virtually uniformly asymptomatic, and even the HIV data that we're deriving from these plasma donor panels, these are people who gave those high-titer window phase units and got through full screening. So they were asymptomatic, afebrile, and if you actually look at data from, you know, high-risk clinics where people present with the acute

syndrome, they'll have orders of magnitude higher viral load and peak viremia than we've documented with these plasma panels. So it's really remarkable the levels of virus that people walk around with and give blood.

Dr. Brecher?

DR. BRECHER: Mark Brecher, UNC. Dr. Klein already said much of what I wanted to say, but it seems to me when it comes to labeling these processes, we're going to--you have to separate out testing for--well, inactivation of viruses for which testing has already been done versus the unknown viruses that are coming down the pike for which many of these companies, you know, wish to make a claim that we will catch all these next viruses or unknown viruses. And to do that, you really have to look--according to Mike's data, it seems like you have to get in some cases a ten-log reduction if you're going to try to make a claim for efficacy.

DR. BUSCH: Roger?

DR. DODD: I think that this conversation is extremely important because I think that many people feel the real promise of viral reduction, viral inactivation is for the next virus. And yet I think we've already seen data with our existing viruses that, in the absence of testing, a process may well be pushing the limits of its capability to eliminate all potentially infectious virus.

I think that we've also seen data that show that some other viruses that we know about but don't test for can exist at highly--extremely high titers, B19, for example. And I think it's reasonable to suppose that some viruses will be inactivated, but there will be some that cannot. I would also go back to the issue of models behaving differently in different systems and not necessarily being predictable.

So I think that one has to use extremely caution in raising hopes about the inevitable ability of these processes to eliminate the next virus. In some cases it will; in some cases it won't. And that's the only thing that I think is predictable.

DR. BUSCH: Dr. Blajchman?

DR. BLAJCHMAN: Mo Blajchman, Hamilton. Harvey Klein asked an important question relating to the need to define what a log kill is or six-log kill. I think this is very important. But having said that it's very important, I think we need to define--there is a very important paper in a recent issue of Lancet relating to HIV sitting on the red cell. Jay Levy wrote an accompanying editorial, and what seems to be--some of you may not have seen this paper because it only recently appeared, but basically the message of that paper is that HIV--even in individuals who are on treatment so that the plasma levels are undetectable

seem to have high levels of HIV-immune complex associated with the red cell surface as a form of immune complex.

So not only do we need to worry about the titer and the log kill, but we also need to be very concerned for some of these viruses, and the paper only describes HIV, HIV on the red cell surface. And there's no correlation shown in 80 patients or thereabouts between the plasma level and the level of infective HIV virus on the surface of red cells. And I wonder how the panel would deal with that, and I think while it's early days about the significance on--one of the things that I was concerned about when I read the paper is maybe some of the window period and some of the inconsistency, Mike, that you showed during your talk about the plasma level and infection may be related to HIV on the surface of the red cells.

But I still think we need to start with a definition of log, as Harvey Klein said.

DR. BUSCH: I'd just comment that, in addition to these agents binding to red cells, platelets are actually excellent scavengers for a number of infectious agents, and high levels of platelet-associated virus have been documented in many of these systems.

Dr. Corash?

T4A DR. CORASH: Yes, Larry Corash from San Francisco. You know, having wrestled with a lot of these

assays for more than ten years now, I think there are some important distinctions to be made.

First of all, a lot of the data that's been presented this morning on titer of virus is genome equivalent, and I think Mike did point that out in his slides. And that is different than infectivity.

I think everybody would agree that we would like to have infectivity assays that test the entire unit of a component that gets transfused, and in some cases, we've been able to do that, primarily in models like the chimp infectivity model, but we're putting a human pathogen into another animal system. We use animal models for the duck, which I think is an excellent model for DHBV, but it is not exactly the same virus as HBV. And we use murine CMV in immunodeficient mice, but it's not exactly the same as human CMV.

And these are the problems, I think, that people are really struggling with, and you can even take a laboratory-adapted strain of HIV, which we grow up to be able to have extraordinarily high titers, and there are people sitting on that panel who've done exactly that work. But that's not the same as a wild-type isolate from a first-time donor who's newly infected where you have a virus which will not even plaque in the infectivity assays that we have to use to show large dynamic ranges.

So, you know, the challenges here are very considerable, and I think there are some limitations in what can, in fact, be demonstrated. And I don't think you can claim something unless you can demonstrate it. And I think that's what everybody in this field, you know, has been struggling with, and I would encourage the panel and obviously the agencies to--you have to think about, you know, the nature of these problems and what, in fact, can be validated.

DR. BUSCH: Steve?

DR. WAGNER: With all these comments, I think there's probably pretty much consensus that it's impossible to promise about the future virus because of all the complexities involved. Even the viruses that we know how to deal with, as Larry says, there are strain differences, there's differences in infection models. And there are infectivity models that can be used, and at best, the most we're going to learn is a certain level of certainty but not complete certainty whether or not that virus is going to be cleared or not. And you're going to have to implement the process and then do surveillance to really know how effective some of these maneuvers are going to be.

DR. BUSCH: And one of the things that I suggest in terms of these common non-pathogenic agents, in terms of the HGV, SENV, that are being transfused, and the clinical

trials that have been and are being done with these inactivation methods, you know, there should be specimens from the donations that were treated and the recipients pre and post. There's other viruses, enteroviruses that Peter Simmons has shown that are quite common in donors and can be transmitted.

I'd actually ask Harvey, who's worked a lot on these, whether he thinks evaluating the efficacy of these compounds using those as sort of models.

DR. ALTER: Well, it would be nice, but the problem with all these agents--HGV, TTV, SEN--is that we have no cultured system. We have no titered inocula. And we have no disease model so you can transmit it to the chimp, but all you show is that you can infect. So you could do a plus-minus. You could say it infects or it doesn't infect. But it's not the ideal system to work with because you don't have a culture, a particular--

DR. BUSCH: Right. What I was suggesting is, for example, in the trials that are being done of the Helinx methodology, the people that have been enrolled have been exposed to many donors who undoubtedly a subset of those donors were carriers of these viruses, and we could just use the human data to evaluate effectiveness.

DR. ALTER: We, in fact, will be doing that in this new prospective study we're doing. And the rates will

be high enough that we'll see whether an infectious donor--and the REDS study could do the same--whether an infectious donor will actually transmit to the recipients. Yes, so in that sense, it could be done.

In fact, can I keep--it seems to me that this whole field of viral inactivation can be viewed as good or bad. There are people who think it's good because it's close to the magic bullet. It gets beyond testing. It may kill the next agent. It will kill bacteria, kill viruses. We don't know how much it will do, but it has a lot of good things.

Others see it as bad because it is expensive, time-consuming, and it may not kill everything to the nth degree.

But if we consider that it is more good than bad, which is my opinion, and if there are no major toxicities demonstrated, then I think we need to get off the dime, that we need to use the NAT model and say this is something we want to do, how can we get there fast, or faster? I think NAT came exceedingly fast when a major effort was put out to do it, and it got validated.

So I think the first decision is do we really want viral inactivation. But if we want it, then I think we have to not get every piece. We've already been ten years in this process, and if we go to Mahmood's model,

which is a very good model, but if you go to that, it will be another ten years before anything could ever get out. And I think we have to say what's realistic, how can we measure it, who can do it, and really set a task to do it if we believe it adds something to the safety of the blood supply, which I believe.

DR. BUSCH: Steve?

DR. WAGNER: If I could respond to that, if we do indeed implement it, I would hope that we also implement sufficient surveillance of recipients to make sure that we're not causing a problem that's bigger than what we have right now.

DR. BUSCH: So really you're talking about IND--or post-licensure, continue sort of Phase IV surveillance. And right now the clinical trials that are being done, the formal trials do not have efficacy of bacterial or pathogen kill built into them. That's all done through these model studies, in at least the large trials that seem to be being reported, they're not--they're just demonstrating product efficacy, not really demonstrating kill, because you couldn't power the studies to demonstrate that with the major agents now.

But to do, you know, an efficacy of kill study, I think that's going to require millions of people for the major agents unless we use these other surrogate common

pathogens. And then your other concern I think is the concern around toxicity, low-level toxicity. So enrolling these people in long-term studies is...

DR. WAGNER: Yes, I think, you know, we're going to have to do infectious disease transmission work like we always do after the implementation of something and look at its impact, and we'd also have to look at--and this is what I keep on stressing. I think that we're going to have to report adverse events in a different way than we do now to make sure that we're catching everything so that we're not creating more harm than good.

DR. HEIDEN: I do not see so much the problems if we decided to accept this pathogen inactivated blood components to balance between the costs and so on. I think these inactivated blood components, even if they do not inactivate all pathogens, will be, of course, anyway, will be a plus in virus inactivation, a plus in safety of our blood components.

The most important thing in my opinion is we have to be really sure that this win in any kind of safety--and it will be a win, even if not any kind of species is inactivated. If this win in safety will be counteracted by any problem in tolerability, by any problem in mutagenicity and so on. And this is my most problem. I do not have any problem if it's expensive. All the testing and all the

things we have introduced in the last years are incredibly expensive. I think it's more important really to know if we do have any mutagenicity, any other serious side effect counteracted by this win in safety.

DR. BUSCH: Dr. Bianco?

DR. BIANCO: This is a fascinating discussion, but I'd like to go back initially to one things that Hannelore started, that is, what is really the objective of the viral inactivation that we are looking for? And I'd like to hear the opinion.

The other thing that I'd like to hear the opinion from the panel and starting from what Steve has raised about long-term surveillance and to answer your question about what side effects, what toxicology could be there, what is the benchmark? That is, what is the control group? What will we look at as the background that we will decide that there is an increase or not of adverse events? That I think is a very difficult question to answer. But I'm sure that you--at least Harvey has the answer.

[Laughter.]

DR. DODD: I can take a shot at your first question, Celso, because I think that the objective of viral reduction, viral inactivation was very clear 15 or 12 years ago or 10 years ago, and this is when the effort really started. But we've been pushed into other

mechanisms, and these other mechanisms have eventually been probably successful beyond our wildest dreams. So we're now left in a situation where we have the technology, and perhaps we're finding some difficult ways to justify it at this point.

I think it's very clear from the data that we've seen already that we shouldn't be overwhelmed by the issues relating to viruses at this point. I think in the context of viruses, we should probably ask ourselves a much broader, and that would be: Is this good public health? And in that comment, I include the question of: Is this appropriate use of resources?

If this was a vaccine that's going to cost however much per shot, would we actually promote using it? I'm not sure.

As to your other question, I'm going to leave that to someone else.

DR. WAGNER: I think that you have to have more complete adverse event reporting, and the sorts of things that you might want to look at might be cancer rates. You may need to look at hypersensitivity, anaphylactic shock. You may need to look at antibodies that occur and what their specificities might be.

It's a lot of work to run down transfusion reactions, but, unfortunately, I think you would have to do

that in order to be able to answer the question of whether or not you're doing more harm than good.

DR. ALTER: Celso, I think what I'm saying is, you know, this is a very difficult algorithm, and ten years ago, the advantage of this method, as Roger said, would have been much more dramatic than it is today. However, ten years ago, we weren't worried about bacteria; we weren't worried about Chagas; we weren't worried about introducing tests for HAV, parvo, and bacterial decontamination methods. So this potentially--while it's not going to get rid of tests like HIV, HCV, HBV, it potentially could prevent the introduction of tests for Chagas, for bacteria, for--and give some protection against the unknown.

But what I'm just--but my real point is, you know, you can argue yes or no, but if we're feeling yes or sort of yes or mostly yes, then I think this meeting is a good start. And I think we need to set a time frame, a schedule. What is the minimum information you need to bring this to light? What are the real toxicity issues? Are they real? Are they not real? Because there's a lot of things you will not be able to answer in any reasonable lifetime, certainly not in mine. And you got to bite the bullet on some of this, and that's where Steve's stuff comes in. If you've bitten the bullet--and FDA has a model

for this. You can't answer everything, so you follow what happens afterwards. We're not anticipating any major trouble.

But I just think FDA needs to set up the criteria, set up the panels, if possible. Can you inactivate this, this, and this? And can you show us reasonable safety? And then follow the people afterwards.

DR. BUSCH: Dr. Sayers?

DR. SAYERS: Merlyn Sayers, Dallas-Fort Worth. Inevitably, this is a question for Harvey. You know, we've heard about safety spoken in a number of different terms. Bernie spoke about absolute safety. Roger spoke about eliminating viruses. You spoke about reasonable safety just a minute ago. And Dr. Heiden spoke about acceptable safety.

So my question really is: Who defines safety? And by what standards are we going to meet this goal? And is the identification of a publishable event some transfusion-transmitted virus yet to be discovered? Does the understanding that that might indeed be possible imply that we are not meeting safety standards?

So I'm just wondering, Harvey, how we set about the definition of safety and how we move to that goal.

DR. ALTER: Basically, I'll define safety.

[Laughter.]

mc

DR. ALTER: No, I mean, we know what things have been transmitted. We know what things are still being transmitted. And so we have some measures to say, you know, you're starting out with an incredibly safe product, but, for instance, bacteria, I think we're unsafe in terms of bacteria. I think your presentation was beautiful this morning, and I think this is a major thing. I think we have to do something about bacteria.

Do we introduce universal culturing? Or might inactivation take care of that at the same time? But something has to be done. That's one safety measure.

Chagas is another concern we have now. I think that's a real thing. We're talking about introducing testing for Chagas. Will these methods kill trypanosomes? If so, that's an additional safety measure.

I'm not worried much about hepatitis and AIDS viruses now, but there still is a risk. We've seen cases, three or four cases now of those, despite NAT testing. Could we stay with mini-pool NAT testing if we had inactivation and not have to go to single-donor NAT testing?

So there are balances on the costs, and I think there--though we're so much safer than we ever were, we're not absolutely safe. We never will be. But this would bring us a step closer and probably a major step closer.

DR. SILVERMAN: Toby Silverman, Food and Drug. I have a question for the panel. Safety does not just include viral or bacterial safety. It also includes safety as measured by lack of efficacy of a product. What balance will you set between your viral or bacterial inactivation or reduction and the effect of the product for the patient, the efficacy of the product?

DR. BUSCH: Actually, I think that's a topic for a later panel.

[Laughter.]

DR. BUSCH: I have one question for our European colleagues. The CE mark that the SIRUS (?) system has obtained, is that--within Germany, is that sufficient to allow use of that product? I mean, one issue around this demonstration of, you know, lack of toxicity would be for these methods to be implemented in Europe for a year or two and we can watch and see what happens.

[Laughter.]

DR. HEIDEN: I do not really know how the other countries handle with the CE mark, because the CE mark for the bags and for the medical devices is mainly accepted. And now exactly in this moment, there had been some difficulties with the acceptance of the CE mark for medical devices which are coated with blood derivatives. And nowadays there are some congruence between the medical

product agencies and the medical device notified bodies. And I think--I have spoken very often with people from these notified bodies, and, of course, they learned a lot in the last six years because it's a new law, a new medical device law, and they look for a lot of--for really Dutch--a number of experts of production of this compound, of the production of the bag system, of the homogeneity of the production, of all these aspects, and they look very well on the pre-clinical studies, and they make also assessments by physicians and so on and so on. They have to make a risk assessment on the basis of the clinical data, and they make a lot of clinical studies, pre-clinical studies. And they are doing a lot of in vitro experiments to show the biocompatibility of the systems or to show the quality of the--not only of the compound which inactivates but also of the platelets itself, of course.

And in Germany, it is so that we have over this system of medical device CE marking, we have to make a marketing authorization for the blood components, for the blood--yes, blood components also. And we have--we're now in the position that we look again to all these documents, which had already been looked by the notified body. And perhaps we will have some more ideas, but a very large part of the work is already done. And I do not know how the other European countries regulate it because we have a very

parliamentary system, and we--okay. For instance, in France, in Austria, and in--not in the Netherlands, but in Spain, they have a more central directed system, and they have more force to regulate some aspects of this licensing. And we have to--we really have to look for our laws, for our guidelines, for to make our assessments, to give the marketing authorization, and they have to take it to town, the CE mark, which has been given by the notified body for this medical device, for this compound.

DR. WILLKOMMEN: May I add? I think it is very clear. The CE mark is for the blood bag which contains these components. In Germany, we have the drug law, and the drug law covers also the blood components. And so the blood components which are inactivated, of course, needs a license. And the license has to be given by the Paul-Ehrlich-Institut. And so it cannot be brought directly on the market, but the situation is, again, different in other European countries because not all of them handle blood components as a drug. And so maybe that in some countries it can be used so, but I think that all the other agencies will also consider something, and I don't believe that it is really completely sufficient to have this CE mark.

DR. BINION: Steve Binion from Baxter. Just a clarification to your question, Dr. Busch. As the recipient of the CE mark in question, by current European

legislation and according to drug-device demarcation guidelines, any of the pathogen inactivation systems by definition fall into a Class III medical device category and, in fact, are being regulated at the EU level as drug-device combinations.

So in our case, the overall system regulatory approval was as a highest-risk medical device, a Class III medical device. But the amotosalen hydrochloride solution as a new chemical entity was the subject of a separate review and submission process as a new chemical entity by a medicines-competent authority. The drug-device combination approval process to obtain the CE mark requires the notified body to consult with a recognize drug authority, a medicines-competent authority, and obtain a favorable opinion as to the approvable nature of, in this case, the amotosalen hydrochloride as a new chemical entity.

So, in fact, what, you know, is referred to as the CE mark was a combined drug-Class III medical device approval. Then, as has been previously reviewed by Dr. Willkommen, the individual member states, since they regulate blood components and the manufacturing and other processes for preparing blood components at the national level, are then following the CE mark required to go through their approval process for the blood component.

DR. BUSCH: Thank you.

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One more quick comment, and then we'll break for lunch.

PARTICIPANT: Meyer (?) Germany. I can add to the pathogen inactivation of methylene blue in Europe. I can add, as was mentioned by Dr. Willkommen, CE certification is one basis in England, for example, acceptance was further done by a red book monography which exists, and in France, it will be still an additional national regulatory besides the CE certification.

DR. BUSCH: Okay. Thank you. Thank you to the panel. We're scheduled to reconvene in 45 minutes at 2 o'clock, so hopefully people can eat quickly.

[Luncheon recess.]

AFTERNOON SESSION

[2:15 p.m.]

x DR. BLAJCHMAN: Okay. For the next two hours, we're going to talk about bacteria and bacterial contamination of blood products, primarily cellular blood products.

As we're starting this concept--and, again, this will be the third FDA meeting on bacterial testing, and so far we have not taken any action around the world, and to quote that famous American icon, Yogi Berra, "It's deja-vu all over again."

I think we heard this morning and we're in a bit of a bind because we're in the conflict area where we're in the conflict between using the precautionary principle to make decisions and evidence-based medicine. And for years, we had a problem because we didn't have the evidence relating to bacterial testing. I think the evidence has been there, and as you'll hear from the next four speakers, the evidence is really there that bacteria in blood products do significantly harm and even kill people, and I think hopefully we will, as a result of this meeting, see some action relating to this.

I just want to make one other point, that it seems--or maybe I'll make it a little later--that the FDA,

using the precautionary principle, have taken two decisions relating to variant CJD, but despite the fact that there's a considerable amount of evidence with regard to bacteria, have not taken a decision with regard to bacteria. And I think it's time we did that.

So, with that as an introduction, I'd like to ask our first speaker, who is Betsy Poindexter from the FDA, to provide a list from CBER of the bacteria for testing decontamination. Betsy?

x MS. POINDEXTER: Good afternoon. This is going to be a presentation based on CBER's list of bacteria for testing pathogen reduction methods, and, in addition, these methods--these bacteria are also recommended for manufacturers who develop and present to us bacterial detection systems.

In establishing this list, we took on the whole ball of wax. We took the universe of bacteria, an all-inclusive list. Then we limited that list to bacteria that were already reported to have contaminated blood products, particularly platelets and red blood cells.

We then limited that list to bacteria implicated in transfusion-associated sepsis and to bacteria that were implicated in transfusion-associated fatalities. There are select model bacteria based on experience as to how difficult they are to eradicate, and I think that was

mentioned this morning. Some of the spore formers are particularly difficult.

In generating this list, we surveyed the literature for bacteria that have been reported to contaminate platelet products and red cell products. We looked at case studies, medical center reports, and review articles.

We had the assistance of consulting with microbiologists at the Center for Device Review at CDRH. Their microbiology consults, Sally Sallapak and her colleagues have been very forthcoming with information on particular bacterial growth patterns and selection of bacteria.

We've also used the PHS Bacterial Contamination Working Group which is a consortium of people from our Division of Emerging and Transmitted Transfusion Disease, the Center for Disease Control, NIH extramural grants, and Clinical Center Department of Transfusion Medicine. So we've incorporated a lot of input.

In 1999, we had a workshop, as Dr. Yomtovian referred to, on bacterial contamination of platelet products. The list that's on the screen right now shows you the number of fatalities, the numbers of cases that were reported, and the percentage of those cases that ended up in fatalities. We had in the red cells Yersinia, the

Clostridium perfringens, and P. acnes, and one case of enterococcus. In platelet products, there's Staph epi, E. coli, Bacillus species, Strep species, Salmonella, and P. mirabilis.

As you can see, if you combine the two groups you have a significant number of fatalities that are reported.

This list gives the Gram-positive and negative groups from this complied list of bacteria that we have been asking the manufacturers to consider when they are using these inactivation processes or bacterial detection processes, as well as a list of the protozoan. Each of these have been reported either to have contaminated a product, to have caused sepsis in a patient, or have actually caused fatalities in the reports that have come to the FDA from 1976 to 1998. Also included at the Rickettsia and the Ehrlichia. Those that are in blue actually caused fatalities in the patients that received the products.

These are those same organisms in a larger format. As you can see, the E. coli, Enterobacter, and Klebsiella, the Proteus mirabilis and Salmonella and the Serratia are the ones that caused fatalities in the reports that we had received through '98.

Among the Gram-positives, the Bacillus species, Staph epi, and Streptococcus pyogenes.

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To date, I don't think that we've had any reports of protozoan fatalities, but I'm not part of that group. People from our compliance group may know that.

And that's it for our list. These are available. Joe Wilczek has a copy of my slides, and if people want copies of the lists, Joe will be able to provide them to you.

DR. BLAJCHMAN: Thank you, Betsy. We'll have time for questions afterwards.

The second speaker of this session is Dr. Matt Kuehnert, who's a medical epidemiologist at CDC, who was deeply involved in the BaCon study, and Matt will talk about some of the results of the BaCon study, focusing primarily on some of the bacteria that were identified within the BaCon study.

Matt?

x DR. KUEHNERT: Thank you very much, Mo.

What I'd like to do today is go over some selected BaCon study results--we've heard a little bit about BaCon throughout the morning--and focus on findings that may help direct intervention, focusing, as I've been directed, on pathogens detected; but, most importantly, to put an emphasis on the clinical context.

I'll start somewhat unconventionally by starting with the punch line, which was to determine the rates of

transfusion-transmitted bacteremia over a three-year time period. And just very briefly, what we reported--and this is in cases per million; people are probably more familiar with the "one per"--it comes about to about one per 5 million red cells with one per 8 million of fatalities. And between single-donor platelets and pooled platelets were not statistically significant and came out to be about one per 100,000 cases, one case per 100,000 and one case per 500,000 for fatalities.

But there's a couple things to consider here. One is that the number of cases were relatively small. There were wide confidence intervals. When you consider platelets, the lower limit the confidence interval went to about one case per 60,000. The other to consider, I think, which is much more important, is to consider what the goal was as far as patient outcome. And since we're just after lunch and I have to sort of wake people up, I made the font as large as possible, which is that BaCon describes reaction rates and etiologic pathogens for documented septic reactions and not for all reactions due to bacterial contamination. And I think that explains some of the difference between BaCon rates and some of the other studies.

And I think that's important also when you consider the pathogen list because these are pathogens that

actually caused bacteremia or sepsis in recipients. So when we look at reports, we have to consider whether the reports are talking about either rates or pathogens in units causing a positive culture but no reaction in the recipient, caused a fever or some otherwise minor clinical symptom, overt sepsis, or fatality.

I just needed to show this slide. I think if Dr. AuBuchon got a royalty every time this slide was shown, he'd probably fairly wealthy by now. But basically it's showing that even the conservative estimate of sepsis, we get down to the point where viral pathogens are below the rate of bacteria. And since I've seen the updates this morning, I could update the curve and it would be even more of a disparity at this point as far as bacteria being a more common cause.

So what does clinically important reaction mean? I just wanted to briefly go over the BaCon study criteria as far as what that actually meant. A recipient needed to have any of the following signs and symptoms: fever, rigors, tachycardia, or change in systolic blood pressure. And also some laboratory criteria in addition to the clinical criteria: culture-positive blood product, recipient blood culture growing the same organism as that recovered from the blood product, and I think that's an important point as far as our confirmed cases; and,

finally, that the organism pair in the product and the recipient be identical by pulse-field gel electrophoresis for molecular fingerprinting.

So these are fairly rigorous criteria, but I think it's important when you distill it down to those confirmed cases, you have what your--I think we can be relative--well, now very confident that these organisms were causative in causing the patient's reaction. And, again, making the point that this is a multi-factorial organizational process, we cast a wide net with the clinical service in that any of a number of symptoms could trigger the start of it, but in the final confirmation, there had to be a fair amount of laboratory evidence that this was an actual case.

So here's what we finally came up with. All submissions were not included. Out of the 56 that were evaluable as far as symptoms and availability of product in blood cultures; 79 percent met clinical criteria, and 61 percent were confirmed by molecular typing.

So that left us with 34 reports which were confirmed--19 single-donor platelets, 10 pooled platelets, and 5 red blood cells. Recipient median age was 48, range 3 to 90 years. Gender was split evenly, and the most common underlying diagnoses were malignancy, by far, and GI

bleed was second most common. And nine, or 27 percent, had a fatal outcome.

Here are the bacteria implicated. It was fairly evenly split between Gram-negative and Gram-positive, which, again, I think differs from many other studies, and, again, to emphasize, we're looking at recipients who had bacteremia or overt sepsis. The Gram-negative organisms included E. coli, Serratia species, Enterobacter species, Providencia, and one Yersinia. And this was a bit of a change from what I think had prompted the study, which were a cluster of Yersinia cases in red blood cells, and we only saw one. And I think this is somewhat interesting, the change in epidemiology.

And the Gram-positives saw a fair amount of Staph epi, also Staph aureus, Streptococcus, and an assortment of other Gram-positives, including Streptococcus pneumoniae.

So one important part of the analysis I wanted to emphasize was what the risk factors were for fatal transfusion reaction. Now, on the univariate analysis, what we found was, first of all, that Gram-negative organism in a product was strongly associated with fatality, and also if the recipient was older, they were more likely to die.

Then there were some other things that made us scratch our heads and made us think, well, do we have the

variables coded the wrong way around, because it didn't seem to make any sense. One was recipient was more at risk for death if they had a shorter time from start of transfusion to reaction, a smaller volume transfused, and a shorter platelet storage time.

And I think what it's all about here is it's all about the Gram-negatives, which overwhelms every other variable. And I'll have an example here of storage time.

You have for platelets, the median storage time was four, relatively prolonged; overall, the range being from two to five. But when you split it up between Gram-negatives and Gram-positives, you have a great disparity: 2.5 days of storage for Gram-negatives versus Gram-positives, five days, which is the conventional wisdom, longer platelet storage is associated with bacterial contamination.

When we see a p value like this, it's certainly something we take notice of. So in the multivariable model, we took all of these things into consideration, and the only thing that fell out was the appearance of a Gram-negative organism. A recipient was almost eight times as likely to die if they had a Gram-negative organism in the product. Recipient age also came out--it nearly was significant, but not quite.

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What I wanted to provide here, I've gotten a number of inquiries about, you know, certain specifics about organisms and type of product storage time and fatality, and just go over basically what was seen in the analysis, which is that fatality was very unusual in Gram-positives and very usual in Gram-negatives. So you see very few fatalities here. No fatalities amongst the single-donor platelets. And here I'm going to introduce a concept which I think by now is familiar to everybody who was here in the morning, which is that it's the endotoxin that kills people.

In every case in which a recipient died and we were able to have the product sent to us and were able to measure endotoxin, endotoxin was present in every one of those cases in significant numbers, as you can see here, in the thousands. So I think it's relatively clear that endotoxin is very important in causing significant reactions.

One thing the BaCon study was not able to do is to find the sources of contamination, which I think is very important in trying to design interventions. And, you know, for most of the event they're unknown, but there have--of course, there are reports in the literature, and generally it can be divided into donor bacteremia in which Gram-negatives mostly, such as Yersinia enterocolitica and

E. coli, are implicated; and, rarely, Gram-positives, such as Strep pneumoniae. Skin contamination is almost always Gram-positive organisms, with some exceptions, but most staphylococcal species; and, finally, processing, in which both Gram-positives and Gram-negatives have been implicated.

I wanted to close my presentation by perhaps stimulating some discussion about changing epidemiology and the need to really look on a regular basis at the epidemiology to see if it's changing.

One issue that came up in the middle of the study was whether there was an association of Gram-negative organisms with single-donor platelets, and that's something that we looked at closely, and in the final analysis it didn't come out as significant. There was a difference in proportion, but it was not statistically significant in comparing single-donor platelets versus pooled platelets.

But it's also interesting to look at the distribution per year, and although the association with Gram-negatives in single-donor platelets did not pan out, it's interesting to note this increase in the last year, which it was almost all Gram-positives in single-donor platelets. So it sort of tended to even out, although it was very choppy in the three years.

So I think, you know, the years we chose in which there was, I think, a fairly fundamental change in the denominator in the use of single-donor versus pooled platelets, I think was somewhat influential and needs to be taken into consideration in designing surveillance in the future.

In summary, the data suggests, both BaCon and otherwise, that platelets are the problem by orders of magnitude; that the most common organisms causing bacterial contamination are staphylococcal species, E. coli, Serratia species; and in red blood cells, now Serratia species are the most common, not Yersinia anymore.

As far as fatalities, platelet reactions are significantly associated with Gram-negative organisms, associated with high levels of endotoxin. These are the bugs that kill people, and they're associated with very short storage times, which I think indicates a minimal time required for the lethal effect of endotoxin producing Gram-negative organisms--not only organisms, but I want to emphasize the endotoxin itself. The bugs don't have to be alive. It's just the endotoxin that can kill people.

And the epidemiology may be changing. There certainly is a shifting use of platelet product types, and whether there's a predilection for certain organisms depending on the product is not clear. We don't know. I

don't think the BaCon study had enough cases or was done long enough to be able to tease that out.

And as I said, the interventions with the greatest impact, as far as fatality, I believe will be ones that prevent transfusion of products contaminated by endotoxin and target at processing--collection and processing steps in which this contamination may occur.

And, finally, the final plug, that the measurement of intervention impact will be critical to evaluate the effectiveness of any intervention that takes place.

Just a reminder that this was a multi-organizational effort with a lot of collaborators, and I thank them for their effort in this study.

Thank you.

[Applause.]

DR. BLAJCHMAN: As you can see from the overhead, our next speaker is Dr. Paul Ness, who is director of the Transfusion Medicine Service at Johns Hopkins Medical Institutions, and Paul will talk to us about the bacteriology of septic platelet transfusion reactions, and these represent his experience at Hopkins.

Paul?

x DR. NESS: Thank you, Mo, and I'd like to thank the organizers of the meeting for inviting me to come here

to present our data, which is really the result of a lot of people's collaboration--our microbiology lab, our Transfusion Medicine Division--and I want to particularly acknowledge the contributions of Dr. Hayden Brain, who's an oncologist, who taught me early on that this is a problem that is very important and worth preventing.

I'd also like to thank somebody for allowing me to survive my lunch of NIH pizza.

[Laughter.]

DR. NESS: What I'm going to talk to you about is a cohort which began in 1986 where we started to investigate all febrile transfusion reactions to platelets and studied them prospectively by culture of the platelet bag.

The febrile transfusion reactions were defined by the presence of a fever rise of greater than one degree Centigrade, or chills, or rigors, and we had a high index of suspicion when a reaction occurred that this might be a contaminated reaction and would then do a culture.

Our definition of what was aseptic platelet transfusion reaction is a little different than the BaCon study, and perhaps more inclusive, but we said that confirmed septic platelet transfusion reactions were identified by isolation of the same bacteria from the bag and the patient's blood, which occurred in about a third of

the cases, but in about a third of the cases probably because the patient was on antibiotics or we just couldn't have enough bacteria to grow from the bag, we confirmed a positive culture in the bag by a positive Gram stain in the bag at the same time. And this allowed us to eliminate a number of the false positive cultures you get if you routinely send bags of platelets down to the microbiology lab.

Around the same time, we also, because of some reports of reactions we had encountered before all of this started, initiated a program to convert platelet transfusions to single-donor platelets. On this slide, you see the overall experience. At Hopkins we transfuse a lot of platelets. We looked at it in two-year periods because towards the end of the periods, as you'll see, the number of events gets very small. But in 1987 and 1988, when we started, we gave out 11,641 pools of random-donor platelets, 12,451 single-donor platelets, a total platelet transfusion exposure of 25,000 platelets over two years and a percent of single-donor platelets of about 51.7 percent.

Over time, as a result of our intention to try to reduce the numbers of random-donor platelets and to increase the number of single-donor platelets, you can see that eventually in the period '97-98, we ended up with 30,000 single-donor platelets, only 193 pools of random-

donor platelets, and a percent of single-donor platelets at Hopkins of 99.4 percent.

In terms of the reactions, here you see the same last column from the previous slide. In the first year, when we were using 51 percent single-donor platelets, we had five reactions due to random-donor platelets and none due to single-donor platelets, for an incidence of reactions of 1 in 4,818 platelet transfusions. And, gradually, as you can see, as the percent of single-donor platelets went down, the numbers of septic reactions due to randoms obviously went down. We weren't using them. And the numbers of single-donor platelets causing the reactions went up. But the number of septic platelet transfusion reactions went from originally about 1 in 4,818, when we started at 50 percent single-donor platelets, to 1 in 15,098.

I show this graphically here. This is 23 reactions which occurred during the 12-year period. The ones in this orange color are due to the random-donor platelets, the ones in this sort of light blue color due to the single-donor platelets. And you see that originally there was all randoms and gradually shifted to single-donor platelets.

Here is the same curve only looking at those that were fatal versus those where patients survived. We had 23

reactions. We had four fatalities out of 23. So of the patients who had these septic platelet transfusion reactions, 23 percent were fatal, and usually within 12 hours of the transfusion, which led us to believe this is a very serious event.

This is a slide which I think brings a lot of internal control to the data. What I show here is the total numbers of donors who provided the platelet products at Hopkins in this two-year period, which, as a result of our going from random platelets to single-donor platelets, is decreasing. So we started it in the first two-year period with about 80,000 donor exposures to transfuse our patients and ended up with about 30,000 donor exposures.

What I think is very interesting and serves as a very nice internal control is this bottom line here, which shows the number of reactions per donor exposure, which stays constant right around 1 in 15,000. So that as you see, as you diminish the number of random-donor platelets and donor exposures, the number of reactions goes down, but the reactions per donor exposure stay constant.

Now, in terms of the bacteriology, these are the 23 cases we talked about. We had 15 cases where we believe that the organism which was involved was a skin contaminant, 10 cases of Staph epidermidis, 3 cases of B. subtilis, two cases of other skin flora that I didn't have

room to spell out here, and I thought the names would change by the time I got back.

Eight cases, however, were due to bacteremic donors, and I think this is something that we need to take cognizance of because a lot of the efforts we do to sterilize skin or think about diverting the first part of the blood collection would not handle these cases, and these were 8 cases: 3 of Staph aureus, 2 of Strep pyogenes, 2 of E. coli, and 1 of Strep pneumo.

Here I show you the reactions to the four bacteria that caused the fatal septic platelet transfusion reactions. We had one case--actually, two cases of Staph aureus, one case of Staph epi, one case with E. coli. As you can see, two of these reactions came from platelet pools; two of these fatal reactions came from single-donor platelets. One of these reactions, we could actually grow the organism in the patient. Three of them, we grew the organism in the bag and confirmed, as I said, from the earlier definition that the Gram stain of the bag at the time of the culture was also positive.

I also, because of the previous remarks, added another column which I haven't shown you here in terms of the length of storage. This Staph aureus here was five days. This E. coli was stored for three days. This Staph aureus was four days. And this Staph epi was two days.

So unlike the BaCon study, three of the four fatal reactions we had were actually Gram-positive organisms, and the periods of storage tended to be relatively long as opposed to the BaCon study for our fatal reactions.

So, in conclusion, I've shown that with single-donor platelets, the incidence in our setting fell in terms of septic platelet reactions to 1 in 15,000. Now, as you'll remember, when we started the first year when we were at about 50 percent, the rate was about 1 in 5,000. And if you back-calculate what the risk would have been if we shifted, instead of going to single donors, back to all random-donor platelets, the risk would have been 1 in 1,606 reactions. The reactions were caused by skin contaminants in two-thirds of our cases, but bacteremic donors can cause one-third of the septic platelet transfusion reactions, and I've already remarked as to what I think this implies in terms of our attempts to prevent these. And three of the four fatal reactions actually arose from bacteremic donors.

The final comment I want to make is that I've heard a number of my colleagues because of the skin contaminant say that Staph epidermidis is benign, and I don't think Staph epidermidis gets enough respect. I don't even have enough respect to spell it correctly.

[Laughter.]

DR. NESS: Which I apologize for, and my microbiology colleagues will get on me for it. But I think it's important to recognize that Gram-negatives--I'm sorry, Gram-positives in these skin contaminants are important pathogens in platelet transfusion and need to be handled appropriately.

And just a final slide, we have two publications on much of this work: an earlier publication from JAMA by John Morrow et al., and then a later publication in Transfusion 2001, where most of the data I just showed you are published.

Thank you.

[Applause.]

DR. BLAJCHMAN: Okay. The fourth speaker of this session is Dr. Mindy Goldman, who's the associate medical director from Hema-Quebec, and Mindy is going to be presenting the Canadian data that will include data both from Hema-Quebec and Canadian Blood Services. These two services, most of you know, provide all the blood products that are distributed in Canada, and her talk is based on the hemosurveillance system that's in place in Canada, and she will review the data that's currently available on bacterial contamination.

Mindy?

DR. GOLDMAN: Thank you very much, Mo.

Some people may know that I trained with Mo. It's been a few years now. And he infected me with his interest in bacterial contamination, and everybody who knows Mo knows that his convictions are very deeply held, and so they tend to be very infectious.

I'd also like to thank the organizers and, as Mo mentioned, my colleagues at Canadian Blood Services, Dr. Hume and Dr. Sher, for sharing their data with me, so this really is a Canadian summary.

Now, what we know about bacterial contamination in Canada comes from surveillance cultures and adverse event reporting.

If you look at surveillance cultures, they're done in three different laboratories across Canada: two using bacterial blood culture systems and one using the old fluid thioglycollate medium. They're not always done at the optimal time, I would say, to find contaminants, so for single-donor platelets, they may be done close to the production time. For the whole blood-derived platelets and for red cells, they are done at the expiry date of the component.

These are the results of the surveillance cultures for the last two years. So for red cell units, there were close to 5,000 products cultured, and there were three positive cultures. Whole blood-derived platelets,

you could see that there were seven positive cultures out of over 4,000 components. And thrombapheresis platelets, there were three positive cultures out of over 6,000 components.

Here you see the percentage contamination, which appears to vary from 0.05 percent for thrombapheresis platelets up to 0.17 percent for whole blood-derived platelets. However, the numbers are very small, and that's why I calculated the 95 percent confidence interval, so you can see that all these numbers are actually overlapping, and there's not really a statistically significant difference in these numbers, so you can't really draw too many conclusions from this.

However, the results have stayed pretty much the same compared to the previous two or three years in Canada. They've dropped a tiny bit, but not, again, in a statistically significant way.

So the overall rate is 0.09 percent, which is a number that I think is found in other studies in the literature.

Now, if we look at what the organisms are in the red cell units, they were pretty much skin contaminants. One is fungal. I guess maybe I shouldn't have included it, but anyway, it was one of the positive cultures.

Here in the whole blood-derived platelets, again, really skin contaminants: Propionibacterium, Staph epi--I'm not going to read all these to save time and also because they're hard to pronounce. You have them in the handout.

Here's the thrombapheresis platelets, and, again, three are really skin contaminants.

Now, if we turn our attention to the adverse event reporting, the circular of information that the hospitals get says that all serious reactions, including bacterial contamination, should be reported to the blood supplier, and then the blood suppliers report these to Health Canada.

There are standardized forms that are used for reporting, but there's not really a standard protocol for investigating the reactions. And in the province of Quebec, which is where I work, there's a hemovigilance network that has been set up over the past couple of years, and this includes 22 safety officers who are in the largest hospitals called the designated hospitals, and one of their functions is adverse event reporting. And these type of programs are being set up in seven other Canadian provinces, but it started a little bit earlier in Quebec than in some of the other provinces.

So I'm going to show you first the reactions, transfusion reactions from Quebec, and then the rest of Canada, and you'll see that there's quite a difference in the frequency of adverse event reporting.

So just to show you--this is over three fiscal years--over the three years what has happened in terms of the number of severe reactions reported in the province of Quebec to Hema-Quebec, so to the blood supplier, and this is not all bacteria, but all severe reactions.

So you could see there were 27 here, 47 here, and 67 here, and I don't think our product has gotten less safe in that period. I still work there, but you could see that the reaction rate has gone up by leaps and bounds because people are looking more closely for reactions.

Now, if we look at the subset of these that are due to bacteria, out of these 17 there were five that were possible bacterial contamination, and out of these five, two were probable bacterial contamination. And I'll just define what I mean by possible and probable.

Probable is something that could be part of the BaCon study; in other words, the same bacteria was isolated from the recipient and from the component, and there were clinical symptoms that were highly suggestive of bacterial contamination.

Possible is the kind of things that we tend to get in the real world when you work in a blood center--that is, the product was cultured and bacteria was found; the recipient was cultured and that was negative; but the recipient is on antibiotics. So I'm not quite sure what to make of that, but I didn't want to discard those, so I put them as possible.

Or, conversely, the recipient was cultured and had a positive culture; the product had a negative culture; but I'm not quite sure what the protocol was at the hospital for culturing that product. In some cases, a segment was cultured, which is obviously inappropriate.

So I've included those, but obviously not all the possible ones are real.

So if we go through them and we start with the platelet pools, we had 11 reactions in this three-year period, and these are the two that I would say were definitely due to bacterial contamination, and they were both fatalities, septic shock and fatality, with the platelet pools. In the first case, it was S. epidermidis, and this actually was written up as a letter to the editor in Transfusion, and it was very clear that this bacteria was present in the component, in the recipient, also in the red cell and plasma component from the same donor and the same species of the organism was on the donor's skin. So

that was definitely a fatality due to bacterial contamination. And the second one was S. aureus that was found in the component and in the recipient, with septic shock starting during transfusion leading to death. And both these patients were fairly elderly, immunocompromised patients with hematologic malignancies.

Now, here are ones that are really possible but not necessarily confirmed, so you have the symptoms that in some cases--such as here where you have just fever and chills--could definitely be febrile non-hemolytic transfusion reactions not due to bacteria. You have others where you have just hypotension, so the symptoms are possible but not necessarily due to bacterial contamination. And you have positive cultures on the component in all these cases, but not--either it wasn't done on the recipient in a few cases, or it was negative in the recipient but the recipient was on antibiotics. So you have Bacillus, Streptococcus, two different bacteria, a few coagulase-negative Staph, and Moraxella.

To continue, these are two reactions with platelet pools where the recipient culture was positive, but the culture of the component was negative. And I'm not sure how well the component was actually cultured.

There was one reaction with the thrombapheresis unit where the same Bacillus was isolated from the

component and the recipient. In terms of plasma, it's surprising but there was one reaction with a plasma unit, an FFP unit, where the same bacteria was isolated from the component and the recipient, and one other plasma reaction where the culture in the recipient was not done because the recipient was on antibiotics.

If you look at the red cell reactions, we didn't have any reactions with red cells where the same bacteria was isolated from the component and from the recipient. So you have several where there was a bacteria in the component, not in the recipient, and then several where it's the other way around. And the symptoms here could be due to bacteria, or it could be this patient had septicemia completely unrelated to their transfusion.

Now, if you look at Canadian Blood Services--and this is where I said there's a big difference because we had 20 possible/probable reactions combined in the three-year period, and Canadian Blood Services had nine. Canadian Blood Services has about over three times our volume, so we transfuse about 100,000 random-donor platelet concentrates per year and about 2,000 single-donor platelet concentrates, and Canadian Blood Services transfuses over three times that. So there's quite a disparity there.

So there were two reactions with platelet pools, and the same bacteria was not shown in the component and in

the recipient in these cases. And there were seven reactions with red cells, and there was only one of the seven where the same bacteria was found in the two, and I think this was a reaction that was reported by Mo. And others, mainly with Gram-positives, there was not a culture done in the recipient.

So just to summarize the organisms that were found and all these reactions--and there's more organisms than reactions because in some cases more than one bacteria was isolated--there were 29 Gram-positive organisms which were mainly the type of organisms you find as part of skin flora, and there were six Gram-negative organisms.

So just to summarize, the overall frequency of bacterial contamination in the surveillance cultures was 0.09 percent. The reporting and the investigation of the adverse reactions was not uniform throughout the country, and that just shows how difficult it is to come up with incidence figures for these kind of reactions and how the more you look, the more you find. And the organisms that were most frequently identified in both settings were either part of skin flora or are organisms such as Bacillus that are sort of ubiquitous in the environment.

Thank you for your attention.

[Applause.]

DR. BLAJCHMAN: We're very much on time, even though we started 15 minutes late. The organizers will be happy with that, I think.

I'd like to start the panel discussion by inviting all the speakers up, and we've also invited several other people other than those who spoke at this symposium: Roslyn Yomtovian, who spoke this morning, David Leiby, who spoke this morning, and Mark Brecher. I'd like those people to come up, please.

We have a series of questions that have been put out by the organizers, but perhaps before we do that, since we didn't have any discussion or questions to each of the speakers, I wonder if people--before we deal with the formal questions, as it were, I'd like to invite people to come to the microphone to ask specific questions to the speakers.

Perhaps I can start while people are making their way to the microphone, if they are. Matt, your data is quite different from most reported data, as you know. I think in my opinion, your data represents the minimal frequency of bacterial contamination. To use the iceberg analogy that Dr. Yomtovian talked about this morning, I suspect that most of the cases that you describe represents the tip of the iceberg, and I'd invite you to comment on that and maybe get Dr. Yomtovian to comment as well.

DR. KUEHNERT: I'd agree that it represents the tip of the iceberg. I'd say instead of the top, though, of the iceberg, it represents the tip of the iceberg that actually spiked the Titanic. So I think it's important to emphasize once again that these cases represent severe recipient morbidity and should not be compared apples to oranges in looking at, for instance, surveillance cultures or even in looking at, you know, single-center programs where they have routine either surveillance or protocols in place where they aren't--they don't have as restrictive criteria on recipient morbidity or have--or take into consideration needing to have matching organisms by pulse-field gel electrophoresis.

So I think that's an important point to make in looking at comparing numbers.

DR. BLAJCHMAN: So with regard to platelets, for example, which is the biggest part of this, 1 in 100,000 rate event is the absolute minimum, and the rate is going to be significantly--the actual rate in practice is going to be significantly higher than that.

DR. KUEHNERT: That's right. I think so. I mean, I think that that represents an estimate of the number of recipients who have severe sepsis, and I think there were limitations to the study because of the design,

but I think that's a good starting point for the incidence of events causing severe sepsis.

DR. BLAJCHMAN: Roslyn, do you want to make a comment?

DR. YOMTOVIAN: Well, I think the BaCon study is a very good study given its own limitations which it set on itself, and if one reads the papers carefully, it clearly states in the papers that this is a minimal estimate and the problem is likely greater. That was clearly stated in the paper that was published.

But another limitation, of course, of the BaCon study, which I think has been largely overlooked, although occasional people have pointed it out, is the lack of recognition clinically of these events. And, you know, it's not just the rigorous criteria which threw out a lot of cases that almost certainly were due to contaminated products. It's clinicians just not recognizing that a reaction may be due to a contaminated product, and that still persists today in spite of numerous articles, workshops, et cetera, on this. That is an incredible challenge, and that was, you know, one of the things that I learned through the years.

We picked up cases with our prospective surveillance where, when you went back and looked at the chart, there were clearly symptoms. But they were

overlooked, and that is a major educational issue that has to be addressed.

Can I ask a question to one of the panelists? Do I get a chance to do that?

DR. BLAJCHMAN: Yes, but maybe we can ask the audience to get audience involvement first, and then we'll come back to you.

DR. KUEHNERT: I just wanted to add one thing to what Dr. Yomtovian said. In reading the excellent publication on sort of what started your interest in this, if I remember right, after an education program was put in place, the detection rate increased, I believe it was 20-fold afterwards. And so I think that's an excellent point, and I think that certainly affected rates, too, as clinical recognition--

DR. BLAJCHMAN: And Dr. Goldman's point of having somebody to actually monitor shows that very clearly when you compare the Hema-Quebec data with the CBS data in Canada.

MS. JACOBS: Mary Beth Jacobs, U.S. FDA. Dr. Yomtovian referred to our not having a systematic approach in the U.S., and my question is for Dr. Goldman. I'm asking whether Hema-Quebec has had a systematic approach to prevention, and have you used your transfusion safety officers in the hospitals for that?

DR. GOLDMAN: Okay. There's really two separate things. One is Hema-Quebec, the blood supplier, and the second is the whole hemovigilance network that the government is setting up. We work very closely with them, but we don't control them or in any way responsible for them.

In terms of Hema-Quebec, what we've done, I think we've optimized our skin disinfection protocol a number of years ago, and it's the same one that's used throughout Canada. We've encouraged our blood supplier to make bags with the derivation pouch, and we've validated those, and they're in production and we'll be switching to all bags with a derivation pouch for whole blood collections in the next few months. And we've also started routine cultures of all thrombapheresis platelets, and we do that on one site now and will be extending that to our other site in the next few months.

I have to say, in all honesty, that the majority of our platelets are not thrombapheresis platelets. We're increasing our percentage of thrombapheresis platelets. They're now about 20 percent of our production. What we would like to do is we would like to target those platelets to the sickest patients, the bone marrow transplant and hematologic malignancy patients. And then we'd like to gradually increase our thrombapheresis program so that more

patients would be getting products that had had bacterial cultures. So that's been our approach.

In terms of the government and the hemovigilance network, I think what that has allowed hospitals to do is to see where the actual problems are, and problems at the hospital end, because here we're talking about residual risks that are very small and that actually people never see. I mean, we don't see HIV or hepatitis C in the hospitals anymore. But when you start looking you see a lot of ABO errors and you see a lot of anaphylactic transfusion reactions and a lot of volume overload and things that they could then take action on the hospital level. And I think that if you don't collate those things, they never really get any press, sort of. So that's the other part of the approach.

T5A DR. BLAJCHMAN: Dr. Goodrich?

DR. GOODRICH: Ray Goodrich. I'm with Gambro BCT. This may be a variation on Question 2 that's up there, but I wanted to ask in a slightly different way because it is something that--

DR. BLAJCHMAN: Can you get closer to the microphone? I'm not sure we can here.

DR. GOODRICH: Certainly. It is something that I've asked several of you, I think, independently, and I'd like to get the broader perspective.

When you're dealing with a product that is bacterially contaminated at the point of donation, is there a way of estimating what the titer of bacteria may be in that product? And does it differ if it originates from a skin plug versus an enteric infection? Is that known?

DR. BRECHER: I don't think anyone really knows. You can estimate backwards for a given organism if you wanted to. There have been some papers that have looked at the time it takes to positivity on a culture machine. So if you picked it up on a culture machine and you know how many hours it's taken positive, you can extrapolate back down on that particular organism. That's a little laborious. Whether the growth curves are different coming out of a skin plug or whether there's a bacteremia, I don't think there's any hard data on that. But you would expect that probably in the bacteremic state, the bacteria is getting out sooner and may have a bit of a jump-start on growth. But I don't think anyone really knows.

DR. BLAJCHMAN: Anybody else on the panel have any opinion on that?

[No response.]

DR. BLAJCHMAN: Celso?

DR. BIANCO: Yes, I have a quick comment about BaCon and then a question for you.

BaCon was a voluntary reporting study, and that's the major problem. I was associated with a large institution in a large metropolitan area. None of the 200 hospitals in that area participated in the study because they said they did not have the resources to do the additional culturing and study for each one of the patients that had a change in temperature of 2 degrees Fahrenheit, that I remember. So that's, I think, a major problem. So you are seeing the minuscule tip of the iceberg.

My question for you, Mo, because you were the first speaker, is: You challenged us that we've talked three times about bacterial contamination but have not done anything about it. And maybe the challenge is that we don't know exactly what to do about it.

There are some approaches, like the diversion bags, that we will know--and particularly after we look at all the numbers today--would apply to a limited fraction of the bacterial contamination and would not solve the problem. There is a culture approach that you have used and several here have used, but not routinely. A big investment, when to do it, how to do it, and those are the types of questions that we wanted to hear--answers, not questions. And now we have another approach that is pathogen reduction. Should we abandon all the other approaches? Should we focus on this one?

DR. BLAJCHMAN: I welcome that challenge, and I'd like to get the panel to comment on that. But perhaps I could, since I raised the issue, I'd like to respond.

I think it's very--for more than ten years, I have been speaking at these sorts of meetings, not only at FDA meetings but elsewhere, saying that we need to do something about bacterial testing.

Well, we don't have a perfect test, as we all agree. The problem is that if we're waiting for the perfect HIV test or the perfect HCV test, we still wouldn't be testing for HIV or HCV because we're still getting patients--two, three recent patients in the United States got HIV.

So I think it's important to start, and I think if you wait for the blood industry to make a decision to start, it's not going to happen. And I have challenged Jay, and I've done the same challenging in Canada, to say that the regulatory agencies need to sort of say we need to do something.

Now, a strange thing has happened that may surprise people, but I think--and I felt that we should do some culturing; direct culturing is the approach, and there are a variety of these techniques available.

I, however, now feel that direct culturing is probably not the way to go. I think the way to go at this

point in time, because we have techniques that are available for pathogen reduction, that rather than come to the point of pathogen reduction and say, well, we don't need it because we're 90 percent effective, assuming it's 90 percent, therefore, we don't need it. So let's not spend our money on doing bacterial testing by culture. Let's take the jump from bacterial testing, and let's do license--assuming the sorts of issues that were clarified this morning. But even the pathogen reduction technique that is close to being licensing, I suspect, is not a perfect technique. It's the first generation of the way we're going to be doing things over the next five or ten years from now. And I think it's important that we start doing that.

So I think we--the point I'm trying to make is I'm trying to challenge people is to say we need to do something and we need to do something before we kill that many more people. We've been waiting for evidence-based data. Well, the evidence-based data has been--you've just heard a lot of it. There's a lot of it that's out there that hasn't been heard. And I think it's important that we start doing that. And the only way to get this done in either the United States or Canada or elsewhere in the world, in my view, effectively is for the regulatory people to say this needs to be done. And to wait for a perfect

solution is not the way to go because we're not going to see a perfect solution in the very foreseeable future.

I'd like to get people on the panel to comment as well. Mark? Ros?

DR. YOMTOVIAN: Okay. Let me follow up a little on what Mo just said and make a couple of points.

First of all, I don't think we'll go home from this meeting and pathogen reduction, even if everyone agreed here that it was wonderful, would be implemented tomorrow. It will take, I believe still, significant time to deal with a lot of the issues that have been raised by several people.

So while we're still waiting for that, which I think many of us would agree is closest to the ideal that there might be to destroy the pathogens, what do we do in the interim? And as I was sitting listening and tabulating the data that all the speakers presented in this afternoon's session, I counted that one-fourth to nearly one-half of all cases of platelet bacterial contamination are due to coagulase-negative Staph or Staph epi. And some of those have been fatal. Now, granted, not a majority, but some of those have been fatal.

I mentioned in a new paper that just came out that the diversion method seems to be particularly good, which makes sense if one is diverting away a skin plug or

bacteria related to a skin plug, in getting rid of not all but much of the coagulase-negative Staph.

Now, for years, maybe not quite ten, but certainly a large number of years, I've wondered: That's a no-brainer to, you know, redesign a--close to a no-brainer, a blood collection set. Why hasn't that been done and maybe a better question now is why shouldn't it be a standard if you--you know, you know that it is somewhat effective, we'll find out how effective. And it really doesn't involve any risk whatsoever. So that's one point.

Then just a quick second point if I can get this in. In the fatalities reported to the FDA, I notice there was no Pseudomonas aeruginosa, and I know that two cases we've reported through the years that were fatalities were Pseudomonas. So I can talk to you later about that. I don't know what happened to those.

DR. BRECHER: Ros, I also noticed there were some mistakes on the FDA slides. For example, they did not list Staph aureus as a fatality reported to the FDA, and yet it made up 17.3 percent of the fatalities.

That aside, one of the problems that was really emphasized in the '99 meeting, when we were all pounding the table and saying do something and mandate something, was that the FDA came back to us and said, But there's

nothing licensed. We can't mandate anything until something is licensed.

Well, point of fact, there is one bacterial detection system that is now licensed, which is the BioMerieux, formerly Organon Teknika, BacT/ALERT system.

Now, I have to say conflict of interest, I did a lot of the research for them to get that licensed, and I've been a consultant for them. So having said that, we can do something, and we can do it as an interim measure now. If I take the liberty of combining some of my data with Jim AuBuchon's--I don't think Jim would mind--between us we have cultured now over 5,000 apheresis platelets on day two, and we have interceded on five Staph epi-contaminated platelets, and we know Staph epi kills people for a rate of, you know, 1 in 1,000 that we've been able to intercede on.

So we can stop these things, and we don't have to wait for the inactivation to come along. We could roll this out virtually tomorrow.

DR. BLAJCHMAN: Jay?

DR. EPSTEIN: Well, earlier, Mo, you challenged us that it was deja-vu all over again, and Yogi Berra also said, "It isn't over 'til it's over."

My point is that we have been making incremental progress, but it doesn't seem like progress because we're

not where we want to be. And it does echo Mark's remarks. We have been looking within the agency at the feasibility to issue guidance on the skin prep, specifically the use of the tincture of iodine as opposed to the povidone iodine. We have made progress on making available blood bags with diversion systems. As in Canada, we lobbied the industry, and there are bags being made available.

We also brought to the Blood Products Advisory Committee the question whether we needed validation data on reduction of bacterial contamination or whether we could simply approve them for what they actually do, which is divert a part of the collection without breaking a closed system and with unidirectional flow. And we did make the determination that we would approve them based on validating their operational characteristics and without the necessity for the manufacturer to further claim reduction in bacterial contamination of the product.

So, you know, that's the standard under which we will approve diversion systems, and that's been made known publicly, and there has been movement toward making the bags.

With respect to bacterial contamination monitoring, it is, I think, a little bit more complicated. What we approved as a device approval was the use of a system for quality control monitoring of the rate of

bacterial contamination. That's not quite the same thing as a product claim for routine culturing as a release criterion. And the subtle point there is that the validation has not yet come forward, at least to the stage of applications, in terms of when and how you do the culture. So the trick in terms of making a release criterion is to know what you get based on how you do it, and we're not quite there.

But, on the other hand, the system is approved for surveillance monitoring, and we do think that if more routine use is made of surveillance monitoring, the overall contamination rates would fall, because I think we have seen that they vary a lot by center, and there's probably, you know, a training and experience element--for example, the data on the phlebotomists--and that if there were process monitoring making the contamination rates visible, there would be a driver toward lowering them through better practices.

So, again, I think, you know, my message here is that there is incremental progress. It is our current thinking to issue a guidance in all three areas, in fact, on use of a more effective skin preparation, on use of--voluntary use of a diversion pouch, and then we're thinking to issue guidance on use of a culture system for routine contamination rate monitoring. And we look forward to

being able to approve systems for routine culturing as a quality control of products, and we would like to see that linked to extension of platelet dating. And we know that there are studies that are looking at the quality of platelets with extended dating.

So, again, my point is that we are making progress but we aren't at endpoints, and, of course, we want to see endpoints.

DR. BRECHER: Jay, there may have to be a paradigm shift if you're going to do a detection system or a culture system. The way they're doing it in Europe is they're releasing the platelets before the culture is finalized, and if it comes up positive, then you call it back. And that's what they're doing in Quebec.

DR. EPSTEIN: Again, we could dive into the details. There are many scenarios that have been proposed, but the point is that if a product sponsor wants to make a claim, they have to define how it will be used.

DR. BLAJCHMAN: Jay, without sounding disrespectful, I have a problem with this because you invoke--"You" being the FDA--the precautionary principle when decisions are made vis-a-vis variant CJD. You don't take the precautionary principle to its extreme; otherwise, you'd ban everybody who visited Great Britain even for an hour or had a hamburger at Heathrow. But you've taken that

decision when there has never even been a case of variant CJD transmission anywhere in the world. Yet you're taking the position vis-a-vis bacteria, and you have lots of evidence that this harms significant numbers of people and the precautionary principle doesn't apply in that particular--you seem to be bending over backwards, avoiding doing this, but taking the precautionary principle approach and say, well, we need better data, better systems, and all this.

I see a problem with your position on--or an inconsistency, I guess is the right word that I'm looking for, between the position that FDA and, for that matter, Health Canada took vis-a-vis variant CJD position versus bacteria. I wonder if you would respond to that.

DR. EPSTEIN: Well, I think that there have been perhaps inconsistencies in the forces acting on different issues--put it that way--that on any given issue, you know, you have a scientific dimension, there's an economic dimension, there's a political dimension, and then, you know, something stirs the mix. Whether that's public fear or, you know, perceived risk, I'm not sure.

So I think that there's truth in what you say. It goes beyond that. I mean, how do we look at ALT? How do we look at anti-core? How do we look at mini-pool

versus S/D NAT? You could argue in various cases that we aren't coherent in our thinking.

That said, the standard of being proactive that was applied to variant CJD leaves you in a quandary whether you shouldn't just do everything else you can imagine on other fronts.

I think that I would just come back to the point that Celso made. The dilemma about bacterial contamination has not been over whether we ought to do something. I think we all agree that we should. It's been a debate over what exactly we should do. And, you know, no one likes a heavy-handed regulator. If we're going to mandate something, we really think we need strong rationale, and I think that there's been enough of a debate over specific methods and whether they're timely and what's their yield and how good are the validation data, that it's been a debate over what exactly to do.

And perhaps we're past that point, but as has been said, you know, we're now engendering a new debate. You know, do you use old methods when there are new ones right over the horizon? And, you know, how different is that than, say, you know, surrogate markers for HIV before there was a specific serologic test? That's a familiar debate, too.

So I don't know the right answer. I just know that, in fact, we have revved up since the last scientific workshop, that there are things in the works, and that our current thinking is to move forward with at least guidance.

DR. BLAJCHMAN: Thank you for those comments.

Merlyn, you've been standing there patiently.

DR. SAYERS: Mo, I've watched you transition from moderator to prosecutor.

[Laughter.]

DR. SAYERS: Let me ask Paul two questions. Paul, what was your yield in terms of culturing those individuals with febrile transfusion reactions as measured by how many you had with positive cultures?

DR. NESS: In terms of--

DR. SAYERS: And then I've got a question after that.

DR. NESS: --suspect patients who had the febrile reactions?

DR. SAYERS: Right, how many cultures?

DR. NESS: It's probably fairly small, probably on the order of, I would guess, 1 or 2 percent. I haven't looked at that rigorously. We were probably having a couple hundred reports of febrile reactions a year, probably less, probably about a hundred platelet reactions, and we probably had at the peak five that were positive.

DR. SAYERS: Okay. And then the other question has to do with those asymptomatic, ostensibly healthy donors who were, nonetheless, bacteremic. I wonder if there's any evidence about the presence of bacteremia in hospitalized patients who do not obviously have a source of infection. One could argue that that might be a reasonable control group.

DR. NESS: In most of these patients, when we were able to go back to the donor--for instance, we had one that was due to Strep pyogenes--we were able to find a donor who was afebrile, didn't have a sore throat, but next day or so developed a whopping sore throat and had a high ASO titer and clearly we got him--he gave platelets early and they were bacteremic and he then got sick.

So, in most cases, we were able to make a cogent argument that the donor who we think had caused the reaction clearly had caused it--

DR. SAYERS: And that's not in doubt. I was just wondering, if you took a group of non-transfused hospitalized patients who did not have obvious infection, how many of those you might identify as being bacteremic.

DR. NESS: Well, how many who were not transfused and we just cultured--well, I think you heard earlier that there's a fairly high and significant rate of false positive blood cultures, depending on who does it. So I

don't think that's any new news. You know, I think what we tried to do to make sure that our cases were really cases was the use of the simultaneous Gram staining of the bag, which showed organisms physically there even though we couldn't grow them in the culture.

Then, since I have the mike, I just wanted to point out in terms of the BaCon study, of the four fatalities that we had, three of them wouldn't have been eligible. But they clearly died because of contaminated platelets.

DR. BLAJCHMAN: After the next question, I want to turn formally to the formal questions. A question?

DR. DIAMOND: Well, this is probably going to lead into the first question, anyway. Dick Diamond, FDA. One of the comments, certainly it's easy to identify the list of clinically relevant pathogens from cases that have been documented of sepsis, fatal sepsis arising from blood contamination and contaminated transfusion.

On the other hand, there are major intra-species differences in virulence, virulence factors and susceptibility to all kinds of antimicrobial agents. It's certainly well demonstrated for antibiotics, but it's equally well demonstrated--and I have firsthand experience in the laboratory with this--with in vitro oxidant-generating systems where in many cases there are strain

differences that are dependent on the relative balance between--and rates of DNA damage and DNA repair, likewise differences in endotoxin content, endotoxin structure, endotoxin release under certain conditions.

When we test antimicrobial agents of other types, we look at panels. There are different ways of selecting these panels. Sometimes they're arbitrary. It strikes me that we ought to do this in a semi-rational way and discuss how this is done and what requirements there are in the standard list. And I was curious as to whether the panelists come down in that regard.

DR. BLAJCHMAN: Paul, do you want to try--

DR. NESS: Well, I was thinking the same thing because, as I saw these lists go up, I thought of maybe we're all sort of doing bird watching and collecting a list of birds that we've seen, which isn't necessarily useful if we want to figure out how to--I guess we don't want to kill the birds, but it would seem to me that one needs help probably beyond the expertise of most of us in this room, but hopefully not at the FDA, of figuring out if we're going to come up with a list of bacteria to test eradication systems and/or detection systems which have sort of unifying biological principles, so that, you know, a bacteria that metabolizes this way would be killed, you know, by this process, would not be killed, and likewise a

bacteria that has a certain characteristic would be identifiable by this method and not by another.

That seems to be more--I think the incidence reporting is useful to some limited extent, but I don't think that can be the whole answer.

DR. DIAMOND: And if you're defining standard test conditions, growth conditions can have an enormous difference in susceptibility to any antimicrobial agents, including oxidant-generating systems. And they, depending on the oxidant-generating system, the penetrability of oxidants or the relative rates of DNA repair and DNA damage may vary enormously.

DR. YOMTOVIAN: Just to follow up, another conceptual difference between the virus story and bacteria story is when you treat--do an inactivation process to kill or eliminate virus, it doesn't matter when you do it. You can do it, you know, right after the collection. When you're doing it to eradicate bacteria, it may matter very much because if you don't eradicate every last bug, it's possible that if there's a delay in transfusion, it might regrow. So that just becomes another variable or parameter to need to carefully study before one of these technologies is promulgated.

DR. BRECHER: Also, Ros, if we're really worried about a skin core, UV light may not get into that core.

DR. DIAMOND: I'm sorry. I didn't--

DR. BRECHER: If there is a skin core coming up a needle as the source of the bacteria, if that core is sitting in a bag and you UV-radiate it, or whatever energy source, it might not penetrate that skin core.

DR. DIAMOND: And by the same token, neither might any other oxidant-generating system.

DR. BLAJCHMAN: Matt?

DR. KUEHNERT: I wanted to add, I thought--Dr. Diamond, I thought that was a very good point about, you know, different strains within species having different characteristics. We have not done those types of studies at CDC on isolates in the BaCon study, but we never throw anything away as far as isolates. So, you know, I think the idea of having some sort of isolate consortium, not only CDC but other groups who have saved their isolates, it's a very good idea to see what differences in characteristics are.

DR. DIAMOND: I might add, from years of doing research, passage within a laboratory changes all these properties or can change them drastically. So if we're developing standards, we have to be--it doesn't mean it's impossible to do. It just means there are a lot of things we have to be cognizant of if--

DR. BRECHER: There are not a whole lot of organisms, to be honest. I mean, we have this bird-watching list, but if you look at what organisms really kill people in a significant number, 95 percent of them are made up of about eight organisms. It's mainly, just looking at the FDA data, it's 53.5 percent were Enterobacteriaceae and 26.9 percent were Staph, either epi or aureus.

DR. DIAMOND: Yes, you can define the species, but within those species, which representatives are you going to choose? Because the inocula that you have to use to infect an experimental animal or to get killing in vitro is going to vary enormously within different sets of strains and different sets of growth conditions.

DR. BRECHER: And you need plasma-resistant organisms that won't roll over and die when they see plasma, and that's typically a problem with Enterobacteriaceae.

DR. DIAMOND: It's a lot more than just plasma resistance.

DR. BLAJCHMAN: What about the second part of this Question 1? Is a comprehensive list of bacteria necessary, or can a subset of model bacteria be used? Do we get a consensus on that? Do we need every organism that's ever been described to cause septic reactions, or

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can we come up with a subset of--and perhaps even in that subset, a number of different isolates of the same organisms that have been commonly reported to cause reactions? I'm getting a number of heads shaking, so I think the answer to that--the consensus may be yes. Dr. Leiby, you haven't said anything. Do you have any thoughts on this?

DR. LEIBY: Subset.

MS. : Subset.

DR. BLAJCHMAN: Subset. Subset to include maybe a number of different isolates even from the same organisms of the more common variety.

DR. LEIBY: Yes, I would concentrate on the organisms that are known to have caused fatalities in the U.S. For FDA data, I would use the FDA data for FDA submission, essentially.

[Laughter.]

DR. BLAJCHMAN: We'll turn to Question 2, which reads: What level of efficacy is appropriate for a bacterial pathogen reduction process? Is there a time the inactivation must take place in order to prevent bacterial outgrowth, to prevent an adverse outcome from endotoxin? And can inactivated cells continue to produce endotoxin? perhaps the last question is the easiest one. I doubt if--does anybody want to tackle some of those questions?

Anybody in the audience who have experience with pathogen inactivation have any thoughts on Question 2? No comments? Steve, thank you.

DR. WAGNER: Well, I certainly have some star-growing strains in my lab that are able to go from one CFU per mL to 10^8 CFU per mL in a single day. And so if you were to get an apheresis platelet, for example, and store it overnight because you didn't want to be bothered by some inactivation technique, and then the next morning treat, it could very well be very high levels. I would suspect there would be considerable levels of endotoxin in there as well.

DR. BLAJCHMAN: Thank you.

Question 3, should--

DR. YOMTOVIAN: Can I--Mo, sorry.

DR. BLAJCHMAN: I'm sorry. Roslyn?

DR. YOMTOVIAN: I just want to make also a comment on that. That's a very difficult question because there are so many different variables. As Steve just pointed out, you have the endotoxin issue, which, you know, is a big issue, obviously. But from very, very old, old studies on bacterial contamination that I know you're familiar with, you have this lag phase phenomena, and are those bacteria susceptible to being killed?

So, you know, are very low levels susceptible? Is it too late when the level is very high? And I don't

have really the answer to that. I think there needs to be more research done on this. Unless it has been done and people have the answers, I think more work needs to be done.

DR. BLAJCHMAN: Dr. Murphy?

DR. MURPHY: Mo, I think Patrick Hibb (ph), when he was in Calgary, published that in Yersinia the lag phase may be due to the fact that the surviving Yersinia are, in fact, in platelets and also in leukocytes, which brings us back to the story of the skin plug, if you like. You have to be able to address mutualization of Yersinia in platelets or attached to platelets or attached to leukocytes as part of the process, the validation of the process.

The other point I'd like to make is you're going to do the inactivation after leukodepletion if and when you begin universal leukodepletion and before issue, before you place the stuff in inventory.

So that's where it's going to take place, and it's important, if you like, to validate the process. But I think Dr. Yomtovian's point is extremely important. If you're going to store--which we are--products after inactivation, it's important that the efficacy gets them down to zero, particularly if we're going to want to extend

the storage life of platelets on the basis of bacterial inactivation after seven days.

DR. BLAJCHMAN: Dr. Corash, do you want to shed some light on this topic?

DR. CORASH: Well, I just want to respond to the issue that Dr. Yomtovian raised. First of all, I think as everybody who's worked in the area knows, you want to have viable bugs, and so you want to be--you want your inoculum to be obviously in a growth phase so that it's in good condition. But at least I know our own experience is that with very low levels, the technology is very effective.

What one tends to do is put in high levels of bugs because you're looking for that margin of safety.

Now, there's also very, I think, good data in the literature about this lag phase, and although I don't think anybody knows--and I think Mark tried to speak to this--you know, what the level of contamination is immediately at the time of a collection. But people have cultured products early on, and I think some of the work that Don Buchholz did many, many years ago indicates that at least in the early phases of contamination, we're looking at somewhere between 10^1 and 10^2 CFU per mL. Mark is saying maybe a little bit--

DR. BRECHER: Or less.

DR. CORASH: Or potentially less. But, also, I think everybody would agree that to be able to do a process as soon as possible after the time of collection, once you have the product in what would be a closed system, would be the most advantageous way to go.

DR. DIAMOND: Just a comment. In organisms in stationary phase, whether in a small inoculum and in early lag phase, or if they've grown up to stationary phase and are no longer growing, are much more resistant to any antimicrobial mechanism. That includes antibiotics where there is a decided inoculum effect, but also a major effect particularly for cell wall active agents on whether or not they're in active growth.

And there's also increased--a decreased susceptibility to oxidant-mediated damage with non-replicating DNA. So it does matter when in the phase of growth that the organisms would be treated. It doesn't mean that any of these things wouldn't work, but it makes sense to do it during an early growth phase.

DR. BLAJCHMAN: With regard to this question about can inactivated cells product endotoxin or continue to produce endotoxin, does anybody have any experimental data relating to that question? Dr. Diamond?

DR. DIAMOND: The release of endotoxin during the killing of organisms is well demonstrated with antibiotics,

and they're not only cell wall active antibiotics, plus the fact that it's not just peeling off, because there's an unaccountable lag phase between the time of the treatment and the documentation of cell wall damage and the maximum amount of release of endotoxin.

You can also show endotoxin release from oxidant-damaged organisms where presumably DNA fragmentation is the initial event. And, again, the time course doesn't fit totally with breakdown of the cells. So it's a little bit hard to tell.

So while you wouldn't be generating new endotoxin without growth of new organisms, you might be releasing progressive amounts up to a finite limit, depending on the inoculum.

DR. BLAJCHMAN: What about Question 3, spore forming? I don't know--there's Steve Wagner, you suggested this morning that pathogen reduction systems may not be effective--or pathogen reduction may not be effective for spore form--has this been looked at formally, do you know? Or, Larry, has your group done anything in this regard?

DR. WAGNER: We did do an experiment. We've actually now done multiple--

DR. BLAJCHMAN: One experiment?

DR. WAGNER: Well, multiple experiments with Bacillus cereus, which is something that does appear on the

list. And we did it, and it was done by a group that we worked with in Sweden. We didn't do the experiment the right way the first time. We put in a high level of inocula of a spore-forming species and then immediately did the pathogen inactivation. And it was--it shouldn't have been a surprise because spores are very impenetrable, and we had residual viable bugs. We killed between 3.6 and 3.9 CFU per mL, but there were viable bugs that were left.

We then did a study on the time dependency of those bacilli and those spores to enter the vegetative phase, and we found out that within three hours in the platelet products, they all appeared to be in the vegetative phase. And when we did the pathogen--

DR. BLAJCHMAN: After the treatment.

DR. WAGNER: No, no. Before treating.

DR. BLAJCHMAN: Before treating.

DR. WAGNER: So we inoculated the spores, and then did inactivation at various times, and by three hours our observation was that we could kill everything and held the product for five full days and saw no residual bacteria in the product.

So we believe that at least for that one strain, the spores germinated and went into the vegetative phase within a three-hour period of time and were susceptible to pathogen inactivation.

DR. BLAJCHMAN: But spores themselves appear not to be susceptible.

DR. WAGNER: The spores do not appear to be uniformly susceptible. Whether or not some subset are susceptible, we don't know. But the spores themselves do not appear to be susceptible, at least to our technology.

DR. BLAJCHMAN: Having heard that comment, does the panel want to deal with whether there should be required efficacy against spores? Paul?

DR. NESS: I think the question is the other way around, and I don't know the answer to it, but what percent of the--how often do clinical reactions occur because--strictly because a spore on the donor's skin presumably led to an overgrowth and a reaction? I don't know that--I don't know that answer. Maybe somebody else does. But I think that's what you really need to assess to understand--to answer that question.

DR. BLAJCHMAN: The only case that I'm aware of where spores probably were implicated in a fatal septic reaction is a case in London where Clostridium perfringens, presumably the cleansing of the arm did not kill the spores, and that entered into the bag. It's a case of John Barber's.

[Inaudible comment.]

DR. BLAJCHMAN: No, we don't know if it--we don't know if it was spores.

[Laughter.]

DR. BLAJCHMAN: The presumption in the article and the discussion was that it was because the cleansing of the skin did not kill the spores. Do we know that for sure? No.

A question or comment?

MS. SCHILLING: Yes, my name is Susan Schilling. I'm--

DR. BLAJCHMAN: Come closer to the microphone, please.

MS. SCHILLING: Susan Schilling. I'm with bioMerieux, so I'm not going to go into conflict of interest here, but specifically relating to spores, it's a real tricky issue because probably he's right that the spores would become vegetative in the platelets that were incubated at room temperature, so they'd be killed by the inactivation. But in a whole blood unit, I don't know.

And we deal a lot with another industry who had one death due to a Clostridium that it was the spores that were not killed by the process that they treated their tissue with. And it's been a huge investigation ongoing. So that would be something I would definitely consider, but I don't know how frequently you find Clostridium spores,

although they are pretty ubiquitous in nature, so they could be easily on the skin.

DR. BLAJCHMAN: Thank you for those comments.

Question 4 here, will a bacterial reduction process that is ineffective against certain types or forms of bacteria be useful clinically? Anybody want to talk--Mark, you're shaking your head.

DR. BRECHER: I'd just say yes.

DR. BLAJCHMAN: Yes.

DR. BRECHER: Yes.

DR. BLAJCHMAN: A partial effect is--

DR. BRECHER: Going partway is better than not going at all.

DR. BLAJCHMAN: Than not going no effect at all.

It's 4 o'clock--oh, Roslyn, you were going to ask a question, and I didn't--

DR. YOMTOVIAN: I snuck it in. I asked Betsy about the Pseudomonas.

DR. BLAJCHMAN: Okay.

DR. YOMTOVIAN: And so we'll take care of that.

DR. BLAJCHMAN: It's 4 o'clock--two minutes before 4:00. I'll call this session adjourned, and we get back together at 4:20 to hear the industry representatives on Phase I/II studies.

mc

Thank you very much. Thanks to all the participants as well.

[Recess.]

T5B

DR. BIANCO: If you could take your seats, we are going to start the last part of our workshop today, and certainly after all our discussions and points raised, now we'll talk about the meat. The manufacturers of those technologies and products are going to present to us what they have and the data that they have.

It's a great pleasure to invite Dr. Larry Corash from Cerus Baxter to talk to us about these technologies.

DR. CORASH: Thanks very much, Celso.

I am an employee of Cerus Corporation, and I'm also on the faculty of the University of California, still see patients, but don't get paid. So that's my financial disclosure.

We've been working in developing technology for pathogen inactivation of the labile blood components for over ten years now, and in collaboration with Baxter Health Care, and you'll see the reason for that partnership in a moment as to how it applies scientifically.

We've been working on development of systems for all of the labile blood components. We completed work last year, actually, and received approval this year for the CE mark, as you heard this morning, for buffy coat platelets,

and that has now been supplemented with a supplementary application for single-donor platelets in Europe.

In the United States, we've finished Phase III clinical trials with single-donor platelets. The plasma system, which uses the same technology at the platelet system, is nearing completion of its last of three Phase III studies looking at therapeutic plasma exchange, and the red cell program, which uses a slightly different, albeit similar, scientific rationale for technology, has just entered Phase III clinical trials in the United States. And the clinical trials for plasma and for red cells in the United States are being used to support regulatory submissions in Europe.

The rationale for our technology, which is a nucleic acid-targeted technology, is that pathogens and leukocytes require nucleic acids for their replication. We have designed our compounds to target and modify nucleic acids to prevent replication in these pathogens and in contaminating leukocytes.

By and large, red cells and platelets are terminally differentiated cells. They don't contain a nucleus, and they appear not to require nucleic acid for delivering their therapeutic efficacy.

The technology we've developed, we use the nomenclature of Helinx technology as a shorthand

description. In the platelet and plasma system, we use a psoralen compound. You heard about that this morning. It's gone by the name of S-59 for many years. Its official name right now is amotosalen.

S-59 was part of a synthetic development program that was headed by Sue Wallowitz (ph) and grew out of a long experience in psoralen photochemistry that came from the University of California at Berkeley with John Hurst and Steve Isaacs.

In the red cell compound, we use a chemistry that's based on something called frangible anchor linker effectors that was developed at Cerus by Sue Wallowitz and her team. That compound is S-303.

One of the things that attracted us to this technology for psoralen-based technology was that when we started this work back actually in the late '80s, 8-methoxy-psoralen had been in medicinal use to treat chronic refractory psoriasis, and there was an experience, obviously, of human use exposure. It's also the case that the average U.S. citizen eats about two milligrams of psoralen a day. Now, you don't eat S-59. That was synthesize. So there are differences, and there are a lot of psoralens in different foods, and psoralens have different efficacies in terms of pathogen inactivation, and they also have different toxicity profiles. And so the

comments that we're making today are very specific for S-59.

Now, for both of these technologies, just a few key points illustrated on this slide. We have looked for compounds where basically we can have temporal control of reactivity as a way to build safety into the system, and we do that in two ways.

For S-59, which is a photoactivatable compound, the first step is intercalation, and that's a dark process, and it's in dark equilibrium. And there is no covalent addition during that phase. And remember again that this is a treatment process that occurs ex vivo. So it is turned on for S-59 by the addition of long wavelength ultraviolet light. That's light from 320 to 400 nanometers. It is the light which gives you a suntan under the appropriate circumstances.

With the addition of that light, you get covalent addition. You can get a mono-adduct and you can also get a crosslink. You can get a di-adduct.

Based on research that we've done, both mono-adducts and di-adducts are effective in blocking replication because the mono-adducts will block polymerases, and you can make psoralens that will only form mono-adducts, and we've demonstrated they can be effective.

What one strives for is getting a level of modification which overwhelms repair mechanisms, and we believe that we have accomplished that.

In the red cell system, we started out looking at photochemical approaches, but we wanted to stay within the environment of packed red cells, and photochemistry in a bag of packed red cells at a hematocrit of 65 to 80 percent is a very, very major challenge. And so we developed a nucleic acid-targeted chemistry that uses a class of compounds that we developed which are very stable at low pH but when added to blood at neutral pH, rapidly intercalate, form covalent adducts with nucleic acid, and then completely break down. And so that's how we get the temporal control.

At the end of the process--and we'll describe the process in a little more detail later on--we do not find detectable S-303 left. It's converted to a negatively charged breakdown product called S-300, and it's negatively charged and has no covalent addition to nucleic acid.

So, again, that's an ex vivo process, and in a minute you'll see some other aspects of the system which give us further control over safety profiles.

Now, this is the system which has been developed and been through the CE marking process in Europe. It is an integrated container set. As I think Steve Wagner

mentioned this morning, obviously a big part of this chemistry is that you have to have transparency to ultraviolet A light everywhere. And Baxter has great expertise in plastics and manufacturing these types of containers and obviously was a very ideal strategic partner for us in developing this type of technology.

This is a set that requires a single sterile connection here with platelets that are collected, and we use nominally 35 percent plasma and 65 percent of a balanced salt solution that we call InterSol. It is a derivative of a platelet additive solution that's been in use in Europe for some years, but it's has acetate as a substrate, and it's had phosphate added to it as buffering capacity.

We have our amotosalen in a light-protected little pouch. The entire platelet product flows through this pouch into an illumination container. This then gets clipped off.

The illumination container is mixed, and then this cassette of three containers goes into a UVA illumination device. This is microprocessor controlled. It shakes. One of the things that we wanted was shaking so that we'd get even distribution and mixing and we don't get shielding in the product. It's very important, obviously, that you can treat the entire product and not have problems

with shielding. And we have designed experiments to evaluate that as we go through the process.

Now, during the process of photoillumination, something very interesting happens, and that is that S-59, amotosalen, photodegrades into six characteristic photoproducts. Those photoproducts are not reactive with nucleic acid, and there is a residual of about 20 percent of the amotosalen left over after you do the process.

That process is extraordinarily reproducible and, in fact, is in a certain sense an internal dosimeter. In fact, we use it for calibration of these systems.

Our pre-clinical safety program was designed to test S-59, to test the pre-illuminated product, the post-illuminated product, and showed very good safety levels. But in medicine, a number of years ago we decided that less is always better, and so we developed a container that we call the CAD containers, which is a compound absorption device, which is a solid wafer. And after the illumination process, the platelets are transferred into this CAD device. This is clipped again with a heat seal, and the platelets are then incubated for a minimum of four hours, during which time the residual S-59 is further reduced another 100-fold, so that the final amount of S-59 in this container, when it goes into the final transfusion storage

bag, is about 50 micrograms. We start with 15 milligrams, and we end up with about 50 micrograms.

The freed photoproducts are also reduced by the compound absorption device, but as Steve Wagner mentioned this morning, the bound photoproducts--and there are some photoproducts that are, first of all, inside the platelets, because you want to inactivate intracellular pathogens, and some of these photoproducts are dimers of S-59 and they don't come back out of the platelet. And, secondly, you do have complexes primarily with lipids, and those bound molecules are not completely removed by the compound absorption device.

After this incubation process--and it's flexible. You can incubate from four hours up to 20 hours. The platelets are transferred into a final storage container. This is clipped again, and this is what goes into storage and would be sent out to the patient's bedside.

Now, there's an extraordinary amount of pathogen inactivation data that's been gathered over the last ten years, and I think this morning's speakers, you know, raised very relevant issues, and I'm going to try and touch on some of those, and I'm not going to be able to touch on all of those issues.

These are data that have been collected from infectivity assays. We made a decision that infectivity

assays were what we were most interested in, and they were expressed for these log reductions because there are different ways in which you can conduct these experiments. These are PFU or infectious units per mL in terms of log reduction. We're obviously treating all the time full units. All of these studies have been done with full platelet units of approximately 300 mL, with guard band studies for ranges around that. But these are log reductions per mL.

Now, what happens here, though, is that in various types of assay systems, you can analyze the entire bag. And in some systems, it's very difficult to do that because it just scientifically is somewhat overwhelming to try and do that.

In HIV types of assays, though--and these numbers represent the lower limit of the 95 percent confidence interval. So we sort of ask the question: What happens if I go back in and take another sample? And the volumes that are sampled here range all the way from 1 mL up to 300 mLs, where the entire unit has actually been sampled and cultured. And so the array of information, you know, needs to be broken down, obviously. But the 95 percent confidence interval and the use of multiple replicates and multiple experiments I think gives us a reasonably good estimation of what is inactivated.

The limits of detection, when those are reached in assay systems, are indicated by the greater-than sign, and what we can demonstrate is obviously governed by what we can put into a system.

So, for example, HIV-1, which is cell-free--this is a lab-adapted strain because you can grow it to very high infectivity titers. There are many wild isolates that you cannot grow to high infectivity titers, and so you would have very limited dynamic ranges.

Cell-associated comes in two flavors. It can be either a mononuclear cell, which is productively infective and has a cytoplasm full of virus in lots of vacuoles, and that's what this represents; and then you also have the situation where you can have a cell line that has a single integrated copy of the HIV genome which can be stimulated with TNF alpha and induced to express infectious virus. And there, of course, you're inactivating the cell rather than a productively infected virus because you use this assay for that, and you can demonstrate inactivation of large numbers, you know, greater than six logs of these infected cells.

Clinical isolates represent basically community-acquired strains which have not been lab-adapted and have to be assayed not in MT2 plaque-forming assays, because not all wild types will plaque, but have to be assayed in

mononuclear cell culture assays. And so that's why these titers are low because that's basically the dynamic range that you can get.

For the hepatitis viruses, these represent chimpanzee infectivity assays, and Harvey Alter touched on this this morning. These come from his laboratory, and they represent the MS2 strain and the Hutchison strain. These are calibrated chimp inocula that have been used many times in chimpanzee experiments, and these are the infectivity titers of what are available and what have been validated and titered many, many times. So that's what's available to us.

We recently completed studies with HTLV-1 and 2 with the laboratory in Bordeaux, France, because we determined that they had the best assay. And here you don't see a greater-than sign for these infected cells which represent high titers of infected cells because the background was not clean enough to get to a greater-than sign. We feel, based upon some data that I'm going to show you in a few minutes, that we have a very high level of inactivation here, but we can't get to the background which is sufficiently clean to allow us to say greater than.

This is syphilis in a rabbit infectivity assay, looking at testicular lesions showing in two separate series of experiments that were done, $10^{6.8}$ and 10^{7}. This is

data for plasma, and then in the red cell program, there are obviously things that will be filled in here. We have yet to do chimpanzee infectivity studies. We're early in this program for some of these pathogens.

Model viruses have been used very effectively. We like the duck hepatitis B model. It's a very robust model because you inoculate one- and two-day-old ducklings, which are highly susceptible to this virus. And then you raise them up to adulthood, and then you basically probe their livers with DNA blots, and it's very sensitive. If you have infectious agent, it's going to replicate, you're going to pick it up in the liver blot.

DHBV is very hard to inactivate. It's a small genome, and it's only partially double-stranded. So we think it's a very good model.

Hep C, everybody uses the bovine viral diarrhea virus.

CMV, the human strains that we use are cell-associated, and we can inactivate very high titers, again, in full units of platelet concentrate. This is a cell culture system.

We've also used a murine model with cell-associated. This is an infectivity model in mice where you can actually examine tissue, and you can take a very large number of nude mice and put a lot of infected cells into

them. These are infected splenocytes that come from other donor animals.

You are limited, though, in terms of the types of titers that you can get, and so these are two separate series of experiments where we could get a titer of $10^{3.3}$ and a titer of $10^{5.1}$.

We've looked at other Herpes viruses in the red cell program, Herpes Simplex. This morning people mentioned some other viruses. We've done Epstein-Barr virus. The data are not up here for that, but it's very susceptible, and one obviously can do HHV-6 and 8.

Non-enveloped viruses are a challenge. As I think somebody showed this morning, we've done bluetongue. We've done calici as a model for hepatitis E virus. We came across a very interesting discovery in working with parvo B19. We've been trying to crack this virus in an infectivity assay for about six years, and this is the result of inactivation using an erythroid progenitor infectivity assay.

First of all, it's very hard to get infectious B19 inocula that don't contain IgM because the early IgM inhibits infectivity, and we've literally gone to labs all over the world trying to find early infectious material.

Secondly, you want this to be an erythroid infectivity assay, and primary erythroid cell cultures

don't give you the dynamic range you need. We had to spend a lot of time developing an Elispot assay using differentiated erythroid precursors, and we can now demonstrate greater than 4.2 logs. In 35 percent plasma, which is the suspension media for platelets, the problem is that when you dump platelets inside the assay system, they're toxic to the progenitor cells that you need to assay for the endpoint. So we're still working on that piece of it, but we're very encouraged by this data for parvo B19.

This is an infectivity titer. We believe that for a lot of these pathogens you'll find by genomic equivalence obviously higher levels, but for infectivity, this is what we've been able to at least obtain and demonstrate thus far.

Bacteria for platelets are obviously very important. Now, these represent CFU per mL, but here we have a situation where we are able to do the inactivation treatment within the first 24 hours of inoculum, although generally we do it immediately after we've inoculated the whole container of platelets.

For pathogens that will grow in a platelet product or will remain viable in a platelet product, we store the product post-treatment for five days, and then on the fifth day, we culture the entire bag.

For bacteria that will not grow effectively, we will sample, after the post-inactivation period, because if they're going to die off in the bag, then our controls don't remain viable, and so we have to sample early on, and basically we won't necessarily culture the entire bag, we'll culture anywhere between 10 and 30 mLs, but multiple, but multiple replicates again, and again the lower of the 95-percent confidence interval. Where you saw a number without the greater than sign, it means that we did have a viable, you know, organism left after that period of time.

Now looking at what the inoculum load is, it's 10 to the 5.9 or higher than that because we don't reach the endpoint here. We do think that early on, in blood components, the level of infectivity is somewhere in the range of 10 to the 1 to 10 to the 2 CFU per mL. So we think there's a good margin of safety here, but we don't know with absolute surety those initial infectious titers.

This is a list of Gram-positive aerobic organisms. The other point that I would make about these is that these strains have all come from the California Department of Health. These are all clinical isolates, not necessarily from transfusion, but from septic events that have killed patients and the inocula have been sent to the Department of Health. These are virulent pathogens in that setting.

How did we select this list? Basically, when we started this work a number of years ago, we read the literature, tried to obtain all of the samples that were published in the literature at the time that we were initiating this work and have continued to try to add to it.

We talked about this just a few minutes ago--Bacillus cereus. This was a spore former. The first time that we did this, we did the inactivation process virtually immediately after adding this inoculate containing the spores to platelet product.

The same type of experiment was actually done in Sweden by Folke Knudsen (ph) and Claus Hirkman (ph) for buffy coat platelets, and we saw pretty much the same thing. We can get 10 to the 3.6 or 10 to the 3.9 in different experiments, but we have residual infectivity when we go out to five days.

We repeated the experiment allowing time to go into vegetative phase, which in our hands took about three hours for everything to convert to the vegetative phase, and then could get to the limit of detection.

This is a very interesting bug, Deinococcus radiodurans, because it has a very effective repair mechanism, and you cannot kill it with gamma radiation, but

with the S-303 technology in red cells, we can take it to the limit of detection.

We became interested most recently in anaerobic bacteria because those are on the lists, and this is an array of various anaerobes. We were inspired by John Barber's case report in the U.K. about two or three years ago about Clostridium perfringens, and this is a spore former. So that was a spore-forming pathogen. Now this was not stored for five full days, and so that experiment, allowing the vegetative phase to occur, needs to be repeated with five full days of storage.

We think that this technology should be very effective against protozoans and have started an array of work a number of years ago in this area.

For platelets, we have completed work in an infectivity assay with T. cruzi. We recently completed work with falciparum. Now these are parasitized red cells at a rate of a parasitemia of about 45 percent, seeded into a full-unit platelet product at a hematocrit of around 0.2 percent. So it is not red enough to interfere with the pathogen and activation process, and that is one important point.

That is hemoglobin will absorb UVA light, and therefore you have to have a platelet product which has a hematocrit or hemoglobin concentration which is below a

certain level, but it is a level at which you visually would notice that the product had a red coloration.

It's highly effective against malaria. These studies are now in progress for the red cell program. We actually did Babesia microti first for red cells, and demonstrated, using a prototype of S-303, that it was highly efficacious. This is actually a golden hamster model, which is a validated model for infectivity.

We just recently completed Leishmania in platelet product and showed good levels of inactivation. This is a cell culture model. So all of these are infectivity models, but only this one is a whole animal model.

We believe that leukocyte inactivation is highly effective. This is inactivation of T-cells using a greater-than-5-log-clonal expansion assay, the same assay that was used by FDA to actually set the guidance for 2,500 centigrades of inactivation data.

We've been able to measure, actually, with radiolabeled compounds, the number of adducts formed per base pairs, both in platelets, plasma and in red cells. And in comparison, gamma irradiation gives you a strand break in every 37,000 base pairs. This is 1 in every 83, 1 in 150 in the red cells.

We've done work to show we can inhibit cytokine synthesis, and we can also, in animal models, prevent transfusion-associated graft versus host disease.

Preclinical safety is a very important issue, and we've conducted a very extensive program looking at the parent compound, S-59. In the red cell program, we don't look at S-303 because it's an unstable compound, but we look at the breakdown product, which is S-300, and we look at the treated red cells.

We obviously look at the platelets pre-photo illumination, post-photo illumination without CAD and after CAD. This is a very large array of studies. The ones that I would really focus on, I think, are reproductive toxicology that includes perinatal studies, where we have not seen any evidence of toxicity in these model systems, and, lastly, carcinogenicity. Because these are nucleic acid-targeted compounds, a number of years ago Tim McCully in our group met with the Carcinogenicity Assessment Committee at FDA and designed an assay system and a program that uses the heterozygote P-53 mouse model with a transfusion for six months looking for induction of neoplasia beyond background.

And those studies have now been completed for platelets, plasma and red cells, showing that these treated products or their breakdown products are not carcinogens in

this assay system. This obviously also is a chronic transfusion experience because these perinatal animals are transfused for up to six months, and then they're sacrificed and examined.

Phototoxicity studies were done using albino animals that were transfused either once or repetitively and then illuminated with intense ultraviolet light to try and induce phototoxicity. This is the only place we've observed a positive in the system where we get up to 1,000 times the clinical exposure one can see--I'm sorry. At 1,000 times the clinical exposure, we have not seen consistent evidence of phototoxicity. So, even at 1,000 times the clinical exposure dose, we haven't consistently seen phototox.

We have done absorption distribution metabolism and excretion studies in all of these systems, and I don't have time to walk through that data, but S-59 has a relatively short half-life of about 40 minutes.

Neoantigenicity is always an issue, and it's one that people have asked us about for a very long period of time. Starting early in the program, we began to develop assay systems to look at this. We've looked at it in a variety of ways. In the pre-clinical phase, we've attempted to immunize animals with components treated with these products and then basically looked for bound

immunoglobulin and looked for altered proteins, and we have not seen any.

In our toxicology studies, where animals have been repeatedly exposed, we have looked, and we have not seen anything. In our clinical studies with ELISA assays, we have not seen evidence of antibodies in chronically transfused patients.

We have done Phase I studies which looked at recovery and survival with an array of components, and I'll really focus on Phase III studies here where we've looked at therapeutic efficacy and safety of single-donor platelets, buffy coats in Europe and then single-donor platelets in Europe as well.

Summarizing our Phase I studies, this is recovery and lifespan data. These are our products here compared to control. This is an array of published studies, and we have significant differences for five-day product, but we also believe that we fall within a range of studies that have been published, similar type of data for lifespan, and you have these slides in your handout.

Our conclusion, from these Phase I and Phase II studies, was that these platelets had viability that was acceptable. They were tolerated, and we did a study, and I think you might hear something about this tomorrow, it shortened long bleeding times.

I want to switch now and talk about Phase III studies, where we basically have done studies to try and mirror the clinical transfusion experience, where we've randomized patients primarily with hematologic neoplasias, taken them through transfusion cycles of either 28 or 56 days, followed them for a year and re-enrolled them in a second cycle when they've needed more, and we've looked at their adverse events, both in the active phase and during a surveillance phase of either 7 or up to 28 days.

In Europe, we've done count increments as the primary endpoint. In the U.S. studies, we've done bleeding. In the U.S., there was a very large study with 645 patients and 4,719 transfusions; in Europe, 103 patients, with 676 transfusions. Cycle II in the U.S. was as big as Cycle I in Europe.

In Europe, the primary endpoint was the 1-hour count increment, and we used a method called longitudinal regression analysis. In the United States, it was a bleeding endpoint, Grade 2 bleeding. This was a study that was designed in consultation with FDA and used a systematic way of assessing bleeding on a daily basis. We also looked at higher grade bleeding, Grade 3 and 4.

Quickly, in the European study, when one looks at count increment as a function of dose, although we had slight differences in doses, we see basically comparable

response to 1-hour count increment. We still have similar data for 24-hour count increments over a range of doses, and these doses vary depending upon which country the study was done in. We operated in four different countries.

In the United States, for the bleeding study, we were able to demonstrate equivalents for the rate of Grade 2 bleeding and also not a difference in Grade 3 and 4 bleeding. Days of Grade 2 bleeding were significantly different, but we had some outliers when we looked at the mean, but when we looked at the median, we did not see a difference. The duration of platelet support was not different among these groups.

Platelet count increments. We do see differences in the 1-hour CCI, both in Cycle 1 and in Cycle 2 and in the count increments, but we think that these are in ranges, and I think the bleeding studies support that they're in ranges to support these patients.

Twenty-four count increments, we also pick up significant differences in count increment, but again we are in ranges that will support these patients.

Platelet transfusion experience. We do see, in the U.S. study, in Cycle 1, a difference in the interval between transfusions. We did not observe this in Cycle 2. There were some dose differences because there is about a 10-percent processing loss, but these are still very robust

doses of platelets, no differences in red cell transfusions.

Adverse events were very high in the patient populations we studied because these are people undergoing chemotherapy and stem cell transplants, but basically no differences across the groups, and we did have a lower rate of acute transfusion reactions.

The red cell system basically is also a plastic container system with a CAD device to bind the S-300 breakdown product. In vitro studies demonstrated for 42 days of storage, no difference at all in the key in vitro parameters. We've done a series of Phase I studies, and as I mentioned, are now in Phase III studies for acute anemia and chronic anemia.

I think the critical data are looking at red cell half-life for 35-day-old stored red cells. We saw no difference at all in the lifespan of these cells measured as half-life.

In recovery, we had a very highly powered study. We exceeded the 75-percent threshold,b ut we did pick up a 2.8-percent difference in 24-hour recovery at the point .05, just at the .05 level. The competence level barely was above zero here.

Two Phase III studies that are currently running--acute transfusion and CABG and chronic transfusion in

patients with sickle cell anemia and thalassemia. This is really looking at efficacy of chronic support. This is largely a safety study.

Our conclusions are that these platelets are effective in treating and preventing bleeding. A pool of buffy coats give comparable count increments, but the single-donor platelets did have lower count increments. We didn't see a difference in immunologic refractoriness. We have detected no antibodies to new antigens, and we saw no evidence of accumulation.

Our conclusions for red cells are that these have adequate viability, repeated transfusions didn't stimulate immune responses, and full-unit transfusions were well-tolerated. The Phase III studies are currently running.

Just to acknowledge a very large number of investigators who have participated in these trials, and I think you for your attention.

[Applause.]

DR. BIANCO: Thank you, Larry. I wish we had a third day of the meeting to really look at all of this data.

I'd like to invite Bernadette Alford, representing Vitex and their technology here at our meeting.

Bernadette?

DR. ALFORD: Thank you very much. I'm Bernadette Alford with VI Technologies, and we are very pleased to have an opportunity to describe our INACTINE technology for you.

One of the aims we have, obviously, for our technology is to address the transfusion safety of red cells using both microbial and immunological risk factors. The INACTINE chemistry is a family of compounds with nucleic acid-targeted and a triggered chemistry.

The compound we specifically selected for study with red blood cells is called INACTINE PEN110, and we're chemical inactivation and red cell purification.

What I hope to be able to deliver to you today in this presentation is an overview of the methods that we're using and the approaches that we are taking to characterize our technology specific to red cells. So I would briefly go through the chemistry of INACTINE, the inactivation of the pathogens and how that's accomplished, the removal of pathogenic impurities, the quality of the red cells will be reviewed, and finally the product safety profile.

This slide probably looks a little familiar for anybody who was here this morning, and Steve Wagner did a beautiful review of our chemistry, so he saved us all probably the time and effort for me to review to review that.

I do want to just point out for you that it is a targeted triggered chemistry, so that it is not active, our compound is not active when it is added to a solution of red cells. It is activated once it seeks out its target, and that target is nucleic acid.

So the process, as many of you may be familiar with, is a three-stop process, where you start with a conventional red cell unit. INACTINE is delivered. It is a solution. The incubation is overnight or really 24 ours. We remove PEN110 and other types of contaminants by an automated cell washing machine, and at that point, we have a transfusion ready, storage ready pathogen-reduced red cell unit.

So the process itself really can be addressed in two parts. The first part is the chemical inactivation of pathogens, and this is viral inactivation that we've studied in enveloped viruses, nonenveloped viruses, cell-associated viruses, as well as latent viruses.

We've studied bacteria as a point of the outgrowth during a 42-day storage, and we've also conducted some experiments with both protozoan inactivation, as well as lymphocyte inactivation.

The second part of this process is the removal by automated washing. And the automated washing was initially developed to remove PEN110 and any potential PEN110 adducts

to extracellular molecules, But we have found, over the last several months and the last year, that in addition there are other benefits from this washing which includes removal of prion proteins, as well as immunoglobulins that are associated potentially with TRALI antibodies.

We have also shown that we can remove cytokines that may be implicated with nonhemolytic transfusion reactions, and finally we have removed many of the different plasma proteins that are associated with allergens.

What I want to be able to describe for you, and there was some discussion this morning, is the approach that we are taking for these virology studies so that you can help us critically to evaluate the way we are conducting the science for many of our different studies. So these are seven of the major points that we are using in our approach to virology.

First, we do maximize the viral spike, and there was some discussion about this earlier, on how to get the highest spoke possible, and we have done some studies in order to achieve this end.

Secondly, of course, we characterize the viral infectivity assay, and we characterize the cytotoxicity, the interference, and of course we determine what the limit of detection is.

We also characterize the kinetics of inactivation, and this was brought up again this morning I think by our European counterparts. In that regard, we do full-unit-scale studies only. We do not do any studies at a reduced scale.

We demonstrate robustness, and we do this by time, temperature and blood preparation, and of course we use PCR inhabitation as supportive data for all of our infectivity studies, and many of these studies are done in collaboration with a variety of external collaborators.

This depicts the list of several of the viruses that we have already shown to be inactivated by INACTINE. The list on your left is of the enveloped viruses. The list on the right includes nonenveloped viruses. In response to Steve Wagner's question I think this morning, yes, we can inactivate some of the Picornaviruses in foot and mouth disease. A virus which we did in collaboration with Fred Brown at Plum Island is an example of this. We are still trying to see if we can also inactivate HIV, and we are working on that. As of yet, we don't have the inactivation that we would desire.

There is also a variety of other viruses that you'll notice. We no longer have just PPV on this list. We have now inactivation of nonenveloped virus for Human B19 that we've done again with a collaborator. So this is,

instead of using the model, we're using the actual viral particle.

This just shows you, just briefly, some of the kinetics. This shows you three of the nonenveloped viruses. This is foot and mouth disease, BTV, and VESV. They are very susceptible to inactivation. There are others that are not as susceptible to INACTINE and require a longer term incubation with the compound.

Again, as I had mentioned previously, we look for both extracellular as well as viruses that have already been integrated into the DNA, and the HIV is a prime example of this, and that is just depicting what I'm speaking to. In this case, this is HIV that is cell-free, deliberately inoculated in red cells after treatment with INACTINE, and there is no recoverable virus at 18 hours, and we're using the U1HIV model as a cell-associated HIV, and similarly we have no recoverable virus, and in this case after six hours.

We recently have concluded a series of different experiments on Plum Island with Fred Brown. One of them is involving West Nile Virus. If, in fact, this becomes one of the future emerging pathogens that may affect red cells, we really don't know. The point that we are depicting here is that there is an inactivation of this virus. We know that the virus can't stably survive in a unit of red cells

when it is stored out in the cold, and the inactivation of this is very quick. It's within about 15 minutes.

We took a similar approach to our bacterial studies, and in these studies we're looking at the outgrowth of bacteria over a 42-day storage period. We used a panel of both Gram-positive and Gram-negative bacteria. It's a culture endpoint analysis, and again it's full-unit studies.

We also are using large-volume testing, and we use both clinical and ATCC isolates for these studies. This is a list of some of the Gram-negative and Gram-positive bacteria. We anticipate that we will be adding to this list in the months to come. This is just an example of the bacterial outgrowth studies, and in this case I'm depicting Yersinia enterocolitica as an example.

The red line is the control. The INACTINE treat is blue. Both of the units are spiked prior to storage. The control is stored under normal conditions, refrigerated for 42 days. The INACTINE is treated and then stored, and you will notice that there is no outgrowth during this time period.

For the chemical and activation studies, we have devoted efforts to three parasites, and those are the parasites that cause malaria, Babesiosis and Chagas disease.

As an example, I'm showing you some of the results with the inactivation of T. cruzi, which is the causative agents of Chagas disease.

This is a dose response of INACTINE after 24 hours of incubation. I should remind you that the standard concentration that we use of INACTINE is a .1-percent volume-to-volume in a red cell unit. It is not surprising that the larger the genome the less requirement you would either have for the concentration of INACTINE or in the time period. So, in this case, you'll notice that we get the full inactivation of T. cruzi even with a half of that concentration of .05.

Probably the best example of an inactivation would be an in vivo bioassay, and we have done this study with an investigator at Tufts University. And this is the survival mice that have been transfused with T. cruzi-infected blood. The control is shown in green this time. The INACTINE-treated is the blue line. And this is INACTINE treatment of T. cruzi-infected blood for just three hours, using our standard .1-percent concentration.

You'll notice within 44 days all of the animals have died in the control group and all of them have survived in the treated group. In addition, we did look for parasitemia, and you will note that even after just 24 days in the control group, the level of parasitemia is

greater than 8 times 10 to the seventh cells per mL, and in the control there is no parasitemia.

In addition, we also have a specific approach to lymphocyte inactivation, and the effect of the INACTINE PEN110 on lymphocytes, and we did a dual approach. One was studying graft versus host disease and the second was studying alloimmunization.

In graft versus host disease, much of this work was conducted with Loren Fast at Brown University, where he has some beautiful in vitro models to show inhibition of lymphocyte proliferation, and they include mitogen and MLC assays, as well as cytokine inhibition.

We also went forward with him on some in vivo GVHD mouse modeling, lymphocyte survival in mice, and then finally a SCID mouse study that was also done in conjunction with Loren Fast at Brown and John Semple in Canada. Much of this work will be published in the Transfusion issue in October, as well as discussed in the upcoming AABB meeting.

At the same token, we've done a variety of studies to address alloimmunization, and that was an MLC in vitro assay, again, with Loren Fast, as well as antibody response in mouse models.

This is just quickly showing you some of the data that has come out from these studies. This is a comparison

of PEN110 and gamma irradiation on PHA mitogen-induced cell proliferation. You will note that we have a similar response with PEN110-treated and gamma irradiated versus the untreated control.

This is the effect on MCL assays with the treatment of responder cells and is a very similar effect of PEN110 and gamma irradiated responders. What is very interesting to note is that we have still stimulator cells in the gamma irradiated cells, as opposed to the INACTINE treated, where there is no PEN110 stimulators available.

This is a result of the in vivo study in mice demonstrating that INACTINE presents graft versus host disease. In the top line, there's a note treatment control, and notice the spleen weight, and there's a standard very low level of cytolytic activity.

When you use untreated lymphocytes from Strain 1 into Strain 2, there is, as you would anticipate, a major increase in that spleen weight, with a similar increase in cytolytic activity. But when you INACTINE treat the lymphocytes from Strain 1 and inject them into Strain 2, you have a normal spleen weight and the cytolytic activity is as normal.

The second part of our INACTINE process that I alluded to before was the contaminated removal by automated washing, and this was done, as I said, originally, for

PEN110 removal, as well as potential PEN110 adducts to extracellular molecules, as well as prion protein removal, immunoglobulin, cytokines and even plasma proteins.

This slide shows some of the results of the clearance of albumin and prion proteins from red blood cells by the INACTINE process. We're using albumin here as a marker, and it does track with the removal of prion proteins. And in this case, we have used the spikes derived from platelets, as well as from the recombinant forms of both alpha and beta form, and the beta is thought to be maybe very closely associated with what the pathogenic prion protein may look like.

This is the maximum that we were able to spike into our process, and you'll note that in all cases, they were greater than 3.4 log removals. It was really to the level of the sensitivity of a very sensitive DELFIA assay that was developed initially at the Institute of Animal Health that we are using internally at Vitex.

In similar fashion, using the automated washing process, which is the end of our three-step process, we did clearance studies with immunoglobulins that are thought to be associated with other types of contaminants in the red cells. In this case, a control unit of red cells has near 10 mgs per mL of IGG, but the clearance is almost at 5 logs. We're down to a level of about 140 nanograms per mL.

Now with that both removal, if you will, as well as inactivation, there is always a question on what does the quality of the red cell look like and what is that red cell physiology. So we've done extensive studies to characterize the metabiologic integrity, the physical integrity, which includes both morphology, osmotic fragility, ectocytometric assessments. We've used assimilated extracorporeal circuit to do a study. We've characterized the oxygen binding. We've done phenotypic analysis of red cell antigens with or without storage. We've also compared in all cases licensed units and gamma irradiated units, and we've always done our studies with full units, following 42 days of storage.

I will just briefly show you a few of these studies. This is a red cell morphology that was done in conjunction with the director of Hematology at Mass General Hospital. He could not find any differences in the morphology from control, sham control, INACTINE treated over the whole 42-day period.

These are standard osmotic fragility assessments. Again, there is no statistic difference between untreated, INACTINE treated nor fresh cells.

This is the results of one of the experiments that we've done with Dr. Frans Kuypers at the Oakland Research Institute on ectocytometric assessment to look at

deformability index. Again, not only are the treated and the control cells similar, they are identical.

And then finally we've done a series of experiments with the Doctors Renders at Yale University on their extracorporeal circuitry to see if the cells are more fragile following treatment. And this is looking for hemoglobin levels to see if there's any increase in hemolysis, and over this 90-minute period, there is no difference between INACTINE treated, which is the purple and the last which is the control.

We also looked at older age cells, and these are cells that are stored for 35 days. Of course, our process is storage of cells for 42 days, but following a 35-day storage, there have been experiments done with 35-day cells using a solution called Rejuvasol to see that there is an appropriate metabolic response.

We have used Rejuvasol on the control and the INACTINE-treated cells. So this is, in both cases, a 35-day storage cell, either the control or INACTINE treated. We've added Rejuvasol to both at this 35-day point, and you will note in both cases, the 23DPG (ph), the ATP, as well as PIVI 50 (ph) is returned to normal both for the control, as well as for the INACTINE treated, to demonstrate the metabolic responsiveness of our cells.

So, with this, we led to in vivo studies to assess the red blood cell quality. The first major study we did was a primate study with Bob Valari (ph) at the Naval Research Center, and that was in his famous primate model involving a baboon, following a very successful result that showed no difference between the baboon INACTINE-treated cells or the control.

We moved onto a Phase I study to see survival and half-life, a Phase II to really look for full-unit safety, as well as to define our maximum storage in our process, and we have Phase III studies ongoing.

The Phase I study was the first time that INACTINE-treated cells were put into man. So that was done with a 10 mL alloquat, and this study was done at Dartmouth under the auspices and direction of Jim AuBuchon.

The cells were treated at six hours because that's where the science had led us at this time, and we followed the cells for both the 24-hour survival, as well as the 20-day storage. There were no safety effects whatsoever, and there was a standard 24-hour recovery and half-life of the cells, and they weren't affected after storage for 28 days with INACTINE.

We then went on to a Phase II study, and in the Phase II study the science had directed us further. The science said that there is many viruses that need a longer

period of exposure to a chemical like ours because, as was suggested this morning, there are some viral inactivation series that are really two phase. It appears there may be a subpopulation, a subpopulation of cells that take a longer time for that exposure to occur. So, for that reason, we do have a longer exposure to make sure that we are covering potentially every type of pathogen that we're looking at.

And, also, which I'm sorry I didn't speak to before, when we talk about a, and refer to a log, it's always a log removal per mL. It is not a total. So I think Harvey Klein asked that earlier, and every time we do address a log it is on a per mL basis.

So, in this Phase II study, we were looking at INACTINE-treated cells for 24 hours. It was a randomized parallel unpaired controlled study, and in fact, 50 percent of each group, and there are three cohorts. The cohorts represented a period of INACTINE-treated and control cells that were stored for 42 days, another for 35, and finally anticipate storage of a cohort for 28 days.

And half of each group also received a full unit of INACTINE-treated red cells or controls for safety. There was a variety of secondary endpoints. They addressed mainly safety parameters, as you'll note. The primary

endpoint was the maximum storage time, looking at a 24-hour recovery, as well as the level of hemolysis.

This just depicts the 24-hour recovery of Phase I, 28-day, which is the first panel on your left, and the other two are the two cohorts, the 35-day storage cohort and the 42-day storage cohort from the Phase II study. We did not go on for a 28-day because it was obvious that the cells could be stored for either 42 or 35 days.

This is a result of the survival. Although there was no statistical difference between any of the specific time points, the slopes of the lines are different, and there's about a 30-percent difference between these two. So this may suggest that there might be a difference in survival, and for that reason we had carried out a variety of different what we call red cell quality experiments that I've shown you in physiology, and again additional studies will be carried out, as well as will be addressed as we continue with our Phase III program.

So the conclusion of the Phase II study was there was no safety-related effects, there was no evidence of neoantigens, no change in phenotypes, and the primary endpoints suggested that, based on our recovery and hemolysis, that the processing storage period is 42 days.

So, with that, we started our Phase III trial program which has two studies. This has recently been

agreed to, with the design by the agency, and one study involves patients that require acute transfusion support, and that would be about 200 patients. The second is a study with patients requiring chronic transfusion support, and that's 70 patients.

They are both designed as multi-center, double blind, and parallel group studies, and both studies will use the current maximum storage period for red cells of 42 days, and it will be a 24-hour treatment.

Now, in the last couple of minutes, I would like to just address for you briefly the toxicology and our approach to the product safety assessment, and this is to identify any potential adverse effects. We want to be able to understand exposure, and that's exposure to PEN110, versus the dose response relationships. With this, we could potentially predict what the safety parameter would be in man, and that would be our risk assessment.

So, for the safety assessment, we've done a variety of different studies. We've completed genotoxicology, neoantigenicity acute studies, subchronic, a reproductive toxicology study, a fertility study, and we have an ongoing carcinogenicity study currently.

I just want to show you, just briefly, a couple of these studies. We've done a neoantigenicity assessment of PEN110-treated red cells in rabbits, and this was either

PEN110- treated or control red cells that have been fused with about 5 mLs per kg. In this case, there were three doses, followed later with what would amount to be potentially a boost, and there was a bleed at Day 105, and there was no indication of any neoantigen that's formed. In fact, these cells were PEN110 treated, and the PEN110 was not removed prior to infusion in the animals.

This is the results of our, and the design, of our reproductivity toxicity study in rabbits. This was daily PEN110 IV doses between Days 7 and 21. This is the period of organogenesis for the rabbit, and the doses varied obviously from a control to 1 mg per kg.

It shows what the equivalent may be on a red-blood-cell basis, and this would be a red blood cell that we have gone through the process, and it has been washed to the level of 50 nanograms per mL, and that's the residual level of PEN110. So, if there was a residual level of 50 nanograms per mL in one unit, then that dose would represent, for instance, .1 would represent, at .1 mg per kg, 400 units, and that's how the study was determined.

These are results of that study. All of the doses were negative for fetal toxicity at Day 29, and so it is shown for, at these dose levels in the rabbit, the PEN110 is not teratogenic.

We did a similar study with fertility and early embryonic development that we just recently completed. In this case, the males were dosed every other day for four weeks, the females every other day for two, and then they were mated. Dosing continued, and you could see there was a variety of different assessments, both done on the male, the maternal data, as well as uterine data.

In this case, the dose levels were zero to, again, about .5 mgs per kg. There's an equivalent dose on the side, as I explained previously, and we have seen no embryonic toxicity or effect on fertility in any of these levels. This has just been recently completed.

As I said, our carcinogenicity study is in progress. So we feel, today, that we have a technology that represents a promising approach to pathogen-reduced red blood cell concentrates. Our Phase III studies are ongoing. We have just started our, and have a view of what our characterization would look like, and many more studies will be completed within the following several months.

Thank you so much for your time.

[Applause.]

DR. BIANCO: Thank you very much, Bernadette.

I think that we will try to see if we have a few minutes at the end for questions from the audience. And as

we do the technical changes here, I would like to invite Ray Goodrich from Gambro to talk to us about riboflavin.

Ray?

DR. GOODRICH: Thank you very much.

I'd like to thank the organizers of this session for allowing us to have the opportunity to come here and talk about our technology today. There are disadvantages to going last. There are also several advantages. Part of that is getting the wisdom of everyone who has gone before you; the other is that everyone is tired, and they want to go home. I don't know if I count that directly an advantage or a disadvantage.

It was not very long ago that this community was posing the question is it possible to have a zero-risk blood supply? I think that the answer to that question, at that point in time, was a definite no several years ago--perhaps more than I'd like to admit.

It's important I think then to realize that today, after the passage of time, after the expenditures of considerable amounts of money and considerable intellectual power on the part of many individuals working in this field, the answer to that question is still no.

I think perhaps what we're experiencing here today is that we're changing what that question really needs to be or should be or is, and that is can we attain a

safer blood supply? Can we attain a safer product? And there are a lot of ramifications that come out from that, including some of the issues that Steve Wagner mentioned earlier today.

What I'm going to try to do is to talk to you about some of the considerations and where we are in our attempt to move towards a safer blood product.

We've looked at this from the standpoint of designing a pathogen reduction technology development program, and that involves really assessing what the need is. We've heard a lot of talk here today about what the need is and what the performance levels need to be for these products. That's very important. That, hopefully, allows you to identify a balanced pathogen reduction chemistry or process that involves both getting effective kill, acceptable toxicity profile and acceptable blood-component quality. I think, hopefully, what that translates into is the design of a practical process. That's something that we haven't spent a lot of time on today, but I think it's a very important issue.

In assessing the risk, this is our score sheet. We look at bacterial reduction leading to a potential for a significant drop in morbidity and mortality as being a real potential positive of this technology or these technologies.

An incremental risk reduction for window period virus carriers, that might be possible, might be achievable.

Parasite reduction, particularly in the case of red cells, which might offer an important opportunity to improve red cell safety and availability; speaking, for example, in particular, in a case of malaria.

We might be able to eliminate or reduce the effects that are associated with white cell contaminants in platelets, plasma and red cells. That's also a potential positive.

I think where we have real question marks are in talking about viral titers of untested individuals for viruses that we don't test today. It's possible, in some of the presentations you saw earlier today, for example, some of the data that Mike Busch presented, that you can exceeding load the ability of pathogen-reduction technologies to remove or inactivate these levels. I think the questions there are is some reduction better than none, and what is the magnitude of viral reduction that should equal or exceed those found at various points in individual donors post-infection?

I think those are the kinds of studies that would need to be done in order to make these kinds of claims.

Another question mark, a big question mark, is with regard to prions. We heard earlier today about understanding whether or not these agents are even transmitted by blood. So I don't think we could say with certainty, if we don't know that it's transmitted by blood, that something that we do will eliminate its transmission by blood.

I think there is a lot more that needs to be evaluated here with regard to the passage and the transmission of these agents. Many of you go to the same sessions that I go to--people like Bob Rohr and Paul Brown who are looking at these kinds of issues.

This is what we're using as our photosensitizer. It's riboflavin, Vitamin B2. It is an essential nutrient. There is a recommended daily allowance. I'll be quick to point, however, that this is not what you would receive after a product has been treated. You receive both residual riboflavin and riboflavin photo product. It does break down. It does do chemistry when it's exposed to light, in both divisible and UV regions.

We were attracted to this, however, because of the fact that it does exist naturally, and there is a wide body of literature that's available on the metabolism and the action of this compound in the body--many of the same studies that Steve Wagner pointed out today, looking at

adduct formation, looking at the clearance of this metabolically in the body. Not all issues are known, obviously, but at least there's a starting point. That's what we operated from to begin with.

This is a cartoon, basically, that describes the chemistry, and I think it's important to call this a cartoon because it's a reflection of real life. It's not necessarily real life.

It's known that riboflavin interacts with nucleic acids, that it carries out oxidation chemistry, as well as electron transfer chemistry when it's excited with either visible or ultraviolet light, and that that results in things such as DNA breaks, covalent adducts, and changes to the DNA or RNA structures, which we believe lead to pathogen inactivation. There have been several descriptions of this in the literature, and you saw the citation earlier today.

We've talked about processes that involved activation of riboflavin, using both UV and visible light. What we've found is that there are pluses and minuses to both of these approaches and the action of the chemistry that occurs based on the activation light that's being used.

UV and visible light is really separated by a break at about 400 nanometers. When you're above 400,

you're in the visible region; when you're below 400, you're in the UV region. For platelets and plasma, I'll be talking about processes today that relate to using UV light activation. For red cells, I'll be talking about visible light activation.

These are the conditions for all of the data that I'm going to present, both in terms of cell protein quality and performance. It's five joules per centimeter squared of light dose, UV light; 278 mLs of product volume for platelets and plasma; 50 micromolar riboflavin; there's a 90-percent plasma carryover in 10 percent of the solution of riboflavin that's added to the product.

This system does have one-bag transfer step. It's a prototype system. Our goal is to get this into a single container for treatment.

In red blood cells, we're using a light dose of 175 joules per centimeter squared. It's visible light; 500 micromolar riboflavin stock solution; 30- to 40-percent hematocrit; and there is, again, one bag-transfer that's involved in this prototype system.

What are the measurement parameters that we're using as a goal to guide our development efforts? Well, it has to be effective, obviously, in reducing bacteria, viruses, parasites, white cells. It should be safe to avoid toxicity to both patients and handlers. Cell and

protein quality, we'd like to know if they're being preserved, their functionality, and we also feel that it has to be something simple or practical; that it can be something easily practiced in a blood center component laboratory. That's an engineering challenge.

So how about effectiveness? I'm going to be very clear on how we do these studies. Our studies conform with the 1995 revised CPMP guidelines for virus validation studies. We do this both for virus and for bacteria with respect to the following: the validation process, the choice of viruses for validation, the design of validation studies, and the interpretation of data.

Don't repeat this, but I agree with Larry Corash on many of the points regarding these types of assays.

I think that it's very important to take these into consideration because it is a very complex issue in terms of the design of these studies. These are borrowed guidelines from CPMP that relate primarily to processes for inactivation of biologically derived products from human or cell-line origin. They don't apply directly to cell products, but we're applying them so that we have some guidance.

So what does that mean when we say we follow these methods? Well, you take a stock titer of pathogen, and you put it into the product. You then measure the

titer of that pathogen in the spiked product. There is no estimation involved. The reason for that is what I think you heard earlier today, that you can have neutralizing antibodies or other things not directly related to the process that might affect the viral titers from the initial step.

You can see differences of 1.5- to 2-log differences just based on an estimated versus an actually observed value in these assay systems. That measurement is usually made, and it's made in our case, with the TCID 50 assay for infectivity, and the values are reported as logs per mL.

We perform a treatment step, monitor kinetics based on energy dose delivery, remove alloquats at each dose, and measure the titer of pathogens in the alloquat in logs per mL. So the reduction is the starting titer that's measured minus the final titer in that product that's measured at each of the time points.

The idea here is really to find an upper limit of the initial challenge where the system is no longer negative to assay detection limits. That gives you the window where your process is going to be effective. What is the challenge, and how well do you perform, and how far are you above those limits?

For bacteria, with the platelet process, we've looked at several contaminants. You've heard these lists that have been described earlier today: Staph epidermidis, Staph aureus, E. coli, Klebsiella, Bacillus cereus. All are somewhat antibiotic resistant, all commonly found in hospital-related infections, and we've looked at these under two sets of conditions--what we call high-spike titers, where we put in greater than 6 logs per mL and at low titers, where we've put in 1 to 2 logs per mL, and then monitored by BacT/ALERT over 10 days of storage for the low spike and immediately post-treatment for the high-spike samples.

Now I asked this question earlier because I put this line in here saying that normal titers at donation are probably much less than 1 log per mL. I throw that open for discussion.

This is what we found: For Staph epidermidis, Staph aureus, Klebsiella pneumoniae, E. coli, and Bacillus cereus, to the levels that we've been able to detect in these systems, these are the log reductions in logs per mL for each of the species that we've looked at.

In the low-spike samples that we have treated with these products, again at initial spikes of 1 to 2 logs per mL, the samples remain culture negative after 10 days of storage post-treatment. That basically means that we

take the samples and store them for five days. We then remove a 4 mL alloquat, put it into the BCT culture system and monitor for an additional five days.

These are the reductions that we've seen with the red cell process for bacteria:

We've spiked with Yersinia and Pseudomonas fluorescence. You'll notice that there aren't greater than signs on this data. We've been able to inactivate and reduce the levels of bacteria to 2.6 logs per mL and 2.7 logs per mL for these pathogens. Above these levels you begin to see positives or breakthroughs. So there is a more limited reduction in kill in the case of the visible light-driven process with red cells.

These are the values that we've observed with our platelet and plasma process:

We've looked at BVDV, Pseudorabies, HIV, IBR, which is another herpes virus. It's Infectious Bovine Rhinotracheitis, and VSV, envelope viruses.

In the case of porcine parvovirus, canine parvovirus, those are nonenvelope viruses. These are the inactivation levels that we've been able to observe. If you don't see a greater than sign in front of the data, it means that we haven't, at those levels, inactivated to the 95-percent confidence levels above those limits, as Dr. Corash described earlier.

This is the kinetic. These are three different runs that are done three different days with three different products, using this process, and this is porcine parvovirus inactivation kinetics. The results are shown for the three different runs and also describe for the average of those three runs. Again, where the line flattens out, it's basically reaching the limit of detection for the assay system red cell process.

We've looked at BVDV, Pseudorabies, HIV, IBR as envelope viruses and Canine parvovirus and Blue Tongue Virus, nonenvelope viruses. Again, in this case, we've been able to inactivate to limits of detection within the spike titers that we can achieve with these products, in the case of envelope viruses, and with nonenvelope viruses, that reduction is limited to the values that you see here. So there is a lower efficacy with regard to visible light inactivation for nonenvelope viruses with the system.

We've also looked at malaria parasite in red cells. This is some data that's going to be presented at the upcoming ISPT meeting by our collaborators at Walter Reed. What this data shows, basically, is a measurement using parasitic lactate dehydrogenase. I think earlier today Dr. Leiby talked about the difficulty of measuring inactivation levels in these types of products. These are actually malaria-infected red cells that are spiked into a

product, it's treated, and then we take out alloquats at various times and look at the level of expression of this parasitic LDH enzyme, as well as the percentage of ring-stage parasitemia that's present in the products. That's by conventional smear techniques.

The LDH enzyme assay is a measure of metabolic activity. It's a lagging indicator. What you see is that in cases where we've exposed to different levels of light with red cells, we're inactivating to the point where there's no detectable, there's a decrease in the amount of the activity of this assay, and it declines to baseline levels, basically.

In the case of just adding riboflavin, there is a drop in the amount of expression of this enzyme, but then, after storage, for prolonged periods of time, you see these levels go back up--meaning that you haven't completely inactivated the parasite that's present in these red cells. New cells are becoming infected.

This result is actually described in a paper that came out of Northwestern Medical School, looking at riboflavin in the absence of light and its ability to suppress malaria parasite replication processes. This is the citation for that article.

We have done studies with leukocytes. I've just summarized these here. We've looked at, for both the

platelet and red cell process, the effects of riboflavin, plus light, on cytokine production. This is IL2 out of jurkat cells. It prevents cytokine production under these treatment conditions. We've looked at white cell replication using oxygen biosensor data on treated products, and we've also looked at DNA fragmentation patterns using a fluorescence assay for looking at strand breaks after treatment. Hopefully, we'll be able to present some of this data in greater detail in some of the upcoming hematology meetings.

Safety profile of riboflavin. We've carried out these tests in order to support a Phase I study with platelets and with red cells. We have done this with starting product and photo product, an Ames test on the starting material and photo product and on native lumichrome, which is the photo product that forms upon exposure of riboflavin to light. All have been negative.

This is the platelet and plasma process. The mouse micronucleus and chromosome aberration tests are ongoing as we speak. Cytotoxicity is negative. Acute tox in a rodent model, in rats, is negative. Neoantigenicity, which was performed using both the opterlani (ph) method and an ELISA assay, is also negative for this process. We also have studies that are ongoing in dogs to look at the acute toxicity profile.

With the visible light, the red cell process, we have conducted studies looking at Ames test on all materials. All have been negative. The mouse micronucleus test is negative; the chromosome aberration test is negative; acute toxicity in both rats and dogs is negative; cytotoxicity is negative; and neoantigenicity is also negative.

We have used the standard kinds of measurements--I think we'll probably hear a little bit more about these in the session tomorrow--to look at products before and after treatment and during storage.

What I could tell you is that there are differences. We see differences in pH, which is primarily driven by differences in metabolic activity post-treatment.

Morphology scores. Percent HSR, there are some declines. ATP levels show a difference, and the key is what do these differences translate to in an in vivo setting, and that's part of what the clinical trials are meant obviously to answer.

We have also gone one step forward, however, of doing studies in primates using treated cells to get a read ahead of time. We've done that both with UV invisible light, in the case of platelets. We find normal recoveries in the range of 60 to 75 percent and lifespans on the order

of 80 to 100 hours in these animals after treatment and reinfusion of full products.

We have also seen, with infusion of full products, no adverse clinical chemistries or reactions.

We are also carrying out platelet function studies. We found these models to be of particular value in helping guide our development efforts. We have used Dr. Blajchman's bleeding time model in rabbits and Dr. Escolar's method, which primarily involves a Baumgartner profusion model for platelets to look at the effects of treatment on cell quality and cell performance.

For red cells, we followed the standard types of parameters again out to Day 35. We have Day 42 studies that are ongoing. We've looked at hemoglobin content, methemoglobin production, osmotic fragility levels, direct antiglobulin tests during storage at various points of time, up to, and including, Day 35.

There are some differences between treated and controls, and, obviously, again the question is how do those translate to in vivo performance.

Plasma proteins. We followed after-treatment levels of fibrinogen, Factor 2, 5, 8, 10, Antithrombin 3, Protein S, Protein C, and looked at antibody levels such as for tetanus and pneumococcal antibodies for functionality, case of IGGs. These are values that we have observed after

treatment of our product, storage under standard conditions.

So what are the next steps, obviously, for us in a clinical program?

Well, our plan is to initiate these studies with platelets, to be followed by a red cell program. The platelet studies initially would be in vivo radiolabeled survival, red cells as well; two centers, 10 subjects per center; normal volunteers; measuring both recovery and lifespan of treated cells.

Obviously, the pivotal studies with platelets are likely to involve efficacy studies with regard to reduction in bleeding events. Those are broad enrollment, based on multiple underlying morbidities, which means that you are overpowered in some cases and underpowered in others. I think the format for this has pretty much been outlined, again, as I mentioned earlier, by those who have gone before us in many cases.

Finally, I think what has to happen here is, taking these performance values that we're able to achieve, and translate this into a system, again, that's a practical process and what this might look like. How will blood banks do this routinely, and reproducibly and reliably?

As I said, this is an engineering challenge that we take into consideration, as we're moving forward with

our program, equally, along with the performance values that we're able to attain. I think it's a very important factor if this is going to be used in the types of products and over the range of products that these systems are being proposed for.

I'd be happy to answer questions if I can.

[Applause.]

DR. BIANCO: We can have a couple of questions. I would ask that Dr. Corash, Bernadette Alford and Ray to maybe sit at the table, and we can have five or ten minutes of questions.

I would just like to take the prerogative of presenter or MC, the role I played today, just to comment that there is one aspect that we have heard very little about the process; that is, what is the impact that those technologies will have in blood centers and collecting facilities. What it appears, from what we saw, and from what we have learned from the manufacturers, and actually Ray has tried to address this very much in his last slide, is the change from something that is very manual, primitive, preparation of luck by lot, 13/14 million times a year in this country, plus the components, to an industrial process that will still have 14 million lots, but much more complex, and that will have a tremendous impact.

So I would like maybe tomorrow or in the next workshops that besides addressing the scientific issues of toxicity and all of that, that we examine how we're going to adapt or help an entire system adapt to a technology of this type.

Questions?

DR. MARTINEZ: Bill Martinez, Gainesville, Florida.

Perhaps I should save this question for tomorrow, but I was thinking is anyone going to do clinical studies to prove this lack of toxicity in the pediatric patients and neonates?

DR. BIANCO: Well, you can choose Larry to the microphone first.

DR. CORASH: Yes, I think there are two ways to approach that.

In our Phase III clinical trial in the United States, we did enroll children in the platelet study down to the age of six years. In our plasma program, we have transfused a few very young patients--I think one patient below the age of one year.

It's very hard to conduct well-sized clinical studies in those patient populations, and I think what one gathers largely is, you know, anecdotal almost type of experience because the numbers are small. I think in our

Phase III clinical trial, we have 30 or 40 pediatric patients with a fair number of transfusions.

One depends heavily on preclinical safety studies, where you can do focus studies on obviously pregnant animals through the perinatal period and young animals, where you raise them up to adulthood and look at the effects. I think that you have to depend for a lot of information from those types of studies.

In our red cell program, we are enrolling pediatric patients, but again not neonates because you don't find a lot of neonates in chronic transfusion studies. So we are enrolling some children, but not neonatal patients.

DR. BIANCO: I think that Bernadette had something to say.

DR. ALFORD: Yes, thank you.

For our chronic study, also, where we will be enrolling patients as early as eight years old, the neonate population is a very important population for us to try to address. I think between a discussion with our Advisory Board that we have at our company, as well as ongoing discussions that we have to have with the FDA, we'd like to be able to design a program to address specifically that patient population.

Granted, animals provide a lot of information and potentially guidance where we could go clinically, but I think in the coming year we'd like to see if there was some way to specifically address that population with a pathogen-reduction process that would involve INACTINE.

DR. BIANCO: Ray, do you have anything you want to say?

DR. GOODRICH: [Off microphone.] [Inaudible.]

DR. BIANCO: Can you say it a little bit louder?

DR. GOODRICH: [Off microphone.] I said I saw Bernie at the microphone there, and I know this question came up at one point with S-59(?) positive years ago. Maybe he could share what some of these issues were and actions at that time.

DR. HOROWITZ: That's actually not why I came to the microphone, but as best my memory serves, we also had very few young, especially very young patients, in the trial. The few that we had, there were no adverse findings, but ultimately these are very difficult patient populations to study, and at the same time, post-licensure, the questions about neonates didn't disappear, and then there were ongoing studies that individual investigators conducted probably with some trepidation as they put a product, which had a lot of human-use history, never mind just the preclinical history, but a lot of human use

history behind it, and still approached those patients with some degree of trepidation.

So I'm not sure I can add a lot to that. Maybe if Joan Pehta is in the audience, she could add more. She was more involved in the clinical side than I was. I don't know if she's still here or not.

DR. BIANCO: Joan, if you could use the microphone.

DR. PEHTA: When we did studies on congenital factor-deficient patients, about half the study was in patients who were under the age of 18. Neonates, there were about four neonates, I think, that were studied in total out of the 150 subjects that the license is based on.

DR. BIANCO: Bernie, you had a question? Dr. Horowitz.

DR. HOROWITZ: I wanted to return, Celso, to your comment, and the same comment was made this morning, although there was no follow-through with respect to both engineering aspects and process control, and both elements are important.

In a plasma fractionation environment, when you're using a chemical or if you're using a heat methodology where, for instance, moisture is important, on every lot you measure how much chemical you've added to make sure that the appropriate amount was added or, if it

was moisture, what the moisture content was, and then following execution at the removal step, you measure to show that the removal was effective.

Here, we have, as Celso put it, more than 12 million lots of product that are going to be made in the United States. I'm wondering, as my first question, how this is being addressed or how it's envisioned to be addressed following licensure; what type of statistical sampling, if any, is going to occur or will we rely solely on validation?

DR. ALFORD: Maybe I could try that, Celso.

I can't address plasma, as you know, Bernie, but I could maybe just address how we're going to handle, from a red cell perspective, that we will be testing each individual lot, and each individual lot is each unit. So we, of course, will do a process validation, which is standard for any GNP environment, but in addition to that, we would test and release on a unit-by-unit basis.

DR. HOROWITZ: That's more than 12 million tests.

DR. ALFORD: No, to begin with. I think, as you said was how would you start, what statistics would you use. And I think, even with our discussion with the Agency, until we gain appropriate experience that we feel, and the FDA would feel, would be appropriate, at first you'd want to test for a while each unit.

I don't know what that number is, Bernie. I don't know what that number is that we would feel comfortable, someone like yourself would feel comfortable, the rest of the industry, the blood centers would feel comfortable with, as well as the medical and the patient community. Until we could determine what that number is, I would suggest that we'll probably, for a bit, test every single unit.

DR. HOROWITZ: Larry?

DR. BIANCO: Larry?

DR. CORASH: I can speak to the process for platelets because that's what's being introduced into European practice.

The way the system has been set up, it is to basically do process validation, collect information on the handling of every unit. So you have amatocillin (ph) pods, which are obviously a manufactured product, and you have a known and calibrated dose that's in those units. The entire platelet unit is passed through that, and then you record basically the process as you go through the various steps.

The light device records the delivery of the dose of light. It has sensors built into it, and it records for every unit the dose of light that is administered to the product.

There has been process validation, and each of the centers that are going through adoption of the process go through process validation steps to show that they are within certain ranges. Of course, we have characterized the product to show that after, for example, the compound absorption device, there is, on average, less than 0.5 micromolar of S-59 left in the product.

So the way the system has been set up is to basically document various steps taking place as you move through the process and to, obviously, have manufactured components that are within very defined specifications and document that the users can create products within the specifications designed for the product.

DR. HOROWITZ: So will there be statistical sampling of final product and, if so, what measurements will be made?

DR. CORASH: There is no plan right now that there would be statistical sampling of the final product, because I think, as you know, with the labile components in blood centers, that's not the way that these products have been characterized up until this point in time.

So what is being done is a characterization of the components of the system and a recording of the actual processing steps. If you do not deliver the correct light dose, you will not be able to release that product.

DR. BIANCO: Dr. Snyder?

DR. SNYDER: Thank you. Ed Snyder from New Haven. This is more of a statement, I guess, to the Agency than a question.

As a director of a transfusion service, what I don't want to find, myself, and I'm sure the rest of the blood bank directors, similarly, do not want to find themselves, with an approved product on the shelf that has had no track record for neonates, for pregnant mothers, for women who don't even know they're pregnant, as far as toxicity issues are concerned.

Why am I concerned? Because, with the economics of the way things are today, just as we had these deja vu discussions about leukoreduction and at what point does a blood center have to convert all of their production to one type of processing because they can't afford to have two types of products available in all of the various flavors, we could find ourselves with only treated pathogen-reduced product on the shelf, with a child who needs something or a pregnant mother who needs something, and for which there's been no data derived.

So, although I don't expect an answer, I think it's something that should be considered as we look. Some people drive down the highway looking right over the edge

of their car hood, I look way, way down when I drive, it turns out.

So that's what I'm doing now. I'm looking for a time, in perhaps a year or two, when there may be nothing on the shelf but treated product, in a specific type, because that's all the blood center has, and what do I with it? Do we get informed consent? Do we not? These are issues that I think need to be looked at and some answers developed as we move ahead.

Getting back to what Harvey Alter talked about, getting off the dime, there are few things that we have to do, I agree with him, but there are other things that we have to look at as well.

DR. BIANCO: You said you don't expect an answer, but you expect an answer; is that correct?

[Laughter.]

DR. SNYDER: No, just a nod will be fine.

DR. BIANCO: Thank you, Ed.

DR. CORASH: If I could just make one point, in the CE mark approval of the platelet system, there are no patient population restrictions. The preclinical safety studies that were done in those targeted populations were felt to be sufficient to not have patient population restrictions.

Now think about, I mean, you've been engaged in clinical trials with us, what you're interested in are safety issues with your adverse events. So think about how many patients you would have to enroll and what type of exposures you would have to give those patients that are either pregnant or neonates and ask the question whether or not those studies could actually be accomplished. I don't think they can, in fact.

DR. SNYDER: No, I understand the problems in human studies. So it may be that we'd have to look more closely at animal studies, and maybe there's sufficient data. I'm not the person to evaluate the toxicologic evaluations, but we do have pediatricians at our institution who still do not want additive solution given to neonates. They want us to spin it and remove it or wash the cells---we do have our limits to what we'll do--but they are still concerned about the adenine stones that those mice, lo all of those many years ago, developed when 90 percent of their diet was adenine. It's hard to get them to change their minds.

Today, with things being much more litigious, I just raise this as a concern.

DR. BIANCO: Matt?

DR. KUEHNERT: Hopefully, a short and simple question. I guess I have a one-track mind today, but it's on endotoxin.

DR. BIANCO: Could you speak up a little bit.

DR. KUEHNERT: It's about endotoxin.

Have any of you looked--I saw log reductions of bacteria--but have any of you looked at endotoxin-level reductions as an endpoint or any effect on endotoxin with any of your processes for Gram-negatives?

DR. ALFORD: Maybe I'll answer. I'll be glad to start.

DR. KUEHNERT: Did you hear the question or should I repeat it?

If any of you have looked at endotoxin as an outcome or endpoint in your studies. I know some of these processes you performed the procedure at the end, and say you have a multi-log reduction of the bacteria, but that doesn't do anything for the endotoxin. I was just wondering how that addresses that issue for Gram-negative organisms.

DR. ALFORD: Thanks. I do understand your question.

We have not looked for endotoxin, as yet. Trust me, when we get back, we'll be looking.

The other thing is, with the process we have for INACTINE treatment of red cells, it's a two-step process, really, inactivation removal, and we're hopeful that there may be effect, either we want to see first from the inactivation, and then additionally we'd like to see from that removal step.

So, clearly, there may be an advantage with our process. We will be looking at it, because it is important. We did hear your message clearly this morning, and we, in fact, plan to speak with you further on it because it is a very important issue.

DR. KUEHNERT: Thanks.

DR. GOODRICH: [Off microphone.] [Inaudible.] I think that's a good question, and it is something that needs to be tested. One of the points that I think is also very clear is that you can kill eight logs or nine logs or ten logs per mL in these bacteria. If you let it grow to that point before treatment, it's probably not going to help you very much.

I think the key is preventing it from ever getting to that point to begin with. As you heard earlier today, knowing where that cut-off needs to be is maybe not well-defined. Lower is certainly better than higher.

DR. BIANCO: Our last question for the evening. Maybe we should freeze the cells?

[Laughter.]

DR. HOLMBERG: Jerry Holmberg.

As far as the psoralen and the riboflavins, we learned this morning from Steve Wagner that the free radical can affect the membrane and also some fatty acid solesafens (ph). How do you plan to evaluate the damage to the platelet membrane? And then I have another question to follow up on that.

DR. CORASH: Well, we've not been able to detect any membrane damage. S-59 has been designed to have a very low level of oxygen-dependent reactivity, and in fact you get the same level of pathogen inactivation if you do this in an argon environment, as if you do it in a platelet product that is exposed to oxygen.

Now Steve showed some data looking at electron microscopy, and that was early data when we were using a type of CAD device that we know induced some level of lysis by the time he got to Day 5.

We've gone back with a solid-face CAD device and are now seeing much lower levels. So we have not been able to identify any specific membrane damage. We can look at the surface of these platelets and see conservation of all of the key glycoproteins by fluocytometry. We know that they don't accumulate immunoglobulin on their surface. We can use them in a bleeding time assay, both in humans and

in animal models--Mo Blajchman's model--and know that they will shorten long bleeding times.

We do, obviously, see some evidence that there is a difference in viability that we can detect, although we know from our Phase III clinical trial that hemostasis is conserved when we look at multiply transfused patients supported for 28 or 56 days.

So we haven't been able to characterize any specific membrane damage up to this point, and we know that ATP production, by mitochondria, is completely conserved.

DR. GOODRICH: [Off microphone.] [Inaudible.]

DR. HOLMBERG: One last question, a very practical type of question.

Each one of you, what have you done or what is the impact of your substance on the laboratory worker and then also how do you dispose of your waste products? Is there a problem with disposal of your waste products?

DR. ALFORD: Celso, I guess, let me--

DR. BIANCO: Please, Bernadette.

DR. ALFORD: Jerry, I'll start.

Right now, our process, as you know, is a three-step. The first step is the delivery, and that is what you're specifically referring to, because, in conjunction with Hemonetics, our final step is an automated wash enclosed system. The system is in a bag. We anticipate,

from further development, just to be able to have a goal of disposing of that to drain.

So the question is really at the beginning of the process, and we are moving towards, and we already have a prototype to have a completed automated system, and that system not only protects the unit, it delivers the appropriate amount of INACTINE, but also would protect the worker because it will both compound, to formulate it from a concentration, and deliver the appropriate amount of solution to each unit of red cells.

We hope to have, in the next several months, that prototype available for disclosure to individuals like yourself, and to the public, so you could see the automation and the protection that the system offers.

Thank you.

DR. GOODRICH: [Off microphone.] [Inaudible.]

DR. CORASH: For the S-59 system for platelets and plasma, the Occupational Health and Safety Studies have been done. The handling procedures are basically as they are for protection against blood-borne pathogens for people working in the laboratory processing. A very large number of platelet and plasma units have been produced during the conduct of these clinical trials.

In the red cell system, S-303 completely breaks down once it's added to the blood component. But S-303 is

a reactive component. It's in the same category of pharmaceutical agents that are used for cancer chemotherapy, and there are handling precautions, and the system has been put together with an enclosed containment system so that it can be practiced in a blood bank environment.

DR. BIANCO: Eleanor?

ELEANOR: [Last name not identified.]

A very short question, and it is not a new one, but it is a question for Bernadette Alford, and I want to come back to your question.

You said that you test normally each unit because you said that it is a batch for you, and you make batch testing. If I understand that right, you test each red cell concentrate. Can you indicate which parameters you are looking for.

DR. ALFORD: I didn't get the last part of your question. Could I indicate what, please? What the testing is? Very good.

What I suggested to Bernie, and what I offered as a response earlier, is that it is a process that's completely under control, it's documented, and, of course, it's a validated process because, of course, we would put the appropriate controls in place to make sure that you had a controlled, validated, reproducible process.

But in addition, what we're testing for at the end is to verify that we have removed the PEN110, our active component, to a level less than 50 nanograms per mL. So I'm suggesting until--it's a very quick assay that we could do. It doesn't impact with releasing that unit, if you will, because, as I said, as soon as it is removed from the automated washing machine, it's ready either for storage or immediate transfusion.

We take a small sample right now, and until such time, as I alluded to earlier, that we would feel comfortable, the community would feel comfortable, as well as the FDA, we will continue that process until we have a good statistical assessment that we're just qualifying again that the process is in complete control.

DR. BIANCO: Thank you. I thank you all, and I thank the three of you for sharing with us as much as you did. It was very good and informative.

DR. VOSTAL: Thank you, Dr. Bianco.

Well, you've been treated to 10 hours of discussion of pathogen reduction, and I think the day is late. You've already heard the FDA perspective from Jay Epstein, so I will leave it at that, and I will just close for today.

I'd like to thank all of the speakers, and the moderators and the discussants for providing their thoughts on the subject.

[Applause.]

DR. VOSTAL: I hope to see you all tomorrow, when we're going to discuss the dark side of pathogen reduction.

[Whereupon, at 6:13 p.m., the proceedings were recessed to reconvene Thursday, August 8, 2002.]

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