U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

+ + + + +

BACTERIAL CONTAMINATION OF PLATELETS

WORKSHOP

+ + + + +

Friday, September 24, 1999

+ + + + +

The workshop met in the Masur Auditorium, Building 10, National Institutes of Health, Bethesda, Maryland, at 8:15 a.m., Jay S. Epstein, M.D., Center for Biologics Evaluation and Research, presiding.

PRESENT:

JAY S. EPSTEIN, M.D., CBER

CHIANG SYIN, Ph.D., CBER

MORRIS A. BLAJCHMAN, Session I Chair and Speaker

MARK BRECHER, M.D., Session II Chair

LEONARD I. FRIEDMAN, Sc.D., Section II Chair

STEPHEN J. WAGNER, Ph.D., Session III Chair and Speaker

JAMES P. AuBUCHON, M.D., Speaker

JOHN BARBARA, Ph.D., Speaker

MINDY GOLDMAN, M.D., Speaker

PRESENT (Continued):

JONG-HOON LEE, M.D., Speaker

CARL P. McDONALD, M.S., Speaker

PASCAL C. MOREL, M.D., Speaker

VIRGINIA R. ROTH, M.D., Speaker

MARK SEAVER, Ph.D., Speaker

LANCE TRAINOR, M.D., Speaker

ROSLYN A. YOMTOVIAN, M.D., Speaker

EDWARD L. SNYDER, M.D., Workshop Summary

JAROSLAV VOSTAL, M.D., Ph.D., Closing Speaker

C-O-N-T-E-N-T-S

Introduction, Dr. Chiang Syin 4

Keynote Address, Dr. Jay S. Epstein 5

Presentation by Morris A. Blajchman, M.D. 14

Presentation by Virginia Roth, M.D. 29

Presentation by Pascal C. Morel, M.D. 46

Presentation of John Barbara Ph.D. 58

Presentation of John-Hoon Lee, M.D. 71, 85

Presentation of Dr. Mindy Goldman 96

Presentation of Dr. Lance Trainor 101

Presentation of Leonard I. Friedman, Sc.D. 126

Presentation of James P. AuBuchon, M.D. 133

Presentation of Stephen J. Wagner, Ph.D. 164

Presentation of Mark Brecher, M.D. 175

Presentation of Mark Seaver, Ph.D. 191

Presentation of Morris A. Blajchman, M.D. 223

Presentation of Stephen J. Wagner, Ph.D. 231

Presentation of Carl P. McDonald, M.S. 240

Presentation of Lily Lin, Ph.D. 257

Workshop Summary, Edward L. Snyder, M.D. 275

Closing Remarks, Jaroslav Vostal, M.D., Ph.D. ... 287

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

(8:19 a.m.)

DR. SYIN: Hello. Good morning. My name is Chiang Syin. I'm the Chairman for the workshop.

Thank you for coming to this workshop.

Before I introduce the first speaker of the day, I have a couple of announcements. In your handout, we have the final agenda, and there may be a couple of versions circulating around, but in essence, we added two speakers in the first session. That's two immediate presentations that we added before the panel discussion, and we also have one more speaker for the second session.

Because we're adding two more speakers in the first session, so we decide to make an earlier break than what we originally planned. We will probably take a break after Dr. Lee is presenting FDA report.

And before I introduce Dr. Epstein, I would like to take this opportunity to thank our staff, especially our program coordinator Joe Wilczek and the staff in the Division of Emerging and Transfusion Transmitted Diseases for helping out to make this workshop a reality.

And I also would like to thank the members

of the Planning Committee to help me set up this workshop, especially who is not in the original Planning Committee is Dr. Jonathan Lasin (phonetic). He's our Associate Director for Research. He has been with me in the last four and a half months every step of the way to set up this workshop. I take this opportunity to thank all of them.

And right now with great pleasure let me introduce our Office Director, Dr. Jay Epstein. He's doing the opening speech.

Thank you.

DR. EPSTEIN: Thank you very much, Chiang.

And I would like to recognize the very special efforts of Dr. Chiang Syin, who joined the Blood Office only within the last year and has already distinguished himself as a fine meeting organizer and has been helpful to us on many subjects.

So if I could have the next overhead.

I think it's useful to focus on why we're here, and so I've summarized what I think are the key objectives of this workshop, primarily to obtain current information on bacterial contamination of platelets, and then also to encourage research and development efforts to minimize the transfusion risk.

It's of useful historic note that we last

sponsored a workshop -- actually NIH and FDA co-sponsored it -- in 1995 that was entitled "Microbial Contamination of Blood Components." This meeting was summarized in Transfusion in 1997, and those who would like to read it, it was Volume 37, pages 95 to 101.

And that conference, I think, was very well received. It was of excellent scientific quality, but there was perhaps at that time an aura of disappointment because people were hopeful that we could get more out of it in terms of perhaps solutions to the longstanding problem of bacterial contamination.

Merlin Sayers was the meeting summator (phonetic). He noted that many of these issues were not new; that the interest had been provoked by what was called a mini epidemic of Yersinia enterocolitica infection of red cells reported in 1991; that there was a glaring absence of accurate incidence data and the general recognition that reporting based on clinical events was undoubtedly under reported, and there was a need for culture surveillance.

It was felt to be critical to understand most how to recognize and manage transfusion reactions, and that's perhaps where the greatest progress was made in the ensuing years.

And then Dr. Sayers was prescient in

calling for further investigation into novel screening and detection methods, especially because the conference had suggested that chemiluminescence would be promising, although it had been somewhat abandoned, and then he called for judicious and rational use of blood as a general precaution and observed that there was no single strategy that was going to solve this problem, at least at that time.

Also as an outgrowth of the conference, the Transfusion Transmitted Diseases Committee of the American Association of Blood Banks issued a set of recommendations that were published June 19th, '95, and these were as follows:

That major emphasis should be given to the development and evaluation of practical, sensitive, and specific screening assays for the detection of bacteria and platelet concentrates and for the development of methods to decontaminate cellular blood products.

Secondly, that there should be a preference for the use of apheresis platelets compared with random donor platelets because of the reduced risk of bacterial contamination on a statistical basis, single unit versus pooling.

And that there should be strict adherence to existing standards, which included scrupulous

attention to the selection and cleansing of the phlebotomy site, careful attention to the expiration date of platelets, and care in the aseptic handling of blood components, and the observation of the importance of visual inspection of units before transfusion as a useful quality control.

Next, please.

So the concerns over bacterial contamination have been with us as long as there has been transfusion therapy. Sepsis is one of the earliest recognized complications of transfusion. Reporting of fatalities to the FDA since 1976 has identified bacterial contamination as the cause in approximately ten percent, although there was some suggestion in early years that the rate was lower.

Also, among platelets, which stand out, the reports are twice as frequent than for red cells, presumably reflecting on a difference in contamination rate, but a difference in outgrowth, related to the fact that red cells can be stored at four degrees Centigrade, whereas platelets are stored at room temperature, once again highlighting the scientific challenge of trying to achieve cold storage of platelets.

Next, please.

So the current practices that will bear on the risks include the collection; the question of apheresis versus random donor; the true implication of a single donor versus a pooled product.

It was noted in the 1995 conference that there was an increased titre of bacteria in a contaminated platelet transfusion when prestorage pooling of units occurred, not due to an increased risk of single unit contamination, but the fact that there was a greater volume of media in which the bacteria could grow.

As I've said, we store platelets at room temperature, 22 degrees Centigrade; that we have historically lowered the storage period for platelets as an effort to reduce contaminations causing clinical sepsis. We all know that the supply of platelets could be improved if we could increase the storage period, but this is a precarious concept at this time unless we have strategies to exclude contamination in the units that are being stored, and this will be discussed at the conference.

So what I'll briefly do then is outline the topics that will be covered in this workshop. First, we'll focus on the clinical and epidemiological issues, looking at the sources of contamination, the relative

roles of bacteremia as skin contaminations, coring and phlebotomy, skin flaps, breaches of closed system.

The clinical manifestations that are associated with either silent bacterial colonization of a unit or outgrowth associated with sepsis; risk factors for clinical events, such as the relative risk of endotoxin and Gram-negative bacteria versus Gram-positive.

We will review the range of organisms that affect platelets. It's well recognized and remains true that there are a very broad range of pathogens, but particularly the associations with skin flora, skin colonization with enterics, and perhaps less frequent, but no less significant, breaches of closed system's seroprocessing resulting in laboratory contaminations, and then discussion of potential control measures.

We'll then shift gear from the clinical side to surveillance efforts and try to compare and contrast surveillance data that have been now obtained from a number of international sources, including in Canada a recent French hemovigilance system and its earliest reports, and then a surveillance study on serious hazards of blood transfusion, SHOT, in the United Kingdom.

And it will be interesting to try to

compare these estimates and understand the very broad range that's been observed. In the United States, we have engaged surveillance since 1998. This was one of the outgrowths of the 1995 conference, and we will hear reports also on FDA fatality reporting and the estimated prevalence based on error and accident reporting.

Next please.

We'll then turn attention to possible strategies of intervention. These fall into two bins. One is testing strategies to try to identify contamination. The other will be preventing measures.

In the area of testing strategies, we will hear some theoretical discussions about what would constitute an ideal test. We'll hear about variables affecting the sensitivity and specificity of testing, including the sample collection, nature of the material, the volume, frequency, timing, et cetera, and then review the relative merits of various detection technologies which, again, span a broad technology range, from manual procedures of staining and culture, use of automated systems, and some of the emerging, rapid diagnostic methods, including biochemical technologies and nucleic acid amplification technologies.

Next please.

Of course, it's always best to avoid the contamination in the first place, and so we will hear about innovations in the cleansing and disinfection at the phlebotomy site. We will also hear some data pertaining to reduction of risk related to discard of the early volume of whole blood collection; the possibility to augment bacterial clearance by leukoreduction; the benefit or lack of benefit of prolonged room temperature storage before separation.

And then also we will talk about some of the emerging detection methods that are novel, like labeled antibiotics, and then the promise or continued promise and development of photochemical inactivation using ultraviolet light and Psoralen compounds.

So all in all, I think -- slide off -- we expect a very full day; that we will be hearing about many promising advancements, and it's perhaps useful just to keep in mind the overarching goal, which is to see if we can identify useful interventions that could be recommended for implementation at this time.

So in the interest of keeping to schedule, I would just like to invite the moderators of the first session on epidemiology to come up to the podium, and they are Dr. Morris Blajchman, well known to everyone

in this field, who is Professor of Pathology and Medicine at McMaster University in Hamilton, Ontario, and then perhaps a little less well known to the group, but one of the emerging stars is Dr. Matthew Kuehnert, who is a medical epidemiologist in the Hospital Infections Program of the National Center for Infectious Diseases at the U.S. Centers for Disease Control and Prevention in Atlanta.

And with that we'll proceed to the first session.

Thank you very much.

DR. KUEHNERT: Good morning and welcome.

First, I have the pleasure of introducing Dr. Blajchman. He trained at McGill in Montreal and also at University of Pennsylvania, and in hematology in England. He's on the faculty at McMaster and is a professor in the Departments of Pathology and Medicine.

He's been active in research, to say the least, in this field, and I think I can say is an expert in the field and will be speaking today, be giving an overview on the magnitude and mechanism of transfusion associated bacterial sepsis.

Dr. Blajchman.

DR. BLAJCHMAN: Thanks, Matt.

I'm delighted to be here, although I must

say that I'm rather disappointed with the turnout, but that's perhaps not too surprising. It seems to me that if this conference had to do with variance CJD disease where there hasn't been a single case of transfusion transmission, this auditorium would be overflowing. Yet the transfusion transmitted sepsis or bacterial contamination is the first recognized transmitted disease and, in my opinion, the most common microbiological problem in transfusion medicine currently, and aren't really interested and people aren't listening.

We are contributing with bacteria to death of many people unnecessarily, and we're not doing very much about it.

And more importantly, and I hope this falls on the right ears, and it's intended as a criticism, the amount of money being spent in the United States and elsewhere to create for not testing, developing of not testing, and for devising tests for CJD and variant CJD runs in the millions and millions and millions of dollars, and I'd like to indicate that comparably tests or money spent on devising tests or systems to detect bacteria is very paltry at the same time.

And I think this is inappropriate and, I think, is rather dangerous, quite frankly. And so I'm

dismayed about what has happened, but I'm pleased to be at this meeting and to talk to you and to be on my soapbox yet again to talk about this issue, and I'll try over the next 20 minutes to review the epidemiology and give you an idea of what I believe is the magnitude of the problem.

Could I have my first slide, or I just press?

I'm going to start. I'm a clinician. I actually see patients from time to time. That's when I'm not traveling, and I'm going to start this presentation by describing a recent Canadian case. The case is very illustrative for a number of reasons which you'll see in a moment.

I'm going to tell you about the donor history. Earlier this year, a blood donor, age 36, healthy, he had many previous blood donations, and on this date came in to present a whole blood donation, which was subsequently processed into red cells, platelets, and FFP.

Three days after donation -- he was well on the day of donation and previously -- three days after the donation, he presented to the emergency room at the local hospital with a two day history of dizziness, vomiting, fever, and diarrhea. He wasn't feeling very

well, and his temperature at the time was 37.9. The doctors in the emergency room didn't quite know what was going on, but they felt that he shouldn't be sent home. So they kept him overnight in the emergency.

Can you hear me now? At the back? Speak through here still.

They kept him in the emergency room overnight for observation. Overnight he became hypotensive, tachycardiac. When they did a CBC on him -- they had done a CBC the night before, and his platelet count was normal -- his platelet count had dropped 35,000.

He was admitted to the ICU in septic shock, and they did several blood cultures on him, and the blood cultures grew staph. aureus.

His subsequent course, he had a loss of consciousness, and he had a lot of support. His wife, the day after admission to the hospital, remembered that he had given blood five days earlier. So this was reported to the doctors taking care of him, and the information subsequently went to the blood centers to try and pull back the units of blood.

Subsequently he had other investigations, a CT scan. He had cerebral left parietal infarcts, and he had some residual disease, but he's recovering, but

he has some neurologic deficits.

The donor -- sorry -- the recipient. This is a 75 year old male with a mild dysplastic syndrome. He was transfused with a pool of five random donor units, including the four day old platelets on day four of the donation, and what is very interesting and very relevant, I think, in view of what's going to be coming later in the day, he had no signs or symptoms associated with the platelet transfusion, nothing. This was done as an out patient. He had no signs or symptoms.

As a result of being informed, his doctor was called, and his doctor decided to check him out, and when he arrived in the hospital, he had chills and fever on arrival, and on admission to the hospital, some cultures were done, and he grew the staph. aureus.

His course in the hospital was deterioration and death eight days after receiving the contaminant platelet unit.

Microbiological testing and other testing, including antibiotic sensitivities, genotyping, genetic fingerprinting show that the donor and recipient isolates are identical. There were no platelet units to be cultured. So we don't have the platelet units, but the donor and recipient isolates are identical.

Now, the importance of this case, and I think the reason I've chosen to present this case, is the following: that this case was identified as being due to transfusion, was only because the donor became ill. There are many donors who carry bacteria or whose donations harbor bacteria that are not recognized. So case would not have been recognized, except for this patient's subsequent presentation to the hospital.

And the other important part of this case is the donor probably had a bacteremia. He was subsequently found to have some cardiac abnormalities. So he probably had at the time of donation sub bacterial endocarditis. So his bacterial level was rather small, and the recipient received a small dose of bacteria which caused no symptoms.

And we'll hear later on, actually in the next presentation, about the BaCon study. The yardstick used in the BaCon study is a two degree rise in temperature. So you can have -- I am sure that the BaCon study, and I think that the people involved will acknowledge this, that the BaCon study is the tip of the iceberg. Cases like this represent the body of the iceberg that took down the Titanic.

So that's why I started with this case, and Roslyn Yomtovian and I were at dinner together last

night, and she and I have other cases that are similar to this where the presentation in the recipient is asymptomatic, essentially asymptomatic. Yet these things contribute to the death of patients, but are not recognized.

So if you just wait for patients to develop high fever, you're going to miss cases.

Now, I'm going to try and give you the magnitude of the bacteria contamination issue. We have good data, plenty of data on the magnitude of contamination in cellular blood products, and I'm going to review this data with you.

There actually have been in the last decade eight prospective studies evaluating the bacterial contamination of random allogeneic donor platelets. Close to 200,000 units in these eight studies have been cultured, and I'm not proposing to go through all of these studies. The references are here, and I think in the handout there's a recent reference in a recent Vox Sanguinis from Hong Kong.

But you can see if you pool all of this data together, the contamination rate in bacterial is something of the order of one in about 3,000, and this is fairly consistent through all the studies that have been done, and I've calculated a 95 percent confidence

intervals, which is indicated here.

So it's clear that one in about 3,000 units of platelets are contaminated, contain bacteria, and when you think that platelets, these units, are regularly pooled, five, six, eight, ten at a time, the incident, the number of contaminated infusates, transfusates of platelets is considerably higher, of the order of one in 500 pooled units has bacteria in them.

This is orders of magnitude higher than any other infectious problem that we deal with, orders of magnitude higher. Yet we're not doing anything about it.

These are similar sort of data. There are fewer studies, five studies looking at apheresis platelets, and the references are shown here. There have been five studies. A smaller number of platelet units have been cultured, but again, the number are very similar. This amounts to about one in about 5,000 units. The confidence units are quite broader because the numbers are smaller.

So, again, platelet, both random donor and apheresis platelets, regularly are contaminated with bacteria.

With regard to red cells, I've been able to

find only two studies, and there's only one red cell unit that was contaminated of some close to 40,000 with a rate something on the order of 50,000. Why this is different, but the confidence intervals here cannot be calculated. They're huge.

So to put this all together, this is the rate of contamination from the prospective studies of random donor, one in about 3,000 units, apheresis, one in about 2,000 units, and RB cells, one in about 30,000 units. So this bacterial contamination occurs, and we've known about this for a century or almost a century because that was, as Jay mentioned earlier, the first problem with blood transfusions.

Now, if you look at -- I'm not going to go, and others will, into these types of flora, but you can see if you break down the flora that are isolated from these various contaminated units, the vast majority of skin flora, with some of them being enteric flora, and some environmental flora, but the majority are skin flora. It's the skin that contributes to the contamination.

Now, what are the mechanisms? I don't have a great deal of time to go into this, but the three possible mechanisms include donor bacteremia, contamination during blood collection, and

contamination during blood processing, and I'll go through this a little bit.

Source of donor bacteremia. Well, you can have asymptomatic bacteremia, for example, as occurs with people with enterocolitica infection, chronic gut infection, and they have transient and often regular bacteremia that is largely asymptomatic.

You have patients with chronic low grade infections, with osteomyelitis, and you can get transient bacteremias following a dental and medical procedure.

And one of the things that always has bothered me is that I wonder whether we also get transient bacteremia when we put the needle or the nurse puts the needle in the donor's arm. People clinch their teeth when they sort of clinch their fists and their teeth, and I wonder. Some of the donors with poor oral hygiene, the clinching of teeth could very easily create a transient bacteremia that can cause a problem, and I wonder if that isn't a factor here.

There's been a rather interesting infection epidemic of sorts or small epidemic of Yersinia enterocolitica in New Zealand. The reference is published in the Australian and New Zealand Journal of Medicine, but they found eight cases of transfusion

transmitted Yersinia over a five year period, of these eight cases, five resulting in death. The calculated incidence rate is one in 65,000, transfusion associated fatality rate, one in 100,000.

This fatality rate appears to be 80 times higher than that appears in the United States. Why that's so, I suspect part of the difference reflects the fact that the reporting rate in the United States is lower than it should be and could be, and the antibody screening was of limited utility. This was reported in an exchange of letters to the editor that's shown here.

Now, you can have contamination during blood collection or blood component manufacture. That may be due to inadequate skin disinfection. Others will talk about this issue this afternoon.

Scarred phlebotomy sites, there are publications showing that if you use the same site, and donors like the nurses to use the same site, this is a source of infection.

There have been reports of contaminated apheresis solutions, contaminated water baths or exteriors of packs, and the issue of skin chlorine, which is my sort of favorite topic.

And just the sources of skin flora specific

contamination is inadequate preparation, scarred site, and skin chlorine, and there's a paper in Lancet way back in 1958, 40 years ago, and these authors, Gibson and Norris, show that regularly when you stick needles into the skin, you get skin fragments. I'm sorry. This is a glass slide, and it broke in travel, but they used different sizes of needles, and showing regular. The n is 50 here, and you can see almost 70, 80 percent of the skin fragments was associated with each stab.

So this occurs regularly. Every time or 80 percent of the time that we put a needle into the skin, we inadvertently or unwittingly do a skin biopsy.

This, to remind those of you, is the sort of diagram of the morphology of the skin. We clean the surface of the skin, but bacteria reside often in the appendages of the skin, the sweat glands, the hair follicles.

When we put a needle and do the skin biopsy, if we go through such an appendage, we inadvertently can take that skin and, become we don't clean that part of the skin, there can be bacteria associated with that appendage.

While there is no direct evidence for this, there's indirect evidence. The people are starting to remove the first aliquot, suggests that you can reduce

the contamination rate by doing that.

The magnitude now of the sepsis problem. I've shown you the magnitude of the contamination problem. Now many, most in fact, and luckily so, most of the units of blood, the cellular blood products, contain low levels of bacteria that are not going to be necessarily pathogenic to the recipient, and we really don't have a good idea, and what we're really trying to prevent is not bacteremia, but septicemia, although bacteremia, as per the case that I presented, can, even though a low level of bacteremia, can cause significant morbidity and mortality, but most probably do not.

And what is the magnitude of the septic problem? Well, we don't really know. We know that bacteremia due to red cells, contaminated red cells, or platelets is of the order of one in 2,000, one in 3,000. I suspect that maybe five to ten percent. So the magnitude of that may be one in 50,000, and we'll hear data from France that may tend to support that number.

And this is the sepsis associated with contaminated platelet concentrates and sepsis due to contaminated red cells, we don't really know, but it may be of the order of one in 500,000.

Now, I want to make the following point. I

don't have time to go into this Paling perspective scale that sets risk on a log scale. We're paying an awful lot of attention to this theoretical disease CJD and variant CJD, and there is yet to be a case of transmitted -- that's why I put it out here with an arrow coming in here. There might very well be a case soon, maybe. It wouldn't surprise me because presumably if you can get CJD from eating a hamburger, there probably is a hematologic -- a period of time where the prion, the infectious agent, is in the blood stream. If you have enough of this, you might get a case.

The HIV, HIV-1, HCV of the order is down here, one in 100,000 to one in million. Bacteria based on my estimates at least and from the literature is somewhere between one in about 2,000 to one in 10,000 or thereabouts, and we're not paying very much attention to this.

We're paying lots of attention to this, lots of attention to this, lots of funds as I've already said devoted to detection tests, lots of funds and narrowing the window, and I'm not saying this is inappropriate.

But what about bacteria? The size of the audience, the amount of money having been spent to

develop systems to prevent this is indicative of a serious problem that we haven't paid enough attention to this issue.

And I want to issue the challenge, and I hope as a result of this meeting that we can see some more action to prevent transfusion associated sepsis, which as I've said, currently in my view is the most problematic, is the biggest problem in microbiology relating to transfusion safety.

I've estimated that probably 20 to 40 deaths per year occur in the United States each year, many of which can be prevented.

Thank you for your attention.

(Applause.)

DR. KUEHNERT: Thank you, Dr. Blajchman.

I wanted to ask you the first question, which was the first case you presented was very interesting, and I wondered what you thought, what intervention could be made to screen donors that look like on the donation at least, at the time of donation that there was nothing that would have indicated a problem, but on follow-up what would you suggest be done to try to capture those events?

DR. BLAJCHMAN: Well, I think the approach that I have suggested and will continue to suggest is a

routine bacterial culture of units of blood. I think we can argue whether this should be done on day one, day two, day three, what methodology, but some sort of screening of units needs to be done.

And if that had been done in this case, it might have prevented that patient's death because the recipient received the platelets on day four, and most, if not all, of the deaths relating to platelets that have been reported have occurred on platelets that were either four or five days old. I think by that time we could have detected the bacteria.

DR. KUEHNERT: And the other I wanted to just clarify is that, as Dr. Roth will go into with the BaCon study criteria, that the signs and symptoms are either/or. So fever does not have to be necessary in order for it to qualify as a case.

DR. BLAJCHMAN: So what's the question?

DR. KUEHNERT: Oh, there is no question. It was just a point of clarification.

Are there any questions otherwise?

(No response.)

DR. KUEHNERT: Well, next I'd like to introduce someone I know well, Virginia Roth. She received her medical degree from the University of Ottawa, certified as a Fellow of the Royal College of

Physicians and Surgeons of Canada in internal medicine and infectious diseases; also trained in England, receiving her diploma of tropical medicine in hygiene; joined CDC in 1998 as an EIS officer in the Hospital Infections Program, and is the co-coordinator for the BaCon study, and she's going to be presenting the preliminary results from the BaCon study.

Virginia.

DR. ROTH: Thank you very much.

It gives me great pleasure and I consider it a great honor to be here to prevent the initial results from the BaCon study, and I want to start by thanking you all for your interest in this study and many of you for your support.

The BaCon study represents the first coordinated, national effort to collect data on episodes of blood component bacterial contamination associated with transfusion reaction in the United States.

Listed on this slide we have the participating organizations: the American Association of Blood Banks, American Red Cross, CDC, and the Department of Defense.

Since the focus of this workshop is platelets, I want to clarify at the outset that the

BaCon study monitors reactions associated with all blood components, not just platelets.

I would like to begin by acknowledging the other members of the BaCon study committee, all of whom have put in an enormous amount of time and effort into the success of this study.

Between 1985 and 1997, CDC received increasing numbers of reports of transfusion associated Yersinia enterocolitica infection with high mortality rates. This heightened concerns about bacterial contamination as a blood safety issue.

We'll be hearing a little later about the FDA reporting system from Dr. Lee, but as most of you are aware, FDA reporting is mandatory only for transfusion reactions involving death. CDC notification of nonfatal events has been inconsistent. Thus, there has been no formal national reporting system to collect data on these nonfatal events.

Under reporting was a widely recognized problem leading to a perceived need for a national effort to coordinate reporting of blood product bacterial contamination.

In 1997, July '97, in response to this perceived need for a national surveillance system, organizations that collect and distribute blood in the

United States approached CDC for assistance. Subsequently, AABB, ARC, and DOD were funded to improve national reporting of data.

CDC also supported this effort by providing technical assistance, including laboratory support and data management.

In August of 1997, AABB, ARC, DOD and CDC met to develop plans for a nationwide effort. A lot of initial effort was directed towards education of clinical and transfusion personnel because it was recognized that one of the most important steps was to heighten awareness among those on the front line, the nurses and the bedside clinicians who were responsible for recognizing a transfusion reaction.

As part of these educational efforts, the study committee provided pocket sized transfusion reaction work-up cards, and these are in your folders. They're laminated cards that are suitable for slipping in a lab coat pocket, and they contain the BaCon study criteria.

The committee also implemented a standard case report form, and that's also included in the handout that you have there for you to look at, and distributed a slide set and script describing the background, methods, and recommended work-up, and this

slide set is suitable to use as a teaching tool.

The purpose of the BaCon study is to determine the rates of bacterial contamination of blood components associated with transfusion reaction, which have only been estimated through extrapolation in the past.

Secondly, to identify the pathogens associated with bacterial contamination, and to estimate their relative frequency; to identify risk factors for bacterial contamination; and last, to identify factors associated with recipient morbidity and mortality. In other words, to describe the characteristics of those recipients who do well and those who do poorly or to on to die.

Here you can see the BaCon study criteria for an adverse transfusion reaction. These criteria were developed by a consensus conference and data accumulated from events before BaCon. The criteria listed here can include one or more, so any of the following signs or symptoms.

And the cutoff time at the onset of the study was that the symptoms had to occur within 90 minutes of the transfusion. Now, Dr. Blajchman just presented a case that would not fit into this criteria. I know Dr. Yomtovian and others have seen somewhat of

a delay in reaction, and I'll talk about some discussion around this 90 minute cutoff in a minute.

But for the purposes of this discussion, the criteria could include one or more of fever, including a temperature of greater than or equal to 39 degrees or greater than or equal to two degrees Celsius rise from baseline; rigors; tachycardia, defined as a heart rate of greater than or equal to 120 beats per minute or a 40 beat per minute rise; or a change in systolic blood pressure, either a rise or drop of 30 or greater millimeters of mercury.

Once a case meets these criteria for an adverse reaction, this flow chart shows how cases are reported to BaCon. The clinical service, transfusion service, and collection facility each have equally important roles in reporting and investigating a transfusion reaction.

The clinical service is asked to report any suspected reactions to the transfusion service. They're asked to obtain the recipient blood culture and to record clinical data on the recipient adverse reaction form.

They are also asked to save the unit, the implicated unit, and the transfusion set and forward them to the transfusion service.

The transfusion service then evaluates and investigates this possible reaction according to their standard operating procedure, including a Gram stain and a culture of the unit. They're also asked to record any manipulations that have been done on the unit, and if the findings are consistent with an episode of bacterial contamination, the transfusion service is asked to notify the collection facility.

The collection facility then compiles the data from the clinical service and the transfusion service, initiates trace-back of co-components and donor review, and then notifies the coordinating organization through whom cases are reported to BaCon.

The BaCon study was launched January 1st, 1998, with an intensive educational effort. BaCon study materials were sent to over 7,000 hospitals through their respective affiliations.

In addition, over 60,000 data cards were distributed to clinicians, and an Internet site was created.

I want to show you now the preliminary results for the first 18 months of the study. So this spans January 1st through June 30th, January 1st, '98, to June 30th, 1999.

During this time period, we received 12

reports that met the BaCon criteria as definite cases, and I use the term "definite" here because these are cases in which the recipient culture was exactly the same by molecular typing as the organism recovered from the blood component. So in these cases, there is no question that bacterial contamination was the cause of the recipient reaction.

I'll show you a little later a summary of reports received which did not meet these stringent criteria as definite cases.

So for these 12 cases, the mean age of the recipient was 57 years. Fifty-eight percent were female. The most common underlying recipient diagnoses were malignancy in five episodes or gastrointestinal bleed in five episodes, and three of the 12 or 25 percent had a fatal outcome.

Here you can see the storage time of the implicated blood product. The first column shows the type of blood product received in these 12 cases, and as you can see, the majority involved platelet. Five episodes involved pooled platelets and six platelet pheresis. In only one of these 12 was a red blood cell unit implicated.

Allowable storage time refers to the number of days that the product could be stored post donation,

and days of storage refers to the number of days that the unit was stored before transfusion.

So for the first line here, platelet pool, all five of these platelet pools were transfused on the last allowable day of storage on day five. On the platelet pheresis, the mean days of storage was four days, with the range of two to five days, and the red blood cell unit was 35 days old at the time of transfusion.

As you can see, a wide variety of organisms were implicated here, the majority of which were Gram positive rather than Gram negative, and again, you can see that a lot of these can be part of skin flora.

We've got two episodes involving staph. aureus, two with Group B strep., two with staph. epi, one Group G strep. and one staph. lugdemensis.

And among the Gram negatives, we had an enterobacter aerogenes, enterobacter cloacae, E. coli, and a serratia liquifaciens.

This shows the recipient signs and symptoms as reported to us. Interestingly, all of them, 100 percent, reported rigors. Of the ten episodes for which information about fever was available, nine of the ten reported fever, and then in decreasing order of frequency, other signs and symptoms included

tachycardia, nausea and vomiting, lumbar pain, shortness of breath, low blood pressure, or high blood pressure.

One of the purposes of the BaCon study was to identify risk factors associated with fatality. Now, before I walk you through this table, I want to caution you that this analysis is based on a small number of cases. We're comparing here three fatal to nine nonfatal events.

Nevertheless, I think it's kind of interesting to look at some of the trends that are starting to emerge, and I wanted to share these trends with you. Keep in mind that as we accrue more cases, this type of analysis will become a lot more meaningful.

If we start with age, looking at age as a possible risk factor, fatal cases tended to be older, with a mean age of 81 compared to 49 in nonfatal cases. This did not quite reach statistical significance.

Gram negative organisms were implicated in all three fatal reactions compared to one of nine nonfatal reactions.

Platelet storage time in days tended to be longer in fatal reactions than nonfatal reactions, 4.8 days versus 2.5 days.

Pretransfusion antibiotics refers to recipients who were already on antibiotics for another indication. We're not advocating prophylactic antibiotics before transfusion here, but these recipients could have been on antibiotics for another reason. None of the fatal cases had received pretransfusion antibiotics compared to two of eight nonfatal cases, and this was not significant.

And lastly, the last factor is not significant either, but it's a very interesting trend. If you look at the time between the onset of symptoms until the time antibiotics were started, it was longer in fatal cases, over six hours from the time the first symptom was recorded compared to 119 minutes in nonfatal cases.

So it makes you question whether there's an association between delay in treatment and a poor outcome.

Here, an additional eight cases in which bacteria contamination could have been related to adverse recipient reaction. Of these eight cases, seven of these were fatal, and these fatalities were reported to us through FDA.

Three of the episodes never had a recipient blood culture done, and you can see the implicated

organisms in these cases. Three episodes the blood product was never cultured, and the last two episodes, the same species, pseudomonas in one episode and staph. aureus in the second, were isolated from the recipient and from the blood product, but the isolates were discarded and the transfusion product was discarded before these could ever be confirmed by molecular typing.

These episodes illustrate that we need to continue to get the message out of the importance of obtaining both the recipient and a blood product culture and of saving these isolates until bacteria contamination can be confirmed or ruled out.

Here is a summary of episodes received by the BaCon study committee during the first 18 months of the study, January '98 through June '99. I already showed you the analysis based on the 12 confirmed cases, and the last slide summarized the eight in which reporting was incomplete.

In addition to these, we received five reports in which the recipient blood culture was negative. Two of these five recipients were on antibiotics at the time the culture was obtained. In an additional two cases, molecular typing by post fill electrophoresis (phonetic) showed that the organisms

were not the same, and one of these was actually asymptomatic, for a total of 27 reports.

In addition to these 27 reports, ARC and AABB have received about three times this number of reports that never met the criteria as a BaCon case.

Has the BaCon study influenced reporting to CDC of transfusion reactions associated with bacterial contamination? This graph shows sort of the pre-BaCon era, so the ten years before BaCon, from '88 through '97, and the last bar here is the first complete year of BaCon that we have data for, which is 1998.

So if you compare the ten years prior, you can see that 31 events were reported to CDC for an average of three events per year. Twenty of these 31, or 64 percent, were fatal.

In the first year of BaCon, in contrast, we had nine events reported in one year. So we've got essentially a tripling of reports, and one of the nine, or 11 percent, were fatal compared to 68 percent in the period before.

This suggests then, in summary, that where BaCon has improved the reporting of nonfatal cases, we received 12 definite cases through the first 18 months of data collection. Rigors and fever appeared to be the most sensitive clinical measures of the transfusion

reaction due to bacteria contamination, and most of our cases were associated with platelet units and with Gram positive organisms.

Although we have only very few cases to look at, we can make some inferences about fatality. Fatality in the cases we've received so far appears to be associated with Gram negative organisms, with platelet units transfused at the end of allowable storage time, older age of the patient, and lack of promptness in initiating antimicrobial therapy.

These last two factors were not statistically significant. However, a trend was seen.

The development of a standardized data collection tool has been a major accomplishment of this study, providing consistent information for risk factor analysis.

We have also been receiving feedback from the participants who feel that BaCon provides important services. About 75 percent of the cases we've gotten so far have been from small facilities who do not have reference laboratories that they can turn to, and they've welcomed the technical support on point of contact provided by BaCon.

BaCon has the ability to detect unusual clusters of organisms. We keep all recovered organisms

in a repository, and when we isolate a causative organism, we can compare its molecular typing profile with those of previously isolated strains implicated in other cases.

In this way we're able to detect any unusual clusters. To date, all of the organisms we have received have been unique. None of them have been the same by molecular typing.

BaCon illustrates what can be accomplished through good interagency cooperation between federal agencies and major coordinating blood banking organizations, and this collaboration has really worked very well.

BaCon serves as a model surveillance system for adverse events associated with blood and blood product transfusion. Other countries who are interested in designing their own surveillance systems are asking questions about our protocol.

And lastly, we believe that BaCon has increased the level of awareness among clinicians and transfusion services. We've received anecdotal feedback that the awareness of the BaCon study has been the impetus for recognizing and reporting some of these cases. We hope that the increased awareness will then, in turn, lead to a timely work-up, making a correct

diagnosis, and initiating effective therapy promptly, which is the ultimate goal of the BaCon study, to improve patient outcome.

BaCon has some well recognized limitations as a national surveillance system. Despite our educational efforts, under recognition and under reporting continue to be problematic, and they're difficult to estimate.

In addition, participation rates are not easily measured, and this includes participation by clinicians, by transfusion services, and by donor centers.

The denominators we will be using to calculate rates will be in terms of the number of units distributed, not the number of units transfused. If anything, this would tend to under estimate the rate.

What is in store for the future of BaCon? Denominator and participation data will be used to calculate U.S. national rates of bacterial contamination at baseline and for comparison over time. Ongoing educational efforts in data collection are crucial in improving recognition, reporting, and prompt work-up and treatment of transfusion related sepsis.

Funding will now provide remuneration for reported cases to compensated participating transfusion

services for the time and effort in reporting these cases to BaCon. This is largely in response to concerns of a high responder burden from the participants.

Also, keep in mind that this is a voluntary reporting system, and so we're hoping that with some incentive we may improve participation and reporting.

CDC has committee to continue working with FDA and coordinating blood banking organizations to improve reporting, investigation, and most importantly, prevention of these events.

And in your handout you will find a document entitled "BaCon Update," and in that update it describes the funding schedules that will be available. It also makes mention that we have agreed to extend the time from symptom onset from 90 minutes to four hours. So we're increasing the window of time in which symptoms can occur.

And finally, I'd like to emphasize that the BaCon study it not just a single year effort. The study is ongoing, and we continue to seek reports of transfusion reaction related to bacterial contamination.

You have in your handouts as one of the slides my contact information. Please feel free to

visit our Web site. There's educational materials on the Web site that you can avail yourself to, slide sets, and the case definition is on the Web site, or feel free to call or E-mail me with any questions or any further case reports.

Thank you.

(Applause.)

DR. KUEHNERT: Thank you, Virginia.

In the interest of time, we're going to be holding questions until the discussion panel later on.

Next I'd like to introduce Dr. Pascal Morel, a medical officer at the Besancon -- excuse me. My French is not the most fluent -- Blood Bank in France. He graduated from University of French Medical School; has received Master's degrees in cell biology and molecular biology; serves as a member of the National Work Group Bacterial Incidence created by the French Blood Agency; and his research program includes the detection of bacteria in blood or blood products.

He's going to be speaking on the French experience in the prevention of transfusion incidence due to bacterial contamination in France.

DR. MOREL: Thank you.

It's time for you to test a little French-English. I will try to do my best.

Good morning, ladies and gentlemen. First, I would like to express our overwhelming thanks to the organizers of this workshop for inviting us and giving us the opportunity to present our experience in the bacterial contamination of the transfusion blood components.

I will talk about TRBC for transfusion reaction due to bacterial contaminations throughout my presentation.

The first slide. The second. The next, please. Oh, it's okay. It's not a good button.

My presentation is divided into four parts. In the first, I suggest to briefly remind you about the organization of the hemovigilance alert system and the organization, our older network, and the implementation of action over the last five years.

Secondly, the findings of the alert system over the five years.

And next, the results of the research program implemented during this period.

This is the organization of the alert system in France. Relatively complicated. Prior to this, the transfusion incidents were not reported at the national level, and now a local level rests on a network of hemovigilance correspondents in the blood

bank and in the hospital, are activated by an information coming from the health workers which an incidence has occurred.

The record system alert aims for, first, preventing other incidents; analyzing the consequences and the origin of each incidence; and too hemovigilance correspondents have to grade the severity and imputability for each cases. They have to send a form to original system in order to activate original plan of action, and they must send notification form to the Agence Francaise du Sang, French Blood Agency, within 48 hours when the case is serious or if more than one blood components is involved.

Concerning the severity and the imputability, the severity is graded on the scale with four levels: minor symptoms, long term death risk, which is not really useful in the bacterial contamination of the blood products, except perhaps with syphilis or some diseases like that, death threats and death.

The imputability or the reliability -- I don't know the exact word in English -- is rated on a scale with five levels: excluded, doubtful, possible, probable, and definite.

It's interesting to note at this time that

when the imputability is graded probable or definite, the bacteria was discovered in the blood products.

It was necessary to improve the quality of the imputability grading because at the beginning of the emerging systems, the imputability grading was analyzed at the time of recording the case in the French Blood Agency over the data gathered by the correspondent of hemovigilance, and only on the data available at this time.

That's why the French Blood Agency created a work group composed of experts in the bacteriology, in hygiene, epidemiology to analyze the level of imputability and to improve it. This group aims to raising physician awareness, to make recommendation, and to suggest modification to the operating procedure.

But it was not enough at this time, and it was necessary to improve the imputability grading, and with the set-up of the BactHem study, on which I will speak later, field investigator helps the correspondents of hemovigilance at the beginning of the investigations and in their data gathering.

Over the last five years, 22 percent of the deaths due to transfusion of blood components were due to the contamination, the bacterial contamination of the products. This is the first cause of death

associated to transfusion over the last five years.

The problem of the imputability is not alone. There is another weakness of the system that is the quality of the quality of the bacterial analysis. This slide shows that more than 23,000 transfusion incidence notification were recorded by the French Blood Agency. Among these, 7,030, suspicion of TRBCs were mentioned, and only 185 cases were confirmed.

What is very interesting is one-third of these 74 unconfirmed suspicions were due effectively to an exclusion because the bacterial contamination was not enclosed (phonetic) or the transfusion in general was not enclosed, but in two -- sorry -- in over two-thirds of cases, of these cases, the exclusion was due to an invalid inquiries or investigation, and notably the bacteriological analysis.

This pie chart presents the number of deaths and number of deaths and death rates and minor reaction over this period. Ten percent had a fatal outcome. Twenty-six percent present vital threats, and 64 percent, minor reactions.

It's interesting to note in this slide that over the two last years the high level of severity increased notably, and it could be explained by better quality of the inquiries.

This slide only to show the part of the platelets in the TRBCs, more than 37 percent. This is a complicated slide, and it's perhaps better to show the bottom on these slides.

For the incidence of TRBC with pooled platelet concentrates, the rate is in France now one TRBC out of 15,000 pooled platelet concentrates, distributed units. For other (phonetic) of these platelets, one death out of 135,000 distributed units.

The incidence concerning the red blood cells is respectively five to 12 less than APC or PPC.

The range for the blue platelet concentrates of the incidence is very, very interesting, between 26.3 and 156 at this confidence interval.

In fact, in France it's possible to consider at this time that for all blood components we can observe one death out of 909,000 and one death threat out of 27 -- 20 -- sorry -- 270,000 and one TRBC out of 135,000 distributed units.

Concerning the recipients/victims of TRBC, the mean age over this period was 62.2 years from birth to 94 years old. The proportion of male is quite different than the BaCon study with the proportion of male more than 73 percent.

The people are previously transfused in 80 percent of cases and are on antibiotics for 50 percent of them, and with disease, the origin of the transfusion with a certain degree of familial (phonetic) efficiency for 83 percent of them and under treatment with a certain degree of immunodeficiency for 58 percent of them.

Are relevant these type of characteristics? That is, this characteristic has a difference between this type of recipient with TRBC and a controlled population without. This is the answers we expect from the BactHem study inference.

This is the frequency of the symptoms reported during these five years. Of course, shivers of rigors. I talk about shivers. I don't know if it's different. Fever are the first and the most important symptoms. More than 70 cases over this period.

The tachycardia is also important in number, and it's important to note that in more than ten percent of the case, the shock was the first symptoms of the case.

The delay in appearance of the first symptoms is quite different between the Gram negative bacteria and the Gram positive bacteria, 15 minutes for the first and 68 minutes for the second.

The delay in appearance of shock when the cases was serious was 90 minutes, with big range between 15 minutes and five hours, and after the first symptoms, quicker with ten minutes only.

This pie chart represents the bacteria reported in TRBCs involving platelet concentrates. It's consistent with the previously published data. A big part of coagulase negative staphylococcus and part important bacillus.

The Gram negative bacteria represent more than 36 percent and all were in severe cases.

It's surprising to note that the bacterial in TRBC involving blood red cells is staphylococcus and streptococcus -- represents in half of the cases, and it could be perhaps explained by the taking into account of all of the cases with minor reactions.

This slide only to show you the list of the bacteria implicated -- involved in the resulting death of the patient. Almost 90 percent of the case were Gram negative bacteria with enterobacter, E. coli, Yersinia enterocolitica, and 51 percent platelets were involved.

When the result was vital threats, the part of the Gram negative bacteria still rests near 50 percent, and the rate is always 50 percent of

platelets.

It's different concerning the TRBC resulting in minor symptoms because in this case the Gram positive represent more than 70 percent of the cases, and the red cells were present more than 60 percent of cases.

The cause, the source of the blood component's contamination is not only for six cases among these 185 cases, and it represents 12 percent when the imputability was three or four. That is when the bacteria was discovered in the blood products.

In one case it was possible to prove a staphylococcus case from the donor's skin flora, and for the five hours cases, two urinary tract, one genital tract infection, and of course, infection with Yersinia enterocolitica in two cases.

I will briefly resume three of the different research programs implemented in France during these last five years. The French Blood Agency sent four proposals out for the research projects in this field, and six studies were supported by grant.

The BactHem study, on which I speak just after, a study of the factors likely to increase the efficiency of cleaning procedure at the phlebotomy sites.

Study on the effects of removing the first milliliters of donated blood after venipuncture.

A study forecasting the combined effects of storage, temperature, and leukocyte filtration, the growth of bacteria.

And two studies were meant to test automated culture systems.

BactHem study consisted in the comparison of the frequency of risk exposure between the case, the patient with TRBC, and the control population without TRBC. Its objectives aims at determining patient related factors in those circumstances.

The secondary objectives aims at standardizing diagnostic criteria, describing the clinical symptoms, and standardizing the minimal information required.

Unfortunately I expected to have these results today, but it's not possible. The statistical analysis will be available only in October.

In order to assess the discovered of the team, the Allouch team of Mr. Allouch, a study, a multi-centric study was implemented in France, and four different blood banks participated.

This study is to assess the potential effects of avoiding the first milliliters of the blood

entering the donation in order to prevent bacterial contamination of the whole blood unit. The method was quite simple. Bacteriological culture of the first 15 milliliters and the next 15; special devices produced by French firm MacoPharma. The cultures were done in BacT/Alert 214, and when it was possible the blood components related to contaminated samples were cultured as well.

More than 33,000 donations were tested, and the cultures were positive in 76 cases, with a range between two and four percent according to the different blood banks.

The new collection procedures avoided the introduction of bacteria in 55 donations. That is a reduction of the risk of contamination of the whole blood units of 72.4 percent.

This is the new collection bag, and this new method of donation is about to be generated in France. It's possible to use this bag for the blood screening.

Two studies were meant to test the automated culture systems. About 7,000 blood platelet concentrates were tested, and no real positives were discovered.

The feasibility was confirmed with a false

level of positive. The false positive control was lower than 0.5 percent. No contamination was confirmed, and at the same time, no TRBCs were recorded at the local hemovigilance system.

In conclusion, it appears essential after notification to rapidly -- and I think that rapidly is real important -- implement procedure to both remove the contaminated blood products from the circuit and investigate the source of contamination. It's necessary to develop standardized methods not only for bacteriological analysis.

And it still remains necessary to improve the public's awareness in order to obtain the notification for old cases.

Contains the research programs. The results of the various studies led to improving practices at the different steps rather than developing a blood testing method. Post donation withdrawal, advantages of systematic donor's blood count, or advantages of temperature measure are studying.

New research programs have been initiated aiming at exploring the behavior of bacteria in blood components in order to explain the poor results of controls by automated culture system in our experience.

And I will finish. This is all people who helped me, and I wish thank them.

Thank you very much.

(Applause.)

DR. KUEHNERT: Thank you, Dr. Morel.

Next I'm going to have Dr. John Barbara, who is currently lead scientist, transfusion microbiology, London and Southeast Zone Blood Service in North London. He was President of the British Blood Transfusion Society from 1995 to 1997; now serves as a microbiology consultant to the Canadian Red Coss; has published over 300 papers, chapters, reviews.

And he's going to be speaking today on the experience in England, "Bacterial Transmission: the U.K. SHOT Analysis."

DR. BARBARA: Thank you, Mr. Chairman.

Ladies and gentlemen, it goes without saying that it's a great honor and certainly a pleasure to be asked to come and speak here. A lovely place to be, excellent weather. The only problem was I arrived last night. I promise that I won't fall asleep halfway through my presentation. I can't guarantee that the same can be said for you in the audience.

(Laughter.)

DR. BARBARA: I shall try and be as rapid

as possible because what I'll be telling you really fits into a context of several systems that have been described here from the States with the BaCon study, from France with the hemovigilance, and indeed, the hemovigilance system is a more complex, more expensive arrangement that is producing some very high grade information, very detailed data.

It's not unusual that in Britain we do things on a shoestring, and really the SHOT analysis, the serious hazards of transfusion -- and, incidentally, we very quickly moved away from calling it "serious hazards in transfusion" --

(Laughter.)

DR. BARBARA: -- the SHOT system is really, I have to say, a poor man's hemovigilance. Those of you who have any knowledge of Britain will understand that this is not unusual.

Having said that, for a very small outlay we believe we can get some quite good returns, and through what I say I hope you'll see that the principles that are emerging from the other more complex initiatives are actually being reflected in what we've found.

I am a fervent disciple of Mo Blajchman. A lot of the things that he's said in the past, some of

those things were intuitive. Currently hard data is being accumulated to prove what he's saying, and again, several of the features that you see will be common to what's already gone before.

I have to acknowledge the help of Kate Soldan, who is a holder of a joint post. One of the really good initiatives we set up in the National Blood Authority in England was to have a joint post between the Public Health Laboratory Service and the Blood Service, and she's a genuine epidemiologist and has a produced a lot of this data.

We have a steering group that runs the SHOT system, and there is a working group that does all of the hard work in this.

It's a purely anonymous system, and we have produced two reports. There's one that's come out this year, one that came out last year.

It's a voluntary system, but it is, in fact, becoming necessary for hospitals for their accreditation to be participating in the system.

In the first year, we didn't have a new reporting set-up. So we didn't know how many hospitals didn't report any complications just because they didn't report. We now know how many can actually actively participate, and although I haven't got the

exact data, it is the majority of hospitals. They will tell us that they are aware of the system, but they have nothing to report.

And the context is that we're talking in the United Kingdom about three million blood donations. The system is U.K.-wide. Now, that means that England and Wales through the Public Health Laboratory Service are directly integrated.

Scotland, although part of the U.K., currently part of the U.K., has their own surveillance and public health system, but they liaise through Kate with us in the U.K.

Our cousins in Ireland are a little bit separate, but they are involved and associated. So, you know, it is a bit complex. You talk about England, Britain, the United Kingdom, the British Isles. I don't really understand it. I don't expect to be able to explain it to yourselves.

Post transfusion infection. I'll just again define here we draw a distinction between PTI, post transfusion infection, and TTI, transfusion transmitted infection. This isn't a play with words. Anything that is temporally associated with transfusion, any infections that occur after transfusion may get reported to us as a post

transfusion infection, and at this stage it's not necessarily defined as due to the transfusion.

When we believe it is due to the transfusion, we'll call it a TTI, a transfusion transmitted infection. So it's a nicety, but it's actually quite important, and I think that a lot of the data you'll see we are trying to concentrate on those events that can be proven to be transfusion transmitted, and they will be minority because undoubtedly because of lack of information reporting or under ascertainment or not having all the work-up completed, there are some cases that can't fit the definition, and we have to be objective about this and ruthlessly exclude them.

But it does mean that we are dealing with Mo's Titanic iceberg.

In this first overhead, just showing you that the whole thing really started to take off seriously in 1995, and it was the association between the blood services and the Public Health Laboratories.

Can I have the next overhead, please?

Although the system overall is anonymous when you're talking about all types of complications, for microbial transmissions it is de-anonymized (phonetic). It is more mandatory to be reporting

because obviously we are worried about infectious donors staying in the system who could subsequently return and infect new patients. We're thinking about other components that may currently be in inventory which could possibly be removed and prevent transmission. So the microbial wing of SHOT is de-anonymized.

I think it's also important to keep in mind the totality of complications, not just to be blinkard (phonetic) and think about the microbial complications, because it will also tell us -- and I think this is important -- what a small percentage of overall complications microbes are involved in.

What does come out after that though, again, as Mo has pointed out, is that of those transmissions bacteria are a very important percentage, and of course, they can often be -- bacterial transmissions can often be -- immediately fatal.

The system fits into the overall pattern of surveillance, which is becoming more and more refined because of the closer working and the organic interactions between blood services and public health services.

And I think this is an important point that everyone can take home. The blood services do not

function, cannot function in isolation. We do have associations with the public health services.

Next slide. Next overhead, please.

For the '96-'97 year, these were the 169 cases that came out as definable transfusion complications, of which we were confident they were transfusion associated, and of course, as I say, in the first year there wasn't a nil return system. So we don't know how many incidents might have occurred and not been reported.

The important point here, as I've already made: transfusion transmitted infections. Despite the huge amount of money that we are spending on these infectious complications, actually only represent a very small percentage of the total complications. Would that we could spend fractions of this money on just making sure that patients were properly tagged; they got the right components; and you didn't have all of these silly errors which are avoidable with just a little bit of time and a little bit of thought.

If I can have the next overhead, which will show you again the summary pie chart for last year or -- sorry -- the current year's SHOT analysis with 215 cases now.

Once the hospitals got over their panic and

stopped having to look over their shoulders about whether they were going to be prosecuted for their sins and actually understood that this was going to be a system that would lead to a better understanding, could enable us to develop ideas about how to reduce risks, they got more comfortable.

But having said that, even then you're still only dealing with two percent of the total number of complications that are microbially associated.

Next please.

As a summary for the microbial risks involved within a period here starting in '95, this is two and three-quarter years' worth. Again, to set the bacteria in the context of the whole of the -- now these are confirmed cases. They may be a bit of a tip of the iceberg, as I've said, because this is where we know we have all the data, and we believe that these cases are validated.

And you're talking about Hep. B, four cases; Hep. C, three cases; HIV, one case with three recipients; HAV. I mean everything is represented here, but notice that the bacteria are really a significant proportion, and again as Mo has said, we do precious little in comparison with all the other risks that are involved.

We also got a fatal malaria transmission in this particular period of time. So all life is seen here, but the bacteria with the usual -- no big surprises, consistent with what people have said before, but at least you see the whole context. These are the sort of bacteria that have been involved.

Next please.

And again, to set context, although you can have a lot of reports, it's only the reports that are put up here in bold, which is where you're actually concluding that there is enough data; you have enough validation to show that these are, indeed, transfusion transmitted cases.

And, again, for this period of time, the bacteria make a hard, distinct proportion of those particular cases.

Next please.

Now, to set context again, you may get a whole lot of reports coming into you, and then as you sift through you find down to a harder core where you can be confident that you've sorted out just what's happening.

So here in this particular period of time, 1995 to 1999, 17 post transfusion bacterial infections; 16 investigations closed; eight probable transfusion

transmitted infections; three investigations concluded not to be associated with transfusion; and then a whole bundle of inconclusive investigations either where you've got suggested bacterial transmissions but there's not enough data to show that it's happened or because you know that you can exclude -- next please -- you can definitively exclude.

Obviously this sort of thing becomes more complex. The more anonymous, the more voluntary the situation is, the more nebulous some of the data is. The more you can mandate, the more you can spend time and money on people rather than the situation in the Britain where you do your day job, and then a few victims are actually doing this as extras. The more resource you can put into it, the more clarity you can get out of it.

But I think the SHOT system is showing principles here, and again, looking at your analyses, eight probable transfusion transmitted infections. This is the range of bacteria that you'll see. You'll see deaths that are directly ascribed to the bacterial transmission. There will also be some cases where although the bacterial transmission didn't directly cause the death, it certainly didn't help in what is an already sick patient.

Next please.

And this is the sort of range of agents where you see that it's decided that they are not transfusion transmitted infections. I believe the organizers are going to be collecting people's slides and overhead. So detailed information will be available to people. In the time allowed I'm only trying to give you a flavor of what we're seeing.

Next please.

And, again, no surprises in the sort of bacteria you're seeing. These are the inconclusive investigations. Some of these may well be due to bacteria, but because of an absence of complete data, one has to be quite strict about this and just state that they are inconclusive.

Next please.

And just to remind you again of the sort of numbers of cases that will be coming through, the sort of reasons why you're getting transmissions when you decide it is bacterial contaminant from the donor's arm, donor culture positive from the same serotype. These are the criteria that you're using, and people will have to work out quite carefully just what criteria you use, whether they're exclusive, whether you're going to be very stringent or not.

And, again, this information will be available for people to read further when there's more time.

Next slide please. I think we'll skip that and go on to the next one, please.

Now, obviously when you start these exercises, you can come up with various conclusions. The first SHOT report concluded that TTIs are now very rare. Actually a disproportionate amount of money being spent on dealing with them, and what money there is being spent isn't being spent on bacteria.

National collation of data arising from these cases is vital, needs to be built over several years so that you get a picture of the extent and nature of infectious complications.

Next please.

You should have standard protocols for investigating post transfusion infections. It's great to see BaCon study in a big country, such as the United States, with a whole lot of agencies involved. The tasks are obviously very much greater, but it's excellent to start seeing coordination and collation, a national awareness, a national approach.

We have produced standard protocols. It's taken us about eight or nine years to do these. The

main sticking point was defining just exactly what should trigger an investigation and a report.

Having produced these first protocols, myself, Carl, and other colleagues were not surprised when, first of all, people weren't interested. Then they became passionately interested.

I don't think we've ever received as much criticism as with these protocols, but at least the process is set in motion. Awareness is higher. People are thinking about it.

Next, please. Very nearly there, Mr. Chairman.

Clinicians should report all post transfusion infections diagnosed in their patients to the blood service for appropriate investigation.

Next please. Next overhead please. And is that the final overhead or is there one more? I think I'll just go straight to the final overhead.

And a somewhat contentious recommendation that we made, which initially people said that they couldn't comply with, and I think slowly we are winning some of our colleagues around.

Hospitals shouldn't destroy blood components implicated in post transfusion reactions expected to be bacterial; should consult the blood

service about the investigation of such cases.

We don't prescribe exactly how it's done and what is done. We just want to make people aware, talk to the blood center, work out systems, know you have a method, and I think we'll gradually get more and more clarification to prove most points.

Thank you very much.

(Applause.)

DR. KUEHNERT: Next, Dr. Jong-Hoon Lee is going to be speaking. He's served as Chief of the Blood and Plasma Branch within the Division of Blood Applications since 1996 and will be speaking today for this talk on the FDA surveillance for bacterial safety of blood.

DR. LEE: Good morning. I'm reminded that I'm rather soft spoken. So I want to make sure that everyone can hear me. I can clip this up higher under my chin if that will help. I believe this is adequate.

Could I have the first slide?

I'm going to try to step through very rapidly a fair amount of slides just to give you an idea, a fairly comprehensive idea of what the FDA surveillance system consists of.

I will first describe the fatality reporting system, and then I will also briefly describe

the error and accident reporting system, and then since my presentation follows that of Dr. Roth, I'll try to just show one slide where we try to kind of reconcile the differences that we perceive between the FDA's fatality reporting system and the data accrued under BaCon.

Next slide please.

In terms of the transfusion fatality reporting, any fatal complication of transfusion is required to be reported as mandated under Title 21 of the Code of Federal Regulations outlined in Part 606.170(b). The report should be made to Office of Compliance and Biologics Quality of Center for Biologics Evaluation and Research within the FDA.

The report consists of the following elements. An initial report should be made as soon as possible, and typically within 24 hours by telephone, and that initial report is then followed by an initial written report, which is to be submitted within seven days.

And as the investigation matures over time, the subsequent follow-up reporting is also forwarded to OCBQ as they are made available.

This system represents a joint oversight among CBER of FDA, the Office of Regional Affairs or

the inspections force of the FDA, and also the Health Care Financing Administration in which the CBER participates as a coordination point and also a center for data compilation and analysis, and the ORA of FDA and HCFA performs additional inspectional follow-up to verify what's been reported and to assess the status at each blood center with respect to their correctable deficiencies in delivering transfusion therapy and also, along with CBER's analysis, to identify some trends on which there may be some new GNP requirements that could be considered.

Just to quickly go over the actual rule, when a complication of blood collection or transfusion is confirmed to be fatal, the Director of CBER shall be notified by telephone or telegraph as soon as possible, and a written report of investigation shall be submitted to Director, CBER, within seven days after the fatality by the collecting facility in the event of a donor reaction or with the facility that performed the compatibility tests in the event of a transfusion reaction.

This graph sort of quickly summarizes the number of reports. This is all reports of transfusion related fatalities reported to the FDA for a period of 23 and a half years, beginning with when the rule was

mandated in 1976 up to the present time, and you can see that there is a trend that is rising upward, and at this point we are projecting approximately between 60 and 70 cases to be reported for this year, and just this prior year it has been as high as 85.

Next slide.

Of these reports, approximately 50 percent, as analyzed through a sampling of eight years of data, approximately 50 percent is reported as a result of hemolytic complication typically due to a clerical error, and of the remaining, the bacterial contamination follows as the second leading cause, but this is also closely followed by transfusion related acute lung injury, non-bacterial infections, and transfusion associated graph versus host disease.

And at present -- just go back real quick -- and at present the bacterial contamination rate appears to be ten percent of the ones that are reported to the FDA, and it's averaging approximately 50 cases per year. I'm sorry. Fifty cases over the total period, about five cases per year.

If we look at the distribution limited to bacterial contamination for the period of approximately 14 years, there is also an indication of a rising trend in reporting, although the numbers are small.

For the first ten years since the reporting was mandated, between '76 and '85, we were averaging a little over two percent, which increased to something like five percent in the next decade, and for the third decade for which you only have three and a half years of data it seems to indicate approximately close to eight percent.

Next.

So this is sort of just a summary in a different form. From 1976 through 1999, for 24 years of data there are a total of 101 cases reported as bacterial contamination, of which only about 84 cases have actual specieation (phonetic) of organisms, and cases per year varies depending upon which set of years you select, but we could say approximately ten percent of all reports is related to bacterial contamination with about four cases on average, but more like seven cases for the recent data, per year in terms of numbers.

So it's clear that the reporting of transfusion fatalities to the FDA is increasing, and whether or not this is related to an actual increase in the fraction of bacterial contamination as a fraction of the units that are transfused, whether that's increasing is unclear, and hopefully not, but it's

probably a reflection of the fact that more blood is being used and also that there is an increased awareness of the mandatory reporting requirement.

And it's also clear that in all of these cases platelets are consistently more frequently reported to us than for red cells, and of the approximately 86 cases that we have fairly good data for, the ratio between platelets and red cells is fairly close to two to one, slightly above that.

Next slide.

Now, this is a listing of the different organisms that were seen. Now, I broke this up into three different categories: the organisms that are seen only with red blood cells; the organisms that are seen only with platelets; and then the organisms that are seen with both platelets and red blood cells.

And for red blood cells you can see that Yersinia enterocolitica tops the list at approximately 17 percent of the ones that were able to be identified, and then this is followed by clostridia perfringens, propionibacterium acnes (phonetic), and enterococcus with only one cases of each of the three.

For platelets only category, staph. epidermidis tops the list at six percent, followed by E. coli, bacillus, streptococcus, salmonella, and

proteus mirabilis, and this list so far appears to be consistent with all of the other reports that identified organisms associated with bacterial contamination.

For the category that I identified as having affected both red blood cells and platelets, klebsiella tops the list, along with staph. aureus and serratia species, to be followed by pseudomonas and enterobacter.

Next slide.

If we categorize into two groups, those that affect platelets and those that affect red blood cells and whole blood, in other words, dismantling the third group, just to make sure that we have a complete picture of what are the organisms that affect platelet components, actually klebsiella tops the list with nine cases reported to the FDA, which is representing 11 percent of all cases for which organism specieation was available.

The similar percentage was true for staph. aureus and serratia, and this is followed by sort of a middle group of staph. epidermidis, streptococcus, salmonella, pseudomonas, and enterobacter and E. coli, and then followed by bacillus and proteus mirabilis species.

Next slide.

So with the transfusion fatality reporting we have some idea of what might be going on, but obviously there's serious problems in under reporting, and we thought that we could get another piece of information by using the error and accident reporting system.

Under the error and accident reporting system, any error or accident that is related for any product that is made available for release should be reported, and this reporting authority stems from Title 21 of the Code of Federal Regulations under Part 600.14, and the report is to be made to also Office of Compliance and Biologics Quality of CBER.

Now, unlike transfusion fatality reporting, the reporting for error and accident is limited to licensed establishments only at this point, although there is a current proposed rule to include all establishments, licensed or unlicensed and registered or unregistered. In other words, all transfusion facilities that handle blood to be included under this rule.

It is worthy of note to say that for every fatality report, once a fatality has been implicated to be stemming from a transfusion, that should be followed

by an error and accident report, and this is not entirely clear, but most of the reports, in fact, the overwhelming majority of the reports under error and accident are after the fact reports. That is, the unit was actually released for clinical use and then some clinical event or some intervention long after the release has triggered an investigation, which led to the assessment as an error or an accident.

So in terms of bacterial contamination, most of the errors and accident reports are actually triggered by a transfusion reaction in which the culture results and the transfusion reaction work-up results implicate the actual bacterial unit used as the cause of the clinical sepsis.

So in a way, this is kind of a poor man's assessment of the rate of clinical sepsis that might be occurring as reported to the FDA.

Now, the role in CBER with all of this is to assess the potential for recall, but often when an error and accident is discovered by a transfusion facility, they have already initiated a recall, and usually FDA need not ask them to initiate a recall and constant notification.

And also, hopefully this type of analysis will recognize trends in product manufacturing

deficiencies.

So if we look at three years for which there is complete data available, you can kind of see that the numbers are pretty consistent from year to year, and on average, about 11,000 error and accident reports are received total, of which 158 reports represent those errors that are at risk for bacterial contamination, and by that I define in orange at the bottom. At risk means that the donor had flu symptoms or the donor had an unacceptable temperature or the unit's sterility was compromised or improper phlebotomy site preparation techniques were recognized or upon use platelet clumps were recognized.

So all of these are conditions that are recognized as an error, which indicate that these results could have resulted from bacterial contamination, but was never confirmed as such.

Now, in contrast to the 158 of the 11,000, only 50 on average per year represent cases that are actually confirmed to be bacterial contamination, that is, these error and accidents were, in the first place, identified through a patient reaction, the investigation of which revealed a bacterial contaminated unit.

Next slide.

So I tried to make some sense out of these data and try to make an assessment of the scope of the problem from the FDA's angle and also at the same time provide an assessment of the accuracy and comprehensive nature of the FDA as it is possible today, since we are getting the world's comprehensive view on the subject.

If we assume that there are approximately 20 million units that are manufactured and transfused in the United States per year, and through error and accident reporting we identify 158 at risk units and 50 confirmed units, which results in seven fatalities, you could generate the following numbers.

At risk units seem to occur in 1.3 times ten to the five units, where the use of these units results in clinical sepsis, in one times four times ten to the five units, and fatality that results from the use of these units occur at a rate of one in three million units.

Next slide.

So just from the angle of transfusion fatalities, to compare this data with that obtained under BaCon, for fatalities that were reported to BaCon between the period of January 1998 and June 1999, for a period of 18 months, there were 16 transfusion fatalities reported to the FDA during the same time

period versus three identified at BaCon.

And Dr. Roth already mentioned that the rigorous nature of the requirement to document the causality probably accounts for most of this -- for all of the deficiency. In eight cases confirmatory cultures were not available to CDC, and in an additional three cases the cause was ruled out or was unable to be confirmed as the cause, and in two cases, FDA inspection discovered the cases rather than proactive reporting from the transfusion facilities.

And in terms of clinical sepsis, the data for BaCon is actually too premature to make any assessments, but if you were to try to calculate some kind of a risk, it would be eight per 12 months, whereas FDA is receiving 50 per 12 months. So once again, there's large discrepancies which indicate levels of sensitivity of surveillance systems more than anything else.

Next slide.

So in conclusion, the fatality rate of roughly five to ten per year or one per three million units transfused is being reported to the FDA, and of the reports, platelets were twice more likely as the cause of fatality than red blood cells or whole blood.

Fatality from plasma use has not been

reported to the FDA, although it has been in the literature.

For platelet fatalities Gram negative and Gram positive organisms were reported at comparable frequency, and although the frequencies may differ as they're associated with rates of clinical sepsis, if you narrow it down to actual fatalities, they become more comparable.

For clinical sepsis rates, it appears to be anywhere from ten to 100-fold more frequent than fatalities, and in terms of clinical sepsis rates and fatality rates, these are both likely to be underestimates the magnitude of which we are unsure.

All of this, the FDA's data, the BaCon data, and all of the results reported in the literature are imperfect complementary surveillance data which, when taken together, might yield the true scope of the picture.

But through all of this it's fairly evident that bacterial contamination is at least as important as any other causes of transfusion complications that have been an issue for transfusion medicine today.

So at this point I would like to pause, and I have the honor of having the break behind me. So I think I'll stop for questions if there are any.

(Applause.)

DR. KUEHNERT: Thank you, Dr. Lee.

We're going to take a break now. We're a little behind, but I think if we reconvene at 10:30 with another of Dr. Lee's presentations, I think hopefully we can get back on schedule.

(Whereupon, the foregoing matter went off the record at 10:20 a.m. and went back on the record at 10:35 a.m.)

DR. BLAJCHMAN: Could everybody take their seats so we can start to try and get back on track?

Our next speaker for this session is again Dr. Lee. Dr. Lee attended a symposium in Heidelberg which dealt with various aspects of microbiological safeties, and he volunteered or we volunteered him to tell us about what news from Heidelberg.

DR. LEE: Only about three weeks ago an international symposium was held on very much nearly the same topic in Heidelberg, Germany. The international symposium was entitled "New Aspects in Microbial Safety of Blood Components," and it was a two day meeting on September 7th and 8th, and it was hosted by the University of Heidelberg and Paul Ehrlich Institute, which represents, I think, the FDA equivalent in Germany for blood oversight.

And what I hope to do in the next ten minutes is to give a brief overview of the symposium and then try to distill what I perceive to be the consensus positions.

Now, we didn't really declare this as consensus positions at the meeting, but clearly as the meeting progressed and discussions evolved, there were certain agreements that people generally recognized.

Next slide, please.

The symposium focused on three areas: the current status of the microbial safety of blood components in Europe, U.S., and Canada; the role of the microbiologic culture; and then the alternative testing strategies, alternatives to the culture, that could be considered in the future.

In terms of the status in Europe, U.S., and Canada, the rates of bacterial contamination varied widely depending upon what blood component you were talking about, the level of hemovigilance applied in assessing that rate, and also with what level you are analyzing the problem. In other words, if you are looking at it from a fatality standpoint, that's obviously a very different situation from the standpoint of clinical sepsis, which is different yet from the contamination rate discovered at the

laboratory which may or may not have anything to do with clinical event.

But overall, a number that sort of kind of emerged was a clinical event rate of something like anywhere between one to 60 per 10,000 units, and depending upon which study and what scenario you analyzed, the numbers were widely different.

The symposium considered not only transfusion blood units, but also considered hematopoietic progenitor cells for which the implication of a contaminated unit is entirely different.

Nonetheless, the contamination rates were far more prevalent in HBC than in transfusion units, and among the transfusion units, pooled units exceeded by the single donor units. This reflected the number of pools that go into -- number of units that go into a pool rather than any increased incidence of contamination per unit, and it was also clear that the contamination rate per unit for platelets was much greater than those for red blood cells and whole blood.

And it was also recognized that the Gram negatives that tended to affect red blood cells and whole blood, although less frequent than those for platelets, actually caused much more severe adverse

clinical events than in platelets.

Surprisingly, Yersinia in red blood cells and whole blood, which obviously had a small epidemic in the '80s about this, seemed not to be as important in Canada and in Europe than it is for United States, and it's unclear why this is given that Yersinia originates typically from donor bacteremia rather than being introduced during collection or processing. Perhaps it represents some difference in donor populations.

In terms of the role of the microbiology culture, this was sort of recognized as the gold standard for the moment anyway, carrying a sensitivity of approximately one to ten organisms per milliliter of blood.

And it was generally recognized at the meeting that the targeted use of the microbiology culture in some standardized format in platelets at appropriate storage time may be a practical alternative that can be implicated today and may, in fact, be also cost effective.

In terms of alternative testing methods, a variety of methods were briefly presented, and the sensitivities difference, and obviously, although these methods with varying levels of sensitivity had other

associated problems of practicality which precluded their use for implication in the immediate future, but holds promise as a method for implementation on a routine basis in the testing of bacteria for contamination.

Next slide.

Now, the meeting participants generally recognized this good manufacturing practice process flow where we tried to identify a sterile donor. Now, it's clear that there is no such thing as a truly sterile donor, and we have to be wary of the appropriate screening processes, particularly when you're dealing with the autologous donation. It's generally perceived that autologous donation is as safe as any blood donation, but that is not true for bacterial contamination. In fact, the complication rates for bacterial contamination in the autologous setting is probably much higher than for the allogeneic setting.

Given a sterile donor hopefully, sterile in terms of bacterial contamination, and given that you have an appropriately validated container collection system which is sterile, the two processes then come together. The two entities then come together in the collection where you try to collect the blood in a

sterile fashion.

And the key areas that were identified at the collection process as being problematic today is the phlebotomy site preparation and the potential of whether or not to divert the first ten to 15 cc's of blood in the hopes of diverting away that skin plug that Dr. Blajchman mentioned, away from the actual container, but divert this potentially to other uses, such as viral testing.

And the processing of that unit once collected leaves some room for improvements towards reduction of the problem. Perhaps there could be a standardized, targeted, culturing method that could be applied to intercept potentially contaminated units.

The introduction of leukoreduction has been recognized as being effective for red blood cells in whole blood, although not necessarily for platelets, and whether or not there could be actual inactivation methods either at the processing stage or actually built into the container systems were methods to be considered for the future.

Next slide.

Now, in Germany a standardized culturing/monitoring scheme was mandated and introduced in the late 1990s, approximately, I believe, introduced

in '97-'98, and it specified the following minimal requirements with respect to testing for bacterial contamination.

It allowed for either a manual or an automated method of bacterial culturing method, but there had to be an aerobic, as well as anaerobic arm with the surveillance, with the requirement that these be sub-cultured at the appropriate time.

The temperatures, storage temperatures, were specified to be between 30 and 37 degrees Centigrade, and the sampling requirements called for at least ten cc's of blood, and these were to be sampled at three days -- that should be plus/minus -- within three days of expiration or shortly thereafter, within three days after expiration.

And the number to be sampled at a particular facility per month was based on this equation, the validation for which I am not able to explain at this time, but a number, 0.4, times the square root of the total number of blood components manufactured at that facility per month was recognized as the number to be sampled. So not every unit is sampled, but there has to be a quality control system.

The incubation time was either 14 days for conventional culture systems or seven days for

automated culture systems, and the interpretation required confirmation/identification of all growths.

When this was instituted, two surveys were conducted, one pre-introduction of the requirement and one post, in 1997 without the minimal requirements and '98 with the minimal requirements. And the German experience indicated a fourfold increase in the contamination rate with the introduction of this standardized scheme, and the results suggested a bimodal distribution of blood centers that aligned itself in two distinct groups.

But there were two distinct groups for every blood component looked at, and not necessarily the two groups were the same for all blood components across the board. So it's funny how this bimodal distribution resulted, and the validation aspect of this study and the interpretation as two distinct groups was difficult to follow. Yet it was an interesting phenomenon to be looked at further.

In that study, according to after instituting the main requirements, the red blood cells land whole blood results were not significantly different from those for platelets.

Next slide.

After having reviewed the available current

data with respect to the scope of the problem and after a review of the available current biotechnology to intervene for this scope of the problem, a certain set of general consensus agreement emerged. Again, this was not declared as consensus, but this is what was readily apparent to the meeting participants.

Number one was phlebotomy site antisepsis where it was well recognized that at many centers the current methods which may already be somewhat insufficient for achieving optimal antisepsis is not even followed.

And in many European centers, this is already being practiced where the diversion of the first of ten to 15 milliliters of blood is delivered away from the blood container for purposes of testing, excluding bacteriologic testing, obviously, to protect the actual blood collected.

The method of surveillance revolved around the culture method, either conventional or automated, but in either case it seemed prudent to standardize the culture method so that the interpretation of the results can be compared across different centers.

And in many European centers, including those in Sweden and the Netherlands, this is already being implemented, where platelets at day three of

storage are subject to culture according to some QC frame, and depending upon the results, I believe every unit is tested, and depending upon the results, the shelf life of the product could be extended from five to seven days.

Now, platelet shelf life had at one point been seven days in the United States as well, although it was cut back to five based on a flurry of bacterial contamination reports. So perhaps this targeted use of the culture method available today allows for increase in shelf life for protection against bacterial contamination while at the same time increasing the blood supply and costs associated with it.

Leukoreduction of whole blood and red blood cells, when done in an appropriate time frame, typically within the first day and more like within the first 12 hours, but not sorely, such as two hours; in other words, there was a window of anywhere between eight hours and the first day, which appeared to give you the optimal effectiveness in terms of intervening bacteria, given that most bacteria that have been implicated for red cell fatalities and reactions typically reside within the granulocytes; that leukoreduction within the appropriate time frame to intercept these granulocytes before they disintegrate

and release the bacterial appear prudent.

So this is being considered by the FDA as a potential way to routinely manufacture all blood anyway, and perhaps we should consider this in a way that also addresses the problem with the bacterial contamination.

And it was apparent that although the numbers are small, the autologous donor needs to be educated appropriately about the need for potential bacteremia that might subject that donor to great dangers of transfusion, if not properly collected.

Next slide.

So as a model, international standards could be generated, which will affect obviously interaction between the regulator and industry, which each produce their set of standards that are consistent with each other.

And then overall it winds up in a good manufacturing practice scheme, and so far it has been strictly processed, QC oriented, but perhaps these processes can be improved, as well as introducing product elements as well to further improve the blood supply with bacterial contamination.

Next slide.

So in summary, the symposium focused on

three areas: the current status, the role of the culture, and a round table discussion on strategies that are available immediately to improve our current situation with respect to the problem.

And the consensus that arose was that bacterial complication was actually more important, much more important than viral complications or other threats that are perceived today as being threats for the blood supply.

And at present, relatively simple, new GMP standards could be introduced to alleviate the problem, and in the future the routine detection and even inactivation methods may be available to further control the problem, and these measures that are available even today may be effective, as well as being also cost effective at the same time.

And I believe the same themes that occurred at that meeting we'll also hear today hopefully, and I hope they're consistent.

(Applause.)

DR. BLAJCHMAN: Thank you, Dr. Lee.

When the organizers put together this program, we wanted to have the opportunity for poster presentations. Because there were only a handful of poster presentations proffered, we decided to put the

posters on the program for a short presentation.

The first one of these is by Mindy Goldman, who is the Senior Director for Medical Affairs for HemoQuebec. Mindy will talk on hemosurveillance of bacterial contamination in Canada over the last three years.

DR. GOLDMAN: Thank you very much.

So hopefully the new technology will come through here as we switch computers.

And I should say that my talk, the equation that summarizes my talk is C minus MB, that is, it's Canada minus Mo Blajchman. So there's not --

(Laughter.)

DR. GOLDMAN: -- that much data there, and the numbers come from the Canadian Red Cross, and then since October of last year from the two new blood suppliers in Canada, who are HemoQuebec, for the province of Quebec, and the Canadian Blood Services for the rest of Canada.

Maybe we should go back to overheads here. Is there a problem? If I could be released from the microphone, I will get my overheads.

(Pause in proceedings.)

DR. GOLDMAN: Can I have the first overhead, please?

Okay. Now I really have to talk very quickly. So the two methods we have in Canada of assessing the frequency of bacterial contamination are surveillance cultures that are done on a fixed number of products that are produced in each blood center and reporting of adverse reactions.

Next please.

The surveillance cultures for Canada are done in three different laboratories.

No, I wasn't -- yeah.

Two of them are using automated blood culture systems, and one is using a fluid thioglycolate medium. For apheresis platelets, the cultures are done on the day of production on a segment that's attached to the product, and for red cell and whole blood derived platelets, the cultures are done by a sample taken directly from the product, and this is a destruction culture with the product having to be discarded after.

Next please.

The circular of information that is distributed to all hospitals in Canada specifies that serious reactions have to be reported to the blood supplier, and it specifies that this includes all bacterial contamination, and then the suppliers have to

provide this information to our regulator, which is Health Canada.

And there are standardized forms, but no standardized investigation protocol for those reactions.

So these are the results of the surveillance cultures over a two year period. You can see for thrombapheresis platelets there were close to 5,000 cultures. Eight were positive for a rate of 0.17 percent, and a 95 percent confidence interval of .05 to .29 percent.

For red cell units, close to 4,000 cultures were done. Nine were positive for a rate of 0.23 percent, and a range of .08 to .38.

And finally for whole blood derived platelets, close to 5,000 cultures were done. Four were positive for a rate of .08 percent, and the confidence interval of .01 to .16 percent.

And this number is very close to that found by Dr. Blajchman in his studies in Hamilton.

The organisms isolated are pretty much what you would expect. There was one red cell unit that had fungal growth; one platelet unit that had prevotella loescheii, which is a Gram negative rod that's usually found in the mouth; and then 19 had bacteria that are

usually part of normal skin flora.

In terms of the transfusion reaction, 11 were reported, seven involving platelet pools and four involving red cell units. I should say that 95 percent of the platelet transfusions in Canada are platelet pools. Only three of the 11 were actually well documented. In two cases involving platelet pools, the same organism was isolated from the pool and from the patient, one involving Group G streptococcus and one involving staph. epi., and Dr. Blajchman has already described the fatal reaction involve S. aureus.

So my conclusions are that surveillance culture rates over the past two years were positive for .08 to .23 percent, depending on the blood components. However, there's a lack of standard methodology and a small number of cultures are done, and so it's very hard to follow if there are any trends with changes that we've made, such as changing our way of skin disinfection, universal leukodepletion of our platelets.

And as Dr. Lee mentioned, I think the guidelines that were developed in Germany would be important to improve the data that we're collecting here.

And lastly, in terms of transfusion

reactions, at least one fatal reaction occurred. Under reporting was likely. There was a marked over representation of the province of Quebec in the data.

Etiology, as other speakers have mentioned, is often difficult to determine, and finally, I think the introduction of transfusion safety officers in hospitals in Quebec this fall, similar to the French hemovigilance system, will lead to improvement in the reporting and investigation of these reactions.

Thank you.

(Applause.)

DR. BLAJCHMAN: Thanks, Mindy.

The last presentation of this session is by Dr. Lance Trainor, who is Director of Apheresis and the Associate Medical Director of Community Blood Center, Community Tissue Services, and he will talk on fatal transfusion or a fatal transfusion reaction due to the transmission of enterobacter cloacae.

Dr. Trainor.

DR. TRAINOR: Good morning. Thank you for having me here today.

I'd like to admit that this is a new interest of mine, bacterial contamination of blood products, and you're about to find out why.

Can I get the first slide, please? Can we

back up one slide?

This is an issue that came to my attention this past May. I was in my office heading out the door to enjoy a long Memorial Day weekend of camping. I had just closed the door. The phone rang. A local clinician called me. He said, "Can you transfuse or can you transmit bacteria from a blood product?"

I said, "Sure, but it's a rare event."

And then rather than saying, "Have a nice weekend," I said, "Why are you asking?"

And then he proceeded to tell me. I'm going to talk about a fatal transfusion reaction due to transmission of enterobacter cloacae from an asymptomatic donor to a recipient by an apheresis platelet product.

The story goes as follows. A 70 year old woman was admitted to the hospital for treatment of advanced small cell carcinoma of the lung. She had previously undergone three cycles of chemotherapy. She was admitted because she was pancytopenic and was suffering from GI bleeding.

She received two units of packed red blood cells uneventfully. Immediately following she received a unit of platelet apheresis. After receiving 72 mLs of the product, she developed rigors, shortness of

breath, elevated blood pressure, elevated heart rate, nausea and vomiting.

Blood cultures were immediately drawn. At the time of blood culture draw, Gram stains were performed. It showed Gram negative organisms in the patient's blood at that time. Empiric antimicrobials were administered. They included Zosyn, Tobramycin, and Diflukin, and the patient expired secondary to Gram negative sepsis 22 hours after the transfusion of the platelet product.

The reaction work-up included a patient blood culture which demonstrated enterobacter cloacae, which was also cultured from the platelet product. A collection record review was performed which was unremarkable. The product had been collected 60 hours prior to transfusion.

The disposables were all quarantined. They included the anticoagulant, the saline, and the apheresis kit, and the manufacturers were contacted, and they reported no other ill effects with the lot number specified.

The machine on which the product was collected was overhauled, and no deficiency was found.

The unit appearance was perfectly normal upon shipping and at issue from the transfusion center.

No clerical error was identified.

The donor was a 57 year old woman in apparent good health. She had recently undergone an unremarkable physical exam that included a negative stool guaiac. She had donated 138 times in the past without problem, 57 whole blood donations and 81 platelet apheresis donations.

Upon extensive questioning of the donor, she did recall passing loose stools on the evening of the collection, but she did say that this is not unusual for her.

Additional work-up, enterobacter cloacae was isolated from the donor stool sample. We subsequently asked the donor to come in, give us another stool sample. We selectively cultured for enterobacter. We did find the organism, and then this was at the suggestion of Dr. Roth from the CDC, who spoke earlier. We sent them that culture, and they did strain analysis, and it was shown that the enterobacter strain from the donor, the recipient, and the product were all indistinguishable by pulse field gel electrophoresis.

We then asked the donor to come in for a subsequent donation. We quarantined the unit. We cultured the unit, and it demonstrated no growth after

five days.

So in summary, we had a fatal transfusion reaction caused by a contaminated apheresis platelet product. Essentially an identical strain of enterobacter cloacae was identified from the recipient, from the product, and from the donor, and there was no equipment disposable or protocol deficiency identified in the collection.

This brings up certain questions for us. What is the mechanism of transmission? Could this be a transient bacteremia or could this be a skin contaminant? And actually more important to myself, this is a committed donor who wishes to donate again. She's called me weekly since this event occurred. She is not aware of the outcome of the recipient, but she does know that there was a bacterial organism found in the platelet product. She desperately wants to donate again. She's a very loyal donor, and the question is: would any of you accept her as a donor again, given a history of 138 prior donations?

And finally, I'd like to give thanks to the people listed here both at our blood center in Dayton and also to the Centers for Disease Control.

Thank you.

(Applause.)

DR. BLAJCHMAN: I'd like to invite all of the people who spoke this morning up to have a panel discussion.

I think we'll see how the discussion goes, but I think we'll only have about a 15, 20 minute discussion rather than the scheduled half hour because we're already short on time.

Perhaps we can start with the very last question that was posed by this interesting case. Perhaps, Matt, do you want to maybe ask? The question relates to would you take this donor again. Put you on the spot.

DR. KUEHNERT: You're asking me?

DR. BLAJCHMAN: Sure.

(Laughter.)

DR. BLAJCHMAN: There's microphones on the table that I hope are on.

DR. KUEHNERT: Oh, are they on?

DR. BLAJCHMAN: Can we have the microphones on the table --

DR. KUEHNERT: I think they're working, yeah.

I think it's a difficult question, but I think, you know, you have to look at what the infection in the donor actually was, and it looked to be that she

had a positive stool culture and perhaps either had bacteremia from that or it contaminated the skin.

But you know, I guess I'd ask a question, which was: was she treated in any way for her what seemed to be a mildly, if any, symptomatic disease?

DR. TRAINOR: No, she did not receive any treatment. She was essentially completely normal, in her normal state of health.

Enterobacter can be found in normal individuals as a colonizing organism. I'm not suggesting that she had an infection with enterobacter, but merely that she was colonizing --

DR. KUEHNERT: Right.

DR. TRAINOR: -- with the same strain.

And I know it's a difficult question to answer. I have E-mailed many people in the field, some of you in this room, with this particular question, and I get variable answers, very extreme answers.

DR. BLAJCHMAN: John Barbara, do you have any thoughts on this?

DR. BARBARA: I suppose one thought is that this was an individual case of bad luck, that if we were to routinely screen and test a whole variety of people, you'd probably come across individuals like this. We wouldn't have any thoughts about taking them

off the panel.

I think probably what you would need to do is with a group of colleagues agree on a protocol whereby you satisfy yourself that this particular individual is not likely to be continually colonized, set up a validation, and at some point decide that she's going to have to be, you know, returned to panel.

You'll have to explain to her to some extent that there was this concern with this particular organism, and this is why you're having to do this, because you cherish her donations and you value what she does for you.

DR. BLAJCHMAN: Perhaps one solution could be to do cultures regularly on her products. We should do it on all products, but perhaps in this case.

Can I invite questions? There's two microphones, and please ask questions to whomever you'd like.

Ed, you start.

DR. SNYDER: Ed Snyder from Yale.

Dr. Goldman, could you give us some background on the transfusion safety officer as to who pays their salary, by what authority, and who they're responsible to, and just a little general background, please?

DR. GOLDMAN: Their introduction is following our whole inquiry that we had in Canada into the blood system. Quebec had its own mini inquiry that came up with a report. In that report it was recommended that the hospitals be grouped and there be sort of hub hospitals that are responsible for the small hospitals in their area, and that there be these special transfusion safety officers in those hubs that would be responsible for educating a staff about transfusion safety and further reporting for transfusion committees in the hospitals and so on.

The money, like all health care bucks in Canada, is coming from the "governement," and they're just starting up, and so we'll just have to wait and see.

And, of course, there's a lot of influence in Quebec with the French model because, you know, there's "une lengage au commune" (phonetic), and so sometimes we catch ideas from them.

MS. HOWLEY: Rebecca Howley, the American Red Cross.

Dr. Lee, I would like to comment on your presentation where you compared the number of bacterial contamination incidences that were reported to the FDA and compared those to the BaCon study.

Since we collect and distribute more blood than anybody else in the U.S., and since we have a very tight system for monitoring, controlling, and reporting our incidences, and since we are some of the organizers and investigators on the BaCon study, I can tell you that what we report to the FDA as bacterial contamination incidences is not at all the same as what we report to the BaCon study. The requirements are very different.

In fact, for deaths that are reported to the BaCon study or even serious cases, it's between one to five and one to ten of the ones that we are required to report to the FDA by our reading of the regulations.

They are not intended to be the same thing. So to say that it's under reported and the people are irresponsible about reporting because they are not the same, I think, is not quite accurate.

DR. LEE: Yes. Point well taken. I apologize if I implied that people were irresponsible. I tried to point out the differences, and I tried to state that the criteria for reporting for CDC was much more strict than those for FDA, and I had intended to say very much the same comment that you just made.

DR. BLAJCHMAN: Virginia.

DR. ROTH: Thanks. Just one other point of

clarification.

If you compare the FDA system with BaCon, we're really looking at two different things. FDA concerns themselves about the unit itself and the safety of that unit. We're looking at the recipient. So our data is coming from what's happening to the recipient and adverse recipient reactions, not manipulation or a problem, a possible problem with the unit.

So they are really two completely different things, but I think complementary, and I hope we can continue to work together in this.

DR. BLAJCHMAN: Question, Roslyn Yomtovian.

DR. YOMTOVIAN: Roslyn Yomtovian from University Hospitals in Cleveland.

And I'm going to direct this question at Dr. Lee. You've mentioned twice, at least twice, that bacterial contamination is at least as important as viral disease. Does that statement indicate that it will be treated as seriously from a regulatory oversight as viral disease?

And as part of that question, I think on one of your last slides, if I'm getting this correctly, you indicated that there are really two mechanisms, kind of a voluntary standards or accreditation or

regulatory.

If things are voluntary, obviously they won't be nearly as effective as they would be if it becomes regulatory. So what are your thoughts on regulations for addressing this problem?

DR. LEE: Well, I believe that's exactly the focus of today's workshop, to recognize the problem, to surface the problem to the public level for an open exchange of ideas, and to hopefully derive some information on which the agency could consider figure requirements about this area.

When I recognize it at least as important or even more important, I guess I was sort of wearing a personal hat, and I did not necessarily reflect the agency's assessment of the problem. I think it's premature to do that, and I have learned over the past three years within the FDA that is not a good thing, that you should always distinguish your opinions from those of the agency's.

And if I didn't make that clear, then I should have.

With respect to the voluntary versus mandatory requirements, you're absolutely right. I think the French data that Dr. Morel presented to us sort of speaks for that. It's a very elegant system,

very comprehensive system, and it's the best available, generating the best available data in the world.

And I think that results from the mandatory, proactive nature of the assessment. Whereas our BaCon study in the U.S. is proactive, but it's voluntary, and the FDA reporting is mandatory, but is retrospective.

So I agree with you that such a regulation, if it materializes, would contribute to a clearer picture and a safer blood supply, but that's why we are here to discuss.

DR. BLAJCHMAN: Dr. Barbara, you had a comment?

DR. BARBARA: Just a quick caution that we maybe ought to do this in two phases. First of all, heightened awareness. Collect data; decide on feasible, practical things that can be regulated before leaping into regulation.

Nothing is worse than having rules and regulations that are hard to work and hard to police and hard to get anything back from. So I think at this stage don't leap into regulation yet.

Now we've increased awareness. Let's start getting science systems that can be well regulated and that will be helpful.

DR. LEE: Thanks for substantiating my initial comment about collecting data and ideas and opinions about this. I agree with you 100 percent.

DR. BLAJCHMAN: Joe.

DR. FRATANTONI: Joe Fratantoni from Rockville.

John, I'm not intending to be piling on, but I have a comment also about something that you presented. You had a comparison between deaths from reported bacterial contamination and then viral infections. I think I just want to point out that with most of the viral diseases, deaths will occur many years after the transfusion, and the connection and the reporting to FDA is probably a small fraction of what it is for the bacterial deaths.

So I think the comparison may not really be one that you could just make as opening as that.

DR. BLAJCHMAN: A corollary to that comment that I would like to make is that one of the reasons, in my view, that bacterial contamination issue has not been dealt with adequately by the government's transfusion medicine community is precisely because of that, that you have with AIDS and with hepatitis, you have a chronic state and, therefore, a set of victims that are alive, that can talk and make comments.

Whereas with bacterial contamination you either die pretty quickly or you recover. So there's no chronic state, and there's no group that will agitate towards doing anything about it. So I think we have to agitate.

Next question.

Is that microphone on? It doesn't sound like it.

PARTICIPANT: You can turn it off.

DR. BLAJCHMAN: There we go.

PARTICIPANT: Okay. Are there any recommendations -- do any of you all have recommendations or is there any data that supports the use of white counts in the donor screening process?

Because at my blood bank now we're only using hematocrits and the platelet counts, and as a hematologist, that's always bothered me just a little bit. Is there anything that supports the use of that?

DR. LEE: I'm not aware of any firm white count cutoff on which to base donor deferral.

PARTICIPANT: I just wonder if any of these bacteremia cases -- if the donor might have had a high white count.

DR. LEE: Yeah.

PARTICIPANT: But there would be no way to

go back and look.

DR. LEE: That's information that's available on retrospective look, but I can't substantiate any actual reports about it.

DR. BLAJCHMAN: And that's not surprising. Well, there are no data, but it would surprise me that the white count would make a difference because in my opinion, and based on the fact that most of the contaminants are skin contaminants, that doesn't induce an elevated white count, and the odd patient who has a transient bacteremia or even a chronic bacteremia, these are patients that are asymptomatic, and I wouldn't expect them often to have an elevated white count because when they're interviewed, they're well. And so I wouldn't expect that to be terribly valuable.

Mark.

DR. BRECHER: I'm a little concerned about the under reporting that I think we're still going to be seeing with the hemovigilance and the BaCon study. It's still somewhat of a passive system. You're not actively going out there and looking for the cases.

And Matt and I have had some discussions in the past about probably the only way to really pin this down is to have a sentinel hospital type of study where cases are reviewed, patients' charts are reviewed 24

hours after the transfusions because many of these reactions are delayed, and having a cutoff of 90 minutes, four hours, six hours is probably not going to be sufficient.

For example, there was, I think, a classic study that came out at the NIH in the '70s that reported a series of salmonella platelet infected units where the time from transfusion to the time of disease was five, six, seven, eight days after the transfusion, and the way that we're currently looking for things, we're losing all of these reactions.

We would have not picked up Mo's case that occurred 24 hours later. So what is the prospect of doing a full prospective, sentinel hospital type study? Is there any chance of doing that to really define what the problem is?

DR. KUEHNERT: Well, I think you bring up some good points, Mark. I think that certainly the surveillance systems are not going to capture every case by a long shot, and I think that the idea of sentinel centers is a good one.

I would have to add that I think that it is crucial to maintain national surveillance at the same time.

Something that was mentioned at an AABB

audio-conference was important in that reports to the BaCon study small centers were most commonly the centers that reported, and I think that sort of was a difference from what people expected.

So I think that keeping the surveillance and keeping it national is critical. That said, I think that there's going to be biases in these reporting systems, and some of those we can deal with and some of them we can't.

The sentinel centers, I think, would solve some problems. I think there would be other problems as well. I think the biggest obstacle, though is funding for those.

I think the idea of having safety officers in every hospital is a great one. Trying to implement that I think would be difficult. Even trying to do that in sentinel centers, I think that would be the only way to go, would be to have these safety officers who bring these events to clinicians' attention because that really is the biggest obstacle, is clinician awareness so that they can initiate reporting. That's the biggest problem we've come up against so far.

But I think that it's a very good idea and, you know, would be a great thing to implement.

DR. BARBARA: I endorse everything you

said, but I would say that certainly in the U.K. there's no way we're going to be funded for anything like this, other than as a specific one of piece of prospective research. It wouldn't be on a routine context.

DR. KUEHNERT: Yeah. I think, you know, I think that on an indefinite basis. I don't think it would be possible here either, but it would give another piece of the puzzle to do that.

Oh, on the second point, as far as the timing of presentation of symptoms, we have wrestled with that issue. We had initially set it at 90 minutes because that was the time that we had seen in previously cases which were dominated by Yersinia cases, which is, you know, one piece of that in Gram negative sepsis.

In looking at our data, there were cases that were reported beyond the 90 minute window. We've extended it, and of course, it's come to our attention on numerous other cases, some of which were associated with, for instance, central lines and probably secondary bacteremia, some maybe not.

I think there are always going to be exceptions to the rule, and I think we have to keep that in mind, and I think we have to remind people that

there are exceptions to the rule, but I think we need to have some rule, and you know, this will be an evolving process not only in the timing of symptoms, but also in the definition of probable cases, which is something that we're looking at very closely and want to include so that we can give a better picture as to how many of these events are occurring.

DR. BLAJCHMAN: A quick question.

DR. HEATON: Yes. Andrew Heaton of Blood Systems.

I have a question and a comment for Dr. Lee. I notice you raised the concept of applying a dating limitation to three days on platelets or some form of bacteriological culture when platelets are transfused after three days, but you know, over the last year we've introduced NAT testing utilizing the pooled approach, and the effect of that is that most platelets are only available for transfusion at about 48 hours and often close to 72 hours.

And that would pose an enormous limitation as a practical matter to supplying platelets for transfusion.

My question to you relates to an alternative strategy, and that is as part of the Best Committee of the International Society of Blood

Transfusions, we've done a comprehensive study on the use of the swirl test or visual inspection of the platelets prior to release, and it's a fact that platelets usually do not swirl when they are bacterially contaminated.

And I believe that the majority of bacterially contaminated platelets could probably be intercepted by a much less technologically complex and a much less expensive visual inspection of the platelets prior to transfusion.

I would appreciate your comments.

DR. LEE: Yes, I believe we're going to hear much more about detection methods, including even simple methods such as the swirl test, in the subsequent sessions to follow.

One comment about bacterial contamination. Intrinsically it's different from viral contamination in that viral contamination, it's there, always there. The best time to detect it is at the earliest possible time.

Not true for bacterial contamination. In fact, every unit is probably contaminated to some degree. It's just that it doesn't amount to much depending upon when you use it.

So for platelets that are used within 72

hours, it's probably contaminated at the laboratory level, but it probably doesn't make any difference from a clinical standpoint.

So what you're really interested in is those units that are clinically going to cause complications, and probably the best time to test, subject them to these for the detection of bacterial contamination is probably at issue. Obviously, with the limitations of the culturing method, you can't do that at issue. So what do you do?

You try to strike a compromise, and three days appears reasonable, where anything below that you need not test and it's probably okay. Anything above that you have some results. That gives you an opportunity to intervene, and also at that point the culture results are sufficient in terms of its sensitivity to allow possible increase in out date from five to seven, which will alleviate the burden that it might place.

So the inherent difference in contamination character between viral diseases and bacteria I think will allow for some of these methods that on the surface seem inappropriate.

DR. BLAJCHMAN: Dr. Barbara, you had a comment.

DR. BARBARA: Yeah, just a slightly different angle that this should be looked at. If we're going to introduce NAT and there's going to be a delay in turnaround time, we might actually use this as an opportunity to help ease in some form of bacterial culture in some way, you know, if we decide exactly how we do it, to extend shelf life, to alleviate -- we use this to alleviate some of the delay pressures that come from the NAT results.

So you know, we may actually have a bit of a silver lining out of what otherwise is just a cloud, in my opinion.

DR. BLAJCHMAN: And the last question of this session, Len, and then you can come up and chair the next session.

DR. FRIEDMAN: Thank you.

Comment for the panel. Len Friedman, American Red Cross.

I'm very concerned about what the Germans are doing, especially since they seem to be promulgating it in Europe. What they've introduced is what I would call statistical process control on their products. It's not product release testing. They're just looking at products, including frozen plasma, for example, where bacteria have never been implicated.

So the question is: if we go ahead and consider what they're doing as part of our overall strategy, we have to be very careful about deciding what do we hope to learn from it. Are we learning the same information other ways, and how can it improve our safety? Because it's not product release.

DR. GOLDMAN: Yeah, maybe I could address that a little bit because I was at that meeting in Heidelberg.

What they're doing basically, as you said, is they're doing destructive cultures on approximately one percent of their production of platelets and red cells, and they have a very fixed protocol as to how they're doing them.

I think what was very interesting about that approach was that they had sufficient statistical power to see if there was a difference from one year to the next when you then introduce some change in your process, like you change the way you disinfect the donor skin or you do universal prestorage leukodepletion and then you wonder have you actually made a difference on your contamination rate or not.

And we've had a lot of discussion about how, you know, there's a lot of trouble collecting the transfusion reactions, and so another way to do it is

to look at these cultures, and because they're doing so many of them by a standardized method, they might actually be able to see differences.

For example, at that Heidelberg meeting, they showed data that the rates seem to be lower after prestorage leukodepletion of their products. Now, they didn't show statistical analysis on that, but it would be very interesting to actually see that.

I'm not saying that I agree with their one percent or that every place has to be doing this, but I could see that there's some very useful information you could get out of that.

DR. BLAJCHMAN: Okay. I think we'll close that session. There are still many other questions that we can answer, but in the interest of time I think we'll go on with Session II. That will be chaired by Len Friedman and Mark Brecher.

So I'd like to invite those two people to the podium, and I thank the speakers of this session for their input and thoughts.

(Applause.)

DR. BRECHER: Okay. We'll start Session II. I'd like to introduce Len Friedman. Len obtained a B.S. in chemical engineering from the Polytechnic Institute of Brooklyn, now Polytechnic University, and

M.S. and a Doctorate in Science in chemical engineering from Columbia.

He established the Biomedical Engineering Laboratory at the American Red Cross in 1973, and this group evolved into the Holland Laboratory, the central research and development facility of the American Red Cross.

He's been mainly interested in applied or translational research, and he is going to be talking on test characteristics and operational implications.

Len.

DR. FRIEDMAN: Thank you, Mark.

If we can have the first slide. We'll see if old technology works. There we go.

This session is detection methods in bacteria.

Next slide.

And what I thought I would do would be to set the stage by discussing some test characteristics and operational implications which you might want to keep in mind as we hear the presentations which follows.

First of all, no one has really talked about what the bacterial load is when we collect a unit. It's not that I know what the answer is, but our

best guess or best estimate is that there might be somewhere between .1 and 1 bacteria per milliliter.

So what are the implications of this? The implications of this is at the time of blood collection, there aren't very many bacterial around. If you take a small sample of blood or platelet, for example, from the bag, what is the likelihood that the sample you take is going to have a bacteria in there, and what is the likelihood that your test is going to have the sensitivity to detect it?

So, therefore, that also implies that if you sample early on when the number of bacteria are going to be relatively low, you're going to need a test with high sensitivity.

That also implies that the test may have a high rate of false positive reactions, which will cause false positives, which will potentially cause other problems.

The next issue is what is the level which leads to sepsis, and once again, if you review the literature you'll find a number in there times ten to the fifth organisms per milliliter. That's essentially saying if it's above that level, there is a chance of leading to sepsis, but there have been no documented cases that I'm aware of where at below that level it

has led to sepsis.

Of course, sepsis is an issue that is not only what the bacterial load is that's being given to the patient, but the patient's immunological status and whether or not the patient is on antibiotics and other factors.

So what does this mean? This means that if we have a test that is going to be implemented, I think -- personal opinion -- that it should have a sensitivity of below ten to the fifth organisms per milliliter, but let's remember there's a time differential between when the sample is taken and when the product is transfused, and bacteria can grow during that time.

So you need some safety margin, and that safety margin will depend upon what the organism is, how fast it grows, and what the time is between sampling and transfusion.

This is just to show you a model of slow, medium and fast growing organisms. If it's a slow growing organism where the doubling rate is perhaps every eight hours, within the normal shelf life of a platelet product, you're never going to get to the ten to the fifth level.

If it's an organism that can double every

four hours, you get there in three days, but if it's a fast growing organism, you can get there very rapidly, within the first 24 hours.

So, once again, the fact that you're testing is not good enough. The question is: how good is your test? And what's the time between testing and transfusion?

Now, we've heard this morning that we have both Gram positive and Gram negative organisms to worry about, and at this point for a, quote, screening test, one to tell us is a product contaminated or not contaminated, do we care?

I personally don't think we care. I want a simple yes/no answer. Is this product below a certain threshold or above a certain threshold? However, at some point if it does lead to a septic event, we do need to know more about it and need to know whether or not it's from the person or whether or not it might be from the bag or the environment.

Another thing I've seen as companies have come into my department to talk about testing with us is they bring in data, and the data are done on very nice culture systems, very nice model systems, and we say, "Gee, this looks good, but what happens in the presence of platelets and residual white cells?"

And that's where there is less information available. So let's hope that the people who will be presenting today use real products after they've developed their system in model products because the model system may not emulate the real system.

Can you focus it, please? Thank you.

And finally, the issue is: where are we going to do the testing? Are we going to do the testing in the regional blood center or are we going to do the testing in the hospital? Because when you're developing a test system, there's usually instrumentation, and there's usually a product definition which is needed.

So the product definition includes the test sensitivity, and I just described the fact that if it's going to be done in a regional blood center, and that's many hours prior to transfusion usually, then it's going to have to be more sensitive.

And if it's more sensitive, it may have a greater false positive rate.

If it's being done in a hospital, my feeling is it should have a test sensitivity of less than ten to the fifth. How much less I don't know. I think the question is let's see what the manufacturers offer us.

The time of sampling, as you'll hear in some of the presentations, typically in the blood center it's going to be 24 or 48 hours after preparation. Why? Because the initial bacterial load is low. You need to give the organism some time to grow so that when you sample you know you're sampling a representative part of the product.

Whereas if it's pretransfusion in the hospital, the bacterial will potentially increase from the time of sampling to the time of transfusion, but that time can be minimized.

The test complexity. If it's in a blood center, blood centers are used to handling many complex things, and while we don't want it too complex, they probably can deal with a system that has some degree of complexity, especially if it's an automated system.

In the hospital, it's got to essentially be turnkey. It can be very sophisticated, but it's got to be transparent to the user.

Test duration. In the blood center, while we don't have a lot of time, we have other things going on during product release. So the test can take an hour, two hours, three hours, maybe even long, but in the hospital where we are doing this as a pretransfusion test, we need a rapid turnaround time.

In terms of throughput, once again, in the regional blood center we're handling large volumes of products, large numbers of tests. We've got to get it in, and we've got to get it out.

Within the hospital environment, that's true. We have to get it in and out, but not at the rate of 1,000 or 2,000 a shift.

And finally, people always say, "Well, how much can this test cost?" Well, I don't know if $5 is a good number, but it is a number to shoot for. Why? Because that's around the price we pay for viral testing today.

So when a company comes in and says, "Gee, I can give you this instrument at $100,000 and this test at $30 a pop," I say: who's going to buy it?

Now, if it's the only thing out there, maybe someone will buy it. Maybe it will be regulated. That I don't know, but I just don't think that's the product definition we should be shooting for.

So with that, I think I'm over, and we're going to have our first speaker, and then we're going to break for lunch, and then we're going to come back and continue after lunch.

Our first speaker is Jim AuBuchon. He's a Professor of Pathology and Medicine at Dartmouth-

Hitchcock Medical Center. He's a nice guy and he's smart.

(Laughter and applause.)

DR. AuBUCHON: Thank you, Len.

That's a lot nicer than the introduce you threatened me with yesterday.

If I could have the first slide, please.

Most all the points that I'd like to make have already been made by other speakers, but I'm going to try to pull them together and put them into a practical context for you looking at bacterial testing and culturing as a means of detecting bacterial contamination of platelets.

Obviously, we know that we have many problems with platelets, and I'm not going to go through this again, but we had the initial low concentration to deal with, and they can be very difficult to detect, particularly if you're trying to pick them out amongst the myriad of little platelets floating by.

Platelets come from a number of different sources and also importantly, as you've seen from other speakers this morning, there are lots of different bugs we have to worry about. So we have to go about this generically. We can't have a test for each one of

these platelets.

And we have to potentially detect them at relatively low levels particularly, as Len said, if you're trying to detect them early on.

We also have a problem in that bacteria disappear from platelets or any blood unit, but they may reappear later. For example, some work that we've done with Yersinia and others have done with other organisms has shown that particularly complement is very effective in clearing Gram negative organisms, and there are other mechanisms for other types of organisms, such that even if you start out at a high level, such as 100 organisms per mL, within an hour or two these bugs are not detectable.

However, if you let this unit sit for weeks or days at room temperature, the organisms can, quote, reappear. So you have to time your culture or other intervention appropriately to pick up what is really lurking in the background.

We also have to recognize that these bugs can disappear from a number of different mechanisms including, in fact, platelets themselves may be bacteriocidal. This bacteriocidal effect may, in part, be due to residual leukocytes in the unit, but in this study, which has been around for a number of years, you

can see that the effect of platelets is greater than the effect of just plasma alone in removing bacteria. In fact, there are some theories that platelets may represent some primordial form of scavaging of bacteria to present them to the immune system or the reticular endothelial system, but that's another talk to for another day.

We also have to recognize that the size of the inoculum can affect the rapidity with which the inoculum grows up, and if you start with a low level inoculum, shown here in the red bars, you may get an apparent lag period before the bacteria begin to grow. If you inject more bugs into a platelet unit, they appear more quickly.

And this isn't just the sensitivity of culture that we're dealing with. It appears that there may be some cooperativity or some other bacteriologic function going on when there are more bugs in the bag.

So we have some other things to compound the problem to consider. We have a shift driven by financial concerns from apheresis platelets back to whole blood derived platelets, at least in this country, and as some studies have shown, these platelets may be involved with bacterial contamination at a higher rate.

With leukoreduction, many of us are interested in performing this in a prestorage fashion, but at the moment there's no FDA approved way of prestorage leukodeleting platelet units derived from whole blood units because one would have to apply an individual unit, individual filter for each one of the six or eight or ten units that one wanted to prestorage leukoreduce, and that gets just incredibly expensive.

And, as Dr. Heaton pointed out, with the implementation of NAT we have delays in release. So platelets are being pushed back further in their inventory holding period, and the potential for further bacterial growth is a concern.

So what do we need? Well, we would like to have, optimally, some means of detecting all contaminated units at the blood center right after collection when the concentration may be as little as one bug in the bag. I don't know if we'll ever get to that.

Minimally we want to detect a lethal dose. What may be acceptable is detection of a clinically significant dose, whether that's ten to the fifth organisms per mL or some number of organisms per transfusion. I don't know exactly what that number is, but it's clearly more than one bug in a bag.

The current technology, as Len said, is going to be maximized in its sensitivity when we move the testing point closer to transfusion, and that's really the focus of my talk today. What are some means that we can do to get there?

A number of different techniques, none of which are either available, such as RNA detection, or which seem to pass the Friedman test of getting down to the level where we're really able to pick up bacteria to prevent all contamination cases.

Well, can we use the bacterial mechanisms of taking glucose and turning it into acid and CO_2 to detect these organisms by other biochemical means, picking up a drop in glucose in the bag, a decrease in pH and the associated disappearance of swirling? Can we detect CO_2 directly, just as the microbiologists do in their culture mechanisms?

And there are a number of different ways that have been tried, looking at automated testing, some biochemical strips that can be used on urine or blood, for example, to determine some of these components, a different framework obviously, and some CO_2 sensitive labels have also been tried to detect CO_2.

For example, Klaus Hogman reported several years ago using these CO_2 sensitive labels the problem

was that you didn't get any change at all with a number of different organisms, and with other organisms, such as the one that was pointed out earlier today, you'd have to have what would probably be a lethal dose before the indicator would turn positive, and it didn't have a great sensitivity.

And of course, the problem is also that platelets can produce CO_2. So you've got some false positives to deal with. That idea looks like its time has not yet come.

Dr. Brecher has been very involved in this field and has published a number of studies looking at the little test strips, biochemical test strips, dip sticks, to pick up changes in glucose or changes in pH after a unit has been intentionally contaminated, and you can indeed see glucose fall.

This looks like it might be useful because clearly as you move out into the time period particularly that we're interested in, days three to five, there appears to be a difference between the control level and the level found in contaminated units.

The problem is that there's a large variance here in terms of what's normal in a control unit, and my apologies to Mark. I had to calculate

what the SD was based on publication, and this may not be exactly correct, but there is a relatively broad range of glucoses or pHs that can be found in any normal, noncontaminated platelet unit, and therefore, we have the problem of false positivity or setting your level of detection at a point where the sensitivity suffers.

Steve Wagner from the Red Cross and Len Friedman's group has also looked at this, and they set their cutoff level based on platelets tested on day six, but again, there was a significant standard deviation to consider, and so really if you're going to set the cutoff level here at maybe around 50 percent of what the glucose was on day zero, a number of normal units are going to fall below that.

And indeed, the false positive rates that they showed looking at glucose levels, pH, or absence of swirling, based on two SD reference levels for the two biochemical tests, showed predictably about five percent false positives. That's what you would expect just from the statistics involved. You're going to get some false positives.

Can we withstand that, particularly at the time when we're very cost conscious and we're cutting back on the blood supply for other safety related

reasons?

As I said, Mark has been involved in a number of attempts to try to figure out a way to prevent bacteria from going undetected, and by using the biochemical test strips in his laboratory at UNC, he's reported greater than 95 percent detection of bacterially contaminated units when the concentration is ten to the seventh bugs per mL.

This varies by organism and is not the same for all organisms, but it looks relatively good. The problem is that this is, you know, well over a lethal dose probably, and we want to pick it up before then.

Steve Wagner has looked at this as well and, again, comes up with an idea that we're probably not meeting the Friedman rule here. He noted that swirling was about as good as glucose changes in terms of picking up the presence of bacterially contaminated units, and he also noted that pH would not be useful for enterobacter because they, and other bacteria, do not produce acid. So you're not going to see the pH change, and you wouldn't also see the swirling change probably, which is pH related.

Swirling is an interesting phenomenon, something that we're all used to seeing in the laboratory, and it depends, of course, on the presence

of the platelet in the discoid form, which then can align perpendicular to flow when we shake the bag back and forth, and as the pH drops or there's some other metabolic disturbance, the platelets don't like that. They become spherical over time, and they obviously can't align along a long axis.

So this is something which has attracted our attention and others as a simple means of trying to pick up those units that may be contaminated.

In performing the same kind of dip stick tests that Dr. Brecher performed and also doing some swirling tests, we're able to essentially recapitulate the data that has been published by others in that you have to have a relatively high contamination rate or bacterial concentration before these tests become positive.

Now, by inoculating on day zero with one organism per mL, we would get up to those levels relatively soon. We could pick up, for example, staph. aureus on day to by the fall in glucose or all of the tests somewhere between day three or day five would become positive for salmonella. Staph. epi., the glucose became abnormal on day two, but there were lots of false positives, and trying to adjust the cutoff level by going to either one SD or three SD didn't

overall improve the accuracy.

We attempted to do this study with blinded observers, our normal bench techs. working with these units along with other units in a paired design to test this out to see exactly how good we could possibly be, and we felt that swirling was probably going to be the best and simplest way to detect.

However, not all bugs cause swirling. Only 25 percent of the staph. epi. contaminated units were detectable by swirling by day five.

The specificity looks pretty good for swirling, but the sensitivity is not what we might like it to be.

Getting back to the Friedman rule, we obviously need something that's going to do better than these levels of sensitivity that I've highlighted here. Can we get any better with swirling by automation? The answer is no.

However, we do swirl testing on all of our units before release because that's the only thing we have available, and in the early part of this year we detected two units that were bacterially contaminated by lack of swirling, one on day two and one on day three. We are not exactly certain where this came from. These all looked like skin contaminants, and we

were happy that we were able to pick them up.

But this caused us great concern because we knew that swirling was not going to be perfect, and we might be missing something. So it brought home to us, even in northern New England, that bacterial contamination was a problem.

So we looked at then bacterial culture. The problem is obviously that it takes time. There is a lag time for the microbiological test to turn positive if it's going to. There are costs, and there's a sensitivity issue that the more you culture, the greater the sensitivity, but then the less you have to transfuse, and we weren't interested in doing destructive testing on all of our units obviously.

So we adopted the following system that we felt was practical for a transfusion service laboratory. All of our units are entered into inventory about on day one, and on day two we perform a bacterial culture using a sterile connecting device.

Whenever the unit happens to be needed by our own protocols, we release that. We culture about five to ten mLs via the SCD.

Now, why did we pick day two? Well, because of our previous work in inoculating on day zero with one bacteria per mL, we found, of course, that the

growth of bacteria over time, on day one only about 80 percent of the units that were cultured five to ten mLs were bacterially positive. However, if we waited until day two, all of the units that we cultured were positive. Therefore, we felt that by waiting to day two, we would be increasing our sensitivity, and knowing also that usually the bacteria are not going to be at such a high level in the first 48 hours, and we don't usually transfuse units within the first 48 hours, that we would not be causing the patient any problems.

Of course, if the positive automated culture is reported to us as being positive at any time, the unit is pulled from inventory, but we don't wait for a negative result. Obviously we don't wait for a seven day culture to release a five day product.

We've been doing this now since early May, and I report here on the first 16 weeks of our experience we cultured 401 units in the automated system. We got three units that were initially positive. On two of them, when we went back to the unit, we could not reculture the organism. This appeared to be a tech. problem. These false positives occurred early on in the first couple of weeks, and we did some more training with our techs. about how to

take the samples, and that problem seemed to have disappeared.

We had two that were positive that were not able to be recultured, one because it had been entered for another reason and one because the unit had already been transfused, and the retention segment was not maintained in a sterile fashion. The patient who received that unit did not show any signs or symptoms. He was on some prophylactic antibiotics, but they weren't very good at dealing with the organism that he was transfused. It was a staph. epi., and he was cultured and had a negative blood culture.

And we had one conundrum, one unit that demonstrated no swirling beyond day three. We cultured that every way our microbiologists could possibly think of and could never get anything to grow. So unless it's some very peculiar mycoplasma, I don't know why this unit was not swirling. It wasn't due to a high white count or abnormally high platelet count.

Now, this costs money. It cost us about $11 for each one of our apheresis units to be cultured. We only use apheresis units so these are the only ones we have to deal with. It takes about seven minutes of tech. time, and when we do get a positive, depending on what we get and how difficult it is for the micro lab

to work up, it does cost us some money.

I'm certainly not interested in spending more money than I have to, despite the fact that I would like to make the blood supply safer, but the payoff here may be in reducing outdates. We outdate about 15 percent of the units that we collect in platelet pheresis. Most of our units are transfused in the latter half of the storage period, but we have a problem with outdates, and we've tried a number of things to beat this down, but it's still with us.

What about taking advantage of the fact that we know that platelets can be stored for seven days in these bags and they work fine with that length of storage? The only reason that we're outdating them at the end of day five is because of bacterial concerns.

If we have a culture on all of these units that's still negative at that point, why not go ahead and transfuse day six or day seven? And you can see that on the days that we did have units expiring, we had an average of two and a half units expiring, and on the next day, day six of their storage period, we had more than enough units requested to use the units that had expired the previous day.

So even being able to use these units one

day more would have brought them back into usable inventory. Does that help the finances? It sure does because if you take out of 100 units that you culture and spend this amount of money in working those units up and then don't outdate 15 of them at, let's say, $500, collection cost, for each one, you have accounted for the bacterial culture costs several fold over while making the blood supply safer at the same time.

Now, others have looked at this as well, and I recommend, you know, you take a look at this report as well because we're looking at pools of platelets, but again, they found that they were able to culture units early on and identify those pools that should not be transfused.

So by going with bacterial culture in the transfusion service, we get around some of the problems of sensitivity because you're then dealing with a time course that's more amenable to picking up low levels of bacteria, and potentially you're using a cost saving means of dealing with this problem, able to both reduce costs and improve the safety of the blood supply.

So what should we do? Well, I'll offer you the hospital transfusionist perspective or at least my own perspective. We need to pay attention to where we're killing people, as Dr. Blajchman opened with this

morning. We're killing them by giving them the wrong units of blood and by giving them units that are bacterially contaminated.

What can we do to prevent that? Well, we can culture these units. We need to do something. It doesn't have to be perfect, and it doesn't have to be done by the blood center. It can be done at the hospital, and it can be done in a cost effective or cost savings manner even better.

And I'll leave you with a quote to consider over lunch, that wisdom consists of knowing when to avoid perfection.

Thank you.

(Applause.)

DR. FRIEDMAN: Thank you, Jim.

We're going to break for lunch now. There's a cafeteria on the second floor, and there's a bigger cafeteria in the basement. If you suffer from senior moments the way I do, you'll know in your pack there are pieces of paper you can write questions on if you have any questions from my presentation or from Jim's or after lunch from the other speakers. Write them down. I don't know if we'll collect them, but at least you'll have notes about them.

And I think we're breaking for lunch now.

Back at one. We're going to start sharp at one.

(Whereupon, at 12:02 p.m., the meeting was recessed for lunch, to reconvene at 1:00 p.m., the same day.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

(1:03 p.m.)

DR. BRECHER: Okay. I'd like to welcome you back to the second part of Session II, detection methods.

Our first speaker this afternoon is Roslyn Yomtovian. Dr. Yomtovian is the Director of the Transfusion Medicine Service at the University Hospitals of Cleveland. She's an associate professor. She's very active in educational efforts, particularly with the American Society of Clinical Pathology, and one of her areas of long interest has been bacteria contamination of blood, particularly that of platelets.

Ros.

DR. YOMTOVIAN: Well, thank you, Mark, and thanks to the organizers and those of you who are in attendance to hear once again about this topic which has interested many of us for quite a long time.

And, let's see, I guess I -- yes. So what I'd like to do today is share with you our experience in Cleveland at University Hospitals on use of the Gram stain and culture surveillance as applied to this problem and tell you about some of our significant findings, focusing to a great extent on the clinical implications.

Now, we entered this foray of studying this problem. Sort of we were victimized in 1991 when we had a mini epidemic of bacterial contamination of platelets which was reported in MMWR. At that time, we were kind of shunned. "Oh, you must be doing something wrong. Why, you know, do you have this problem in Cleveland?"

We invited the CDC to come in. They spent several weeks in Cleveland. We also invited the FDA to come in. They spent significant time, and, lo and behold, they found nothing unusual about our practices that could explain why this occurred, and indeed, there was nothing at the hospital level that was causing this. We did not make any of these platelets. These came from various blood providers. I might add more than one. So it was clearly an issue that was occurring before the blood or the platelets even arrived in our inventory.

We've heard today that bacterial contamination of platelets is the most common cause of transfusion transmitted infectious disorders. It certainly relates to the room temperature storage that characterize platelets, and the risk certainly increases with increasing storage age, and I will come back to that later.

Now, over the eight years that we've been doing surveillance of bacterial contamination, we've really acquired a wealth of information, and some of this information has to do with the rate and incidence of bacterial contamination of platelets, the type of organisms that are involved, the utility of the Gram stain, the clinical significance of contamination, the longitudinal pattern over time, and I'll show you some kind of interesting information on that.

And also we've been able to compare the incidence or risk of random donor platelets versus single donor platelets.

This is our epidemic curve since the beginning, even since our presurveillance time. Actually there should be another case way out here in late 1989, an enterobacter aerogenes. That's what stirred our initial interest, and then we had the epidemic that caused the MMWR report.

And then over time -- and I'll describe these different surveillance periods for you shortly -- we've had different ways of approaching this problem.

You can see just in general most of our isolates have been a staph. epidermidis, coagulase negative staph. We've had a couple of pseudomonas, a couple of bacillus, a couple of staph. aureus, a couple

of strep. veridans group, and one serratia marcescens. We've had mostly random platelets involved, but the stars indicate single donor platelets being involved.

Oh, I should add there's eco. bac. for just a minute. After I made this slide, we did have an additional case this month. So it's even hard for me to keep up with this slide.

Now, this is how our surveillance has evolved over time. In the very beginning when we had the clustering of cases, we initiated a Gram stain and culture from all platelet pools. We, however, did not use these to interdict any transfusions.

As more cases occurred, we then required a negative Gram stain to be issued to us prior to transfusion, and we continued to culture, but did not wait for the culture result.

As time went on and we had data showing that the four and five day old platelets, which I'll call at risk platelets, had a much higher rate of contamination than the one, two, or three day old platelets, we began to do our surveillance only on the at risk platelets. So we evolved to a negative Gram stain required on any pool containing a four or five day old platelet, and we continued for a while to do cultures, however, from all the platelets.

That evolved into studying only the at risk platelets, both negative Gram stain result and cultures from the at risk platelet pools, and finally, in February of this year, we have evolved now into the last interval that we're currently in in which we're not doing the Gram stain anymore, and that was a very difficult decision to make.

Not to preempt myself, I will tell you that three years did go by between the time that we had positive cultures and a positive Gram stain. In other words, all of the cases for three years preceding this decision that were culture positive and confirmed to be true contaminants, all had negative Gram stain. So they were all transfused, and I'll show you what happened to the patients clinically.

So we felt we really weren't doing much by continuing the Gram stain, number one.

Number two, it significantly delayed our issuance of platelets to ever increasing demand from our blood bank for faster service, and that includes a particularly large growing out-patient service.

So we continue to do surveillance, and we certainly reserve the right to go back to Gram staining if it looks like it might help.

I might also say over the last few years,

the epidemic may be changing somewhat in that the number of organisms in general that we're finding in contaminated units are lower than they were earlier in the epidemic, and I have no readily available explanation for that.

I want to emphasize in our culturing methods way back when we developed these, we purposefully developed them, and this was developed with Dr. Michael Jacobs, our microbiologist, to minimize the possibility of false positive results.

Now, I will say, unlike what most people have discussed this morning, we are doing quantitative -- we're doing plates, rather, which allow us to do quantitative cultures if the plate comes up positive. We're only using a .1 mL aliquot. It seems like a vanishingly small amount, and therefore, our sensitivity is a little bit lower than what others have reported, but that, again, was decided intentionally in our particular institution.

We examined the cultures for three days, and we do quantitative cultures if the plate is positive. Of course, we confirm. If we have a pooled positive, we've saved all of the individual units. We know what order they were pooled in. We go in and we culture them, and we've always had a positive

individual unit associated with a positive pool with the same organism.

And this is our methodology for the Gram stain, and our criteria for whether it's negative or positive, and you've already heard that the sensitivity of the Gram stain is right around ten to the fifth or ten to the sixth colony forming units per mL, not very sensitive.

And, again, this is our curve, just to remind you of the cases, and we had another case, and here I might just tell you after we stopped doing the Gram stain, you would expect, of course, that this would happen. We did have this apheresis platelet that was contaminated heavily with a strep. veridans group that was strongly Gram stain positive. So chances are this would have been interdicted.

This was a five day old unit. Chances are it would have been interdicted. This lady did wind up, the recipient of this, did wind up in the intensive care unit with shock. However, it was not involved in her subsequent clinical course. She recovered from that.

Now, this is our data presented a little bit differently. Clinical outcome is on the left-hand column. We've had a total of 32 cases. These are the

different bacteria that we've isolated.

This is the quantitation that I referred to that really we haven't seen yet today, and I want to talk about this shortly. This is obviously very skewed because we're really only studying in a routine, consistent way the four and five day old units. So the ages that are less than that are either patients that have had a transfusion reaction that's been reported to us or occurred in the earlier interval when we were studying all the platelets.

I do want to mention we had one death in a pseudomonas case caused by a three day old platelet, which, by the way, had we been doing Gram stains on all of the platelets rather than just the four and five day days old, that was floridly positive and probably -- of course, you never know -- probably would have prevented that transfusion.

The pink marks here are the cases where the patient also had a positive blood culture, and it's apparent that there are a couple of cases where the patients, even after thoroughly reviewing the chart, talking to the clinician, had no symptoms. One lady had ten to the eighth CFUs per mL transfused, no symptoms. She did have a positive blood culture and was brought back. She was an out patient and treated

with antibiotics.

Another patient with ten to the three CFUs per mL also had a positive blood culture. In fact, three -- two of the three cases we've submitted to BaCon, which haven't been accepted, have had positive blood cultures, but one didn't have symptoms and the other one had delayed fever, and that's the other clinical outcome I just want to mention briefly.

One of these people that had a delayed fever only got -- and the data is there -- ten to the two CFU per mL in the pool, and it was actually 60 CFU per mL in the implicated unit. That lady went home, spiked a fever to 103 degrees several hours later. In between the time she received the platelet and the time we knew the culture was positive and called the clinician, she had donated autologous hematopoietic stem cells, and when we told the clinician her platelet that she got before that collection was positive, they elected to discard her stem cells. I'm not sure that was the right decision, but that's what they did.

So when we ask what is the clinically significant amount of bacteria, the answer is that I have no clue.

I might also emphasize one of our cases is a neonate who got ten to the ninth CFU per mL of

serratia marcescens, had some subtle clinical changes. There was a change in the antibiotic this neonate was on that occurred shortly after the transfusion and some subtle changes in pulse and blood pressure, but it was very subtle and no one even, you know, worried about this child from that viewpoint.

If this had been given to an adult, chances are it would have had a much different outcome. Neonates have a poorly developed cytokine network and probably do not respond the same to endotoxin as adults do. So that's just something else to keep in mind. So outcome is extremely variable.

And you've already heard about the tip of the iceberg from Dr. Blajchman, and you know, in most places that rely on a reaction triggered surveillance, as is the BaCon study, we truly are dealing with the tip of the iceberg. Whereas in our facility for years, we've been trying to look at the entire iceberg, which is pretty massive as we've heard.

Now, just switching gears very slightly and showing you data that's displayed a little bit differently by year, these are Gram stain positive results, 14 total. Several that were read as negative before transfusion in retrospect may have been called positive, but that doesn't do any good when you're

talking about how good a test is. I just put that in for completeness.

There were 32 that were confirmed culture positive, and therefore, we interdicted six transfusion by use of the Gram stain.

And this is some other data that I wanted to share with you on the total incidence of platelet bacterial contamination in our facility over this almost eight year time period.

Over that time we transfused almost 90,000 random donor units that were in pools of generally five. There were 19 positive random donor units for an incidence of contamination of about one in 4,700. Realize that this is mixed data. This includes our culture surveillance data for the older units, at risk units, plus those that are reported to us as reactions which later we document at contamination.

The better number is our incidence in at risk platelets in which we've studied every single one of these, generally in pools of five. They've all been cultured. We've had 14 positives for an incidence of at right contamination of about one in 2,000, similar to numbers you've already heard. This just shows it over different time intervals. This is the raw data.

And more interesting, but I really don't

have time to dwell on it right now, is the fact that there is statistically significant variation over time. So this is not a problem that's occurring uniformly and we're now analyzing some additional time periods to see whether we have yet another mini blurb out here over time, but there is something different that occurred during this time. I don't know exactly what it is, but it's different.

Similarly, through our single donor apheresis platelets, if you only looked at our raw data, you'd think the incidence was lower than that of per unit randoms, that one in 7,700, but when you look only at the four and five day old units and use the appropriate denominator, then you get a remarkably similar rate of contamination unit for unit in the single donor apheresis compared to the random donor.

And actually data I'm not showing today shows that it doesn't make a difference whether they're leukocyte reduced at the time that they're being prepared or not. The rate is the same. There's no difference, although the numbers are very small. So the confidence internals would be probably unreasonable. I mean there are only three cases here. This represents very small numbers, but this is important.

This is the contamination rate of single donor versus random donor platelets on the at risk units, which I've already mentioned, per 10,000, 4.15 for the single donors, 4.97 for the random donors, exactly the same in a statistical sense unit for unit. Obviously you pool the random donor platelets, and that's why they're riskier.

Now, if we look only at our four and five day old random donor platelets, I just summarize this briefly. We had 14 that were positive for bacteria. We had six that were interdicted by a pretransfusion Gram stain, either that had a negative Gram stain, but the culture was positive retrospectively on the unit. What happened to those eight people?

Well, three had absolutely no symptoms or sequelae that we could recognize. Two had no symptoms, but had sequelae, including positive blood culture, and three people had symptoms and sequelae. So, you know, partially based on this data, the fact that the Gram stain has not been very good for the last three years and the delay, we did elect to stop using it for now, although as I say, we reserve the right to start it again if our surveillance shows we need to.

So in summary, bacterial contamination of platelets is a persistent and ongoing problem with

variation over time. Unit contamination rates for random and single donor platelets appear to be identical. They are identical in our facility.

Clinical symptomatology and outcome is variable. The minimum pathologic dose of bacteria is unknown, and I might mention here that the fact that many of these patients have in dwelling catheters allowing for a plastic or artificial surface to interact with even small numbers of bacteria may be important.

And the Gram stain is limited in its utility to interdict contaminated platelet units prior to transfusion.

And I thank you for your attention.

(Applause.)

DR. BRECHER: Thank you.

Our next speaker is Dr. Stephen Wagner. Dr. Wagner is a senior scientist at the American Red Cross Holland Lab; received his B.S. degree in chemistry from the University of Maryland, College Park, and earned an M.S. and Ph.D. in biophysics from Penn State.

He's had a long interest in disease transmission by blood products, particular viruses and bacteria, and he is going to be talking today, and his

topic is automated culture systems.

DR. WAGNER: Thank you very much, Mark.

As if you didn't want to hear more about automated blood culture, but what I'd like to try to do today is get into the guts perhaps of the testing and try to define perhaps what's the best way of dealing with automated blood culture, what might be the best way to think about it.

And automated blood culture detection is usually based on a change which is a color change, which is based on the rate of change of pH in the culture media, and that's generated by CO_2 which are produced by bacteria predominantly, and the reason why I use the word "predominantly" is because we all know that platelets make CO_2, as do white cells, and that's something that I'll come back to a little bit later in the talk.

There are some culture systems, I believe, or there have been some historically that have been based on changes of pressure inside of a culture bottle also.

And this is a schematic of a typical culture system where you have media and a disk which is pH sensitive. A light illuminates the disk, and a photodiode and amplifier detects reflected light, and

when there's a color change, that's indicated as an electronic signal which is dealt with by the instrument.

Many times these instruments make determinations of what the color is on the disk every ten minutes or so.

So what culture media might be appropriate for use with automated blood culture? There's a lot of different culture medias. Here are three that we've investigated.

T-soy, there are some T-soy broths that contain adsorbents, which might adsorb antibiotics because sometimes if a patient is on antibiotics, that can cause interferences with detection of bacteria obviously, and also Brain Heart Infusion media.

And so how does these three look with respect to detecting bacteria? And lots of times when you talk about automated blood culture, you talk of time of first detection. That's a time when the instrument first signals that bacteria is present, and for the most part, these three different media give approximately the same time to detection, 12 hours or so, 13 hours, but there are some interesting differences.

For example, staph. epidermidis, which is a

very slow growing organism, is much slower in a media that contains activated charcoal, for example, and so I'm just giving this out as an illustration that you have to be careful what media that you're going to use, and it might be good to be clear about that because that's clearly going to affect the time at which you can detect an organism.

Well, there's two ways of culturing. You can culture aerobically or you can culture anaerobically, and we're here talking about platelets, which is not quite like talking about red cells, but platelets to me at least are an aerobic environment. Their pO_2s are somewhere between 40 and 100, and so I don't think they really support the growth of anaerobes very well.

You may sometimes find anaerobes in platelets, maybe not strict anaerobes, but they're unlikely to multiply to any great extent.

What should the sample volume be? We start going into more controversial issues. The easiest answer is I don't know, but it's easy to define it if you consider what the initial inoculum is, and this can only really easily be done in experimental systems, and a number of people have done these sorts of studies.

Klaus Hogman's group in Sweden has looked

at this; we've looked at it, and with high inocula, any sample volume is fine because the probability of picking up an organism in a syringe or some other means by which you're accessing the platelet unit, for an organism to be present in the volume is very probable, and so it doesn't matter if you use .5 mL or four mLs, or in our case it doesn't matter if you use .5 mLs or up to two mLs, but you know, that's for fairly large numbers of organisms, ten organisms per mL, two organisms per mL.

What happens when you get to low inoculums, which very well might take place in the blood setting? Well, the same people have investigated this, and basically what happens is when you go to low inoculums, for example, .6 colony forming units per mL, if you take a half mL sample, there's a reasonable chance you will not detect that organism if you take that sample immediately from the platelets.

And we've found the same to be true looking at a tenth of an organism per mL or one organism per mL. You don't see a significant number of organisms, and so that's the rub, is if you sample immediately and your organism level is very, very low, you may not have an organism in your sample. So you're going to end up with a negative test. Yet the unit can grow up during

platelet storage, and it's transfused, and someone would have a severe reaction or die.

So what to do? Well, instead of sampling immediately, one possibility is to delay the sampling, and so therefore, you're letting the platelet container to be your growth media rather than the bottle, and you're letting the organisms grow up in the platelet container before you take a sample.

And we've done work like this. I'll show you examples of actual data later. Our bottom line in looking at a fast growing organism and a slow growing organism was basically you had to wait until day two before you were very, very reasonably assured that if an organism was present, that you could detect it.

And the same sorts of things have been actually done rather than a spiking experiment, but a very nice experiment by Mo Blajchman, which showed in a pilot screening study a comparison of positive cultures in actual non-spiked samples looking at day one and day three, where he roughly saw twice as many positive cultures on day three as he did on day one.

And so now here are actual data. What happens is as you delay the time at which you take a sample from the platelet concentrate, you see a decrease in the time to first detection.

So, for example, E. coli, which is a very fast growing organism, this particular strain in our lab, in some platelet units it grows with a doubling time of one hour. It can be detected at six hours on day zero and about half a day, but after a couple of days it basically takes four hours to detect.

And if you take a large volume rather than a .5 mL inoculum, there's a very, very slight decrease in the time to first detection, but it's not particularly significant, but again, you see this trend of going to shorter and shorter times for actual detection if you delay your sampling.

Now, what happens when you look at a slower growing organism, for example, staph. epidermidis? In our hands, this organism many times has a four hour doubling time, that is, in platelets. If you sample immediately and you're lucky enough to detect it -- and remember this is a low inoculum, and so you have to be incredibly lucky. You do a lot of experiments -- it can take between two or three days to detect it in an automated blood culture system.

But as you delay your time for sampling out to day two, it takes roughly about a half a day, and again, it really doesn't matter whether you take a half a mL inoculum or a 2 mL inoculum, and I'll show you

more data on that later.

So this is not time to first detection, but this is detection frequency, and as I had mentioned before, if you sample immediately, you're not going to detect the organism all of the time. For example, with E. coli, at a very, very low inoculum, out of 12 experiments we didn't detect any with a half a mL inoculum, and we only detected three of 12 with a two mL inoculum.

And you can see by the time we get 12 out of 12 for E. coli, we have to sample at day one, but E. coli, this particular strain, is a very, very fast growing organism, and so to get the real answer, you'd have to look at a slow growing organism that's had some significance for transfusion associated bacterial sepsis, and in that case, we looked at staph. epidermidis.

And you can see -- no, that's E. coli still. Could you advance that? It's stuck. Thank you.

So you can see the same trends, where if you delay sampling, you're detecting at a higher and higher frequency, and for staph. epidermidis you don't detect all of the spiked samples until you go to two days.

And so we seem to be stuck all the time. So you may have to -- could you help me? Well, could you advance it manually, please? Thank you.

So if you look at a composite, and we did a series of experiments with different levels of spiking levels in this case for E. coli, it basically summarizes what I just said. In one day you can detect all organisms.

And the next slide -- no, we're still stuck on E. coli. Could you handle this, please? Next slide please. Unfortunately I didn't bring overheads.

Anyway, with staff epidermidis, basically it takes two days for detection of all samples, and so maybe she'll get it.

I'm going to switch over now, I think, I'm hoping, to a discussion of false positive frequencies because I think that's something of concern, and basically there's been four studies -- not all the names I can remember now -- that I've been able to call data -- oh, does leukoreduction affect detection? No.

Unfortunately we can't move from this slide. Okay.

There's been a report in the literature with high levels of leukocytes affecting detection where you got false positives. There's been a European

study, and this, in particular, has been noticed in peripheral blood stem cell preparations, and these authors found that if they added .5 percent Saponin, which affects leukocytes, they could inhibit the CO_2 productions by white cells, and that reduced their false positive rate.

And so this is something I think that merits consideration and thought when we're thinking about platelets, which also can produce CO_2, and possible ways of improving systems that already exist.

As I said, there are four studies where I can look at the data that's been presented either in abstract form or in scientific reviewed papers where you can come up with false positive rates, and these have varied quite a bit.

There's a cluster around .1 to .2 percent basically, with three authors here, but there's one disturbing result at two percent false positive frequency, which I think would probably cause problems for platelet availability if such a test were done in a global way.

And, you know, you ask yourself questions. For example, is there anything about the white cell level that distinguishes the work that these people did compared to the other people's work? I don't really

think there is.

There are some examples of white cell levels that are the same. One of the things that seems to be distinguished is that these authors used a very high volume of inoculum when you consider the ratio of the inoculum volume to the bottle volume, and I think something like that needs to be investigated further.

And so for the false positive results, the questions that are out there that probably need to be answered are: do leukocyte levels in the platelet preparations affect false positive frequencies?

Do platelets contribute to CO_2 levels and influence false positive frequencies?

And what I just noted, what is the relationship of platelet inocula volume and false positive frequencies?

And the answer to all of these questions are important if you're thinking about a test that might be implemented.

And so in conclusion, automated blood culture is a very sensitive method for detection of a large variety of bacterial species. I didn't show you that, but other people have shown you that.

There are practical limits on sample volume. For example, for a platelet that's derived

from whole blood, you probably don't want to take more than two mLs, and there's a potential for detection for low bacterial loads, in other words, one colony forming unit per mL in terms of limit of detection, suggesting that sampling needs to be performed after day one of storage, and our data at least support the idea that perhaps two days of storage might be appropriate before sample collection.

The results from in vitro spiking experiments with a slow growing organism indicate that all samples were detected within 24 hours after samples were obtained on day of two of platelet storage. So we're into day three, and I think there is the rub, and there's the problem, is is there enough time to provide these products, if they are products, to people how need them.

Think about what happens over weekends and other things. Is it possible to extend the storage time of platelets? Is it possible to introduce a test and simultaneously extend the storage time of platelet from a regulatory perspective?

A three day test may limit availability of platelets with their current five day storage period.

Thank you so much for your interest.

(Applause.)

DR. FRIEDMAN: Thank you, Steve.

Mark Brecher didn't want to introduce himself. He's Professor of Pathology and Laboratory Medicine at the University of North Carolina and will be speaking to us on nucleic acid based tests and cytometry.

DR. BRECHER: Thank you, Len.

Okay. Can we have the first slide?

I was fortunate to be able to attend the NIH transfusion medicine symposium here in this auditorium yesterday, and there was a lot of talk about Jacob-Kruetzfeld disease, and even though this session today is about platelet contamination, it seems like Jacob-Kruetzfeld keeps creeping into the talk. So I wanted to have the final word here.

(Laughter.)

DR. BRECHER: I probably should have shown this before lunch.

(Laughter.)

DR. BRECHER: For those of you who can't read the red on the left it says, "If your cow looks like this, then feel free to fire up the barbecue. If your cow looks like this, may we suggest the fish?"

Okay. I was asked to talk about some of the high tech. methods of rapid bacterial detection,

and I have looked at a lot of different methods in my laboratories over the years, both low tech., mid-tech., and high tech., and I'm going to concentrate on basically three methods today.

Nucleic acid amplification I will only cover briefly because there really isn't very much material out there that relates to platelets and, therefore, abides by the Friedman rules, that if you can do a test, you have to do it in platelets.

I'm going to talk about chemiluminescent ribosomal RNA probes, and a novel, new technique, microvolume cytometry and antibiotic probes, and then try to put all of this into some sort of perspective.

Just briefly -- and I note this is note platelets, Len -- there has been some work, albeit not too much, on polymerase chain reaction to detect bacteria in blood. This is a study that was published in 1992. That's already some time ago, looking at Yersinia enterocolitica, where they were able to get down to five times ten to the third colony forming units per mL.

When we looked at platelets, a method that had a lot of -- I should say "had" a lot of promise. I don't know that it is promising any longer -- was the Gen-Probe technology. Several years ago when I was at

Mayo Clinic, I was working with Dave Persing in the Microbiology Lab on some bacteria experiments, and he had a bright idea.

At the time there was a new diagnostic system that had been licensed for the rapid diagnosis of urinary tract infections that used chemiluminescent linked probes against universal bacterial sites. Now, this was a high tech. test for a low tech. disease. You don't need rRNA probes to tell you whether you have a urinary tract infection, and needless to say, this particular system failed in the market and was pulled.

But Dave had a bright idea that maybe we could take this method and adapt it to bacterial detection in blood products, and basically what this does is you take your crude blood products, and in this case we're going to be talking about platelets, which are these little yellow things here. You subject it to a rapid, crude enzymatic lysis, which gives you the free RNA.

You then add your probe, and the probe will hybridize to the ribosomal RNA. Any non-hybridized probe is not protected for when you add a base. When you add a base, these nonprotected probes hydrolyze, whereas the probes that have linked up with the RNA are protected, and this is referred to as a hybridization

protection assay.

And so these are selectively left intact. So this is a no wash system.

You then put it in a luminometer, and a luminometer looks a lot like a spectrophotometer, except it costs three or four times as much. That automatically adds a little bit of hydrogen peroxide, some more base, and you get a flash of light.

The machine records the light over time and integrates the area under the curve and gives you a result that is outputted as a relative light unit.

Now, the nice thing about using rRNA probes is that in every cell for every copy of DNA you have 10,000 copies of ribosomal RNA. So there is this intrinsic built in amplification that is already there.

The nice thing about using probes in general is that how you design your probes defines the specificity. So you can have a probe that detects all cellular life, for all bacterial life, which is the probes we were using. You can even be kingdom specific, family specific, genus specific, or species specific, which sounds a little redundant.

So what did we do? Well, the initial trials on platelets were done in my lab at the University of North Carolina that looked very

promising, and we rolled this out to a multi-center study that involved University of North Carolina, University Hospital at Cleveland, Sacramento Blood Center, Greater Kansas Blood Center, and the Mayo Clinic, and the different sites are colored differently, but you can see how all of the points overlap each other. So each site had basically similar results.

And you can see that the test is a semi-quantitative test. We have log RLU, relative light units, along the bottom, and then the log of the colony forming units up here, and these were 366 tests done on 120 inoculated platelets, four different organisms: bacillus cereus, pseudomonas aeruginosa, staph. aureus, and staph. epi.

And you can see that there was some piling up of data here. Only one site continued to do the assay once the material had been saturated, and so you can see this pile-up of data here, but it looks relatively good.

And you see that around ten to the fifth, maybe slightly better than that, CFUs per mL are detected with this assay. The assay takes about two hours from start to finish.

Depending on where you choose your cutoff,

this is choosing a cutoff at 30,000 RLU, and what is the range of your bacteric contamination? Ten to the second, ten to the third. You see that the assay picked up about a third of staph. aureus in the ten to the second, ten to the third CFUs.

And then when we get to the ten to the fifth, basically we're picking up almost all organisms. So, in essence, using this cutoff we detected everything greater than five times ten to the fifth, and we had a specificity of 98 percent.

If you lowered the cutoff to 15,000 RLU, you pick up more organisms. You can see here we picked up everything greater than two times ten to the fifth, and we even picked up 75 percent of the cases where the bacteric contamination was between ten to the third and ten to the fourth, and we still get these one-third of staph. aureus down to ten to the second, ten to the third.

We lose some specificity with the initial test, but that can be addressed, and I will show how we might address that here.

In a trial at the University of North Carolina to see how we would implement this in a transfusion service, in the wee hours in the morning we just rolled through all of our apheresis inventory for

six weeks and tested all of our inventory in a batch fashion.

Now, there were 304 apheresis platelets tested. Some of these platelets were tested on multiple days. So there were 509 occasions, but of these 347 platelet bags, 336 tested negative. We cultured all of these units, gave us a specificity of 96.8 percent. So roughly 97 percent specificity in roughly 350 units.

When we retested the units that were initially positive, they were all negative. So no unit was repeatedly reactive, and so in so much of this you can think of having a retest specificity of 100 percent.

Can we go back one?

Unfortunately, while this study looked very promising, and a lot of people were very hopeful that this would be the first nucleotide based test that would come into use in blood banks, the Gen-Probe Company underwent some changes in management. At that point they decided to reshuffle their priorities of the company and decided to shelve this project, despite protestations by several prominent blood bankers.

And so this test is not currently available. Of course, Gen-Probe has gone on to do

other great things. We all know about its work with the NAT testing.

Okay. Now, another interesting technology involves this instrument. This is the IMAGN 2000 from Biometric Imaging. In many ways you can think of this as a desktop flow cytometer.

Unlike flow cytometers which have cells that are essentially dripped past a laser, in this machine there are disposable volumetric capillary tubes. You see each one of these little triangles here is a disposable set, and there were two capillary tubes in here.

A laser scans down the capillary tube and records any fluorescent events, and it has a computer hooked to it, and it actually can draw a scan map of the capillary tube. This is actually a colorized version of a scan map. This is actually with CD-34 enumeration.

This technology is currently licensed for CD-4, CD-8, CD-34 enumerations, and residual white cell testing in leukocyte reduced blood products.

I kind of felt like Ted Turner here. I colorized the spikes so you could see them well, but it makes -- I did this around Christmas time. So green and red.

Now, Richard Rocco had a very bright idea. That's Richard with a little light bulb over his head. He started thinking about the probes that are generally used in flow cytometry and for this volumetric cytometry, which are usually monoclonal antibodies, and so probes are usually raised usually in a murine system. So you have a mouse that you start with, and you get your monoclonal antibody, and this costs big bucks.

Then he thought, well, what about using some other probe, and the pharmaceutical industry has invested large sums of money searching all over the world for exotic chemicals that have high affinity for bacteria. We know of these chemicals as antibiotics. So the difference here is on the end of the word. These are antibodies versus antibiotics, and although they cost a lot of money to isolate these compounds and put them into production, they're actually relatively cheap, cheaply available in the very corner drugstore.

And so the first one that they chose to look at was vancomycin. This is an antibiotic isolated from cultures of streptomyces orientalis, which was originally isolated from soil in India and Borneo. See, I wasn't kidding you. They really did search all over the world, and let me tell you. Drawing this on

my computer was not easy.

(Laughter.)

DR. BRECHER: But we did the first trials in my lab at University of North Carolina about a year ago, and we used a vancomycin conjugated fluorescent probes to look at staph. epidermidis, and we chose as our cutoff the mean of sterile controls, which we had, I think, eight or nine examples, plus three standard deviations. So being very generous.

And you can see that we were able to detect down to at least ten to the fifth CFUs per mL. It's questionable whether we can go any further. I think as the technology is developed, it's going to go much lower.

And we did some preliminary work using a genomycin assay. We just did some dilutional studies here, but it looks pretty good, genomycin for a Gram negative, in this case serratia marcescens.

Now, I was trained as a pathologist at one point in my career, and I've always taken away from that that seeing is believing, and so here are some experiments that Richard did in his lab.

Sterile platelets here under a fluorescent light. Here's your capillary tube. You don't really see anything.

This is serratia marcescens with the use of a polymyxin conjugated antibody, and you can actually see these things glowing back at you. So they really are there, and when you conjugate the antibodies, you don't necessarily inhibit the actions of the antibiotics. This is looking at inhibitory concentrations of bacteria, and this is our vancomycin labeled antibiotic, and you see that as you go to higher concentrations, as you come around the clock face here, that you still inhibit larger areas of the bacteria from growing.

So this is a very promising, novel approach.

Now, to put things into perspective, I've included this figure in the handout that's available in your package, and this is a modification of a figure that we published just a few months ago in Transfusion Medicine Reviews. It sort of summarizes the sensitivity of the various methods in terms of the log, CFUs per mL that you can detect, and the timing.

For examples, cultures, we have it down here down to ten CFUs per mL. Well, if you use a bigger inoculum, maybe you can do a little bit better than that, but it can take up to a day or more to detect versus some of these newer technologies like

ribosome RNA probes where clearly we can get down to around ten to the fifth. It takes an hour or two, and the antibiotic probes, which is very similar, less than an hour or two, and there's promise that these methods can even go much lower under certain circumstances.

Now, this is somewhat similar to some of the slides that Jim AuBuchon has already shown you. From the ribosome RNA experiment where we had inoculated 120 platelets with four different organisms, we looked at how many days it took for them to be culture positive, and we inoculated between either ten to 50 CFUs per mL or 1,000 CFUs per mL. So we were a little on the high side.

But you see one day after we inoculated them, we were able to pick up 90 percent of the bags using culture plates which had a sensitivity of ten CFUs per mL. By day two, 97 percent; by day three, 99 percent of the units were detectable by culture plates.

There was one organism, one bag with staph. epi., however, that did not turn culture positive until day seven. So while you may pick up the majority of cases, there may be a rare bag that you would not pick up.

We've actually been doing very similar experiments in my lab recently to what Steve Wagner

already discussed using automated culture detection system, using the Organae Technica BacT/Alert, and we've already looked at ten different organisms on our way to looking at 16,a nd our results are very similar to Steve's. However, we used a larger inoculum, four mLs of platelets per bag, and so our times are a little bit better.

But for the most part, for these ten organisms we detected them all, and we did replicates of ten; we detected them all in about 11 or 12 hours, except for staph. epi., where we had to go out to about 19 hours, but the standard deviation is very small.

Now, there are some institutions around the world that do not have to answer to the FDA, believe it or not, and this is a study that was presented to the ISBT last year from an institution in Denmark where they used automated screening, and they found a rate of about .23 percent, not too different from what we've seen reported from other studies.

But when they were doing their testing on day three, they said, "Okay. If it's culture negative, we will extend our platelets to seven days," and their outdate prior to doing this was 18.5 percent, which actually is remarkably like Jim AuBuchon's outdate, and they dropped that to 8.8 percent by implementing this

testing so that they could extend their platelets an additional two days.

And in the handout I gave a little cost analysis that based on this sort of data in a transfusion service that transfuses about 2,000 apheresis platelets a year, and I used the data from my institution, which is about $415 a dose. The annual potential savings for a drop in outdate like that would be $72,000 a year, and that's a lot of money, and that would certainly pay for a lot of bacterial testing.

Finally, I just want to remind people about some basic principles. My German, I'm afraid, is not very good. My grandfather would be ashamed of me, I suppose. "Vorsoregprinzip" -- I got the "prinzip" part. It means the precautionary or foresight principle.

When human health or the environment is threatened, precautionary measures are indicated, even if additional scientific evidence is needed to establish certain cause and effect relationships.

We know we had bacterially contaminated platelets. We know that they're going out and they're being transfused, and they are hurting people. We have ways of preventing this. I don't understand why we haven't implemented this.

For example, I recently heard of some data that will be presented soon from another institution where they took urine dip sticks, a method that came out of my lab, and they screened 3,000 random platelets. They found two that were bacterially contaminated with bacillus cereus and pulled them out of inventory.

It can be done. You can stop many of these transfusions.

And finally, I think we really need to remember what happened with HIV in the early '80s and look back at the Institute of Medicine report, and let me remind everybody that the perfect should not be the enemy of the good, and implementation of partial solutions -- and we may not have a perfect one -- but partial solutions, and we have a variety of partial solutions that are currently available and many others that will be shortly available, that have little risk of causing harm should be encouraged.

And I think we need to encourage the use of these methods. Now, I'm not saying that the FDA has to mandate this, but we already have a lot of regulations.

(Laughter.)

DR. BRECHER: But this is an FDA sponsored meeting. So I couldn't resist showing this slide,

which says, "These federal regulations are killing me."

And the sign says, "You must be this tall to attack the postman."

Okay. Thank you.

(Applause.)

DR. FRIEDMAN: Thank you, Mark.

Our final speaker for this session before the panel discussion is Dr. Mark Seaver. He's with the Naval Research Labs, and he has a background in chemical physics. Actually Steve and I networked with him not knowing that the Navy was going to be doing anything that might be useful, and he's going to be talking -- and we gave him platelets. We gave him platelets, you know. What did the Navy have to do with the type of thing we're doing?

So, Mark, it's all yours. Epifluorescence.

DR. SEAVER: Well, I think we're going to kind of talk in between the two previous talks. There's a little bit of high technology here, but living at the edge of high tech., I have this tendency to appreciate how easy it is for high tech. to fail. So trying to come up with a solution that's moderate tech. seems a good way to go.

You can see here's a list of colleagues. Phil Venturelli and Shiva Goel were actual high school

summer students who spent summers in my lab. They did a lot of good computer programming for me.

James Crookston provides our microbiology expertise, and Steve Wagner then is also clearly knowledgeable in the issues of bacterial contamination of platelets, and then lots of talks with Len Friedman and Daniel Robinette providing samples for us, platelet sample for us then to take back home and play with.

I think given what Len said earlier and some of what Steve said earlier I don't need to say much on this one. The only one I guess I would like of point to is this issue of false negatives, and I think, you know, you kind of get the sense looking at today's presentations of what I'm trying to say here, and that is that at a clinically significant level, false negatives is a problem, but if your detection limit is two orders of magnitude lower than what is clinically significant, then you can stand a fair number of false negatives.

And obviously, the standard, everybody would love to have the $5 device that anybody can walk in off the street and use.

Next slide, please.

What we're doing is doing automated microscopy. In doing automated microscopy and image

analysis though, your computer can only do a certain amount. You really need to make most of the good things happen in the sample preparation and how you handle the sample, and a lot of that gets into sample volume issue.

As Steve Wagner spoke about the sample volume really is an issue, because if you have 1,000 CFUs per mL and you only sample a microliter, the statistical probability is that there's going to be most of your samples will have zero in them.

We work with E. coli that was provided by the Red Cross. We work with staph. epidermidis that was provided by a colleague, and what we do is we take and we grow our bacteria overnight, and typically that gives us about ten to the nine CFUs per mL. In the sample we spin and rinse those, and then we spike them into the platelets at a variety of concentrations to try and make sure that our detection methodology is linear in concentration.

Our initial concentrations for some of the earlier work, we started at ten to the fifth, ten to the sixth, and ten to the seventh CFUs per mL. As we got a little bit smarter about how to make these measurements, we wanted to push the detection limit. So then in our most recent sample, we worked on our

preparation methodology, did some sample concentration work, and were able to get down, working down into the ten to the four CFUs per mL.

We, of course, run lots of controls to make sure that what we're seeing has a reasonable chance of being what we think it ought to be. So we both stain platelets only and bacteria only. We do plate counts on the platelet mixtures and all of our spike samples.

Staining takes about 15 minutes, and we haven't really worried about that time. That was kind of an intermediate time. We didn't want to spend too long doing it, but we wanted to make sure the stain got to where it needed to be.

We prepare slides in triplicate for each of our samples, and then we stick that under the microscope and turn on the computer. The computer can handle 100 images and the analysis associated with acquiring those imagines in about ten minutes.

We work at a roughly 200x magnification on the face plate of a digital camera, of a CCD camera, and as I'll talk in the next couple of slides, I'll show you kind of how the automated analysis works.

The automated analysis as we've implemented it at this point in time is not perfect. It takes a significant amount of off-line analysis afterwards to

separate things that we believe are bacteria from interfering particles. Largely in this case we're using information on the morphology of the particles.

Well, here's a picture. This kind of illustrates what you have to do in this case, in the epifluorescence, what your computer has to do in the image analysis. All of these little bright dots are stained staph. epidermidis.

Now, you can see, for example, here. There's a fairly dim one. So one of the problems is ideally you'd like to detect both this one and count that as a bacterium, as well as all the other bright ones.

However, here's something that we think may be a clump of platelets, and you can see there's all of these nice, little bright lumps that are physically about the same size and brightness as the bacteria. Obviously there's a problem there.

Here's a leukocyte, and we're dealing with in this case leukoreduced samples, but there's still significant numbers of leukocytes, and that actually seems to vary quite a bit from platelet sample to platelet sample.

Now, then what we figured out is that by way of an imagine analysis algorithm, it takes it us to

the next slide, and that is the blue ones are the ones that the computer has found and told us that based on the criteria that I've told it, it's regurgitating to me that these are the ones it things are bacteria.

And, for example, we don't see this dim one that was right here. However, you do see -- there's one here that may very well be a bacterium sitting on top of that mass of platelets that you can barely see right around in here, and we don't pick up the leukocyte because we told the computer to ignore anything that's bigger than, you know, a certain size. So the computer can easily separate the leukocyte from the bacteria based strictly on size criteria.

Now, when we're actually doing the analysis, and a lot of times you do, we pick up segments, you know, in a mass like this that the computer tries to tell us are bacteria, but as I said, in the post processing, we can look through those and based on morphology characteristics determine that these are unlike to be bacteria.

In this case we've detected 35 bacteria that I had identified visually from the previous one. There were eight that were too dim to be picked up, and we got lucky here. There was zero interfering particles.

Okay, and the next slide, please.

Here's the results. Like I said, we started out basically trying to demonstrate to ourselves that this could be done, that the image analysis worked, and what we found is that it did work fairly well. We could fairly routinely detect about one and a half to two times ten to the five CFUs per mL, which means that in our 100 frame analysis, we'd get ten to 15 counts.

Now, that gives us about a two to one to three to one ratio of counts in a spiked sample with the counts that we get from the platelet only controls.

And right here is what sets your detection limit. You need to beat these numbers down so you can either analyze more sample, which would increase or improve your detection limit, reduce your detection limit, or, well, I mean, that's the only answer. You've got to get rid of the counts from the platelets.

We see that our count values or count numbers are linear with concentration. They tend to be reproducible at a particular concentration from the triplicate slides at the plus or minus ten to the plus or minus 20 percent level that's indicated here.

Now, the last set of platelets that we had we said, okay, it's time to start pushing the detection

limit. So we spent most of the effort in revising the protocol, the sample handling protocol, allowing ourselves to concentrate the sample somewhat, and with that, we were able to push our detection limit down into the low ten to the four CFUs per mL for the E. coli.

And here's the actual count numbers from our three slides. In the case of staph. epi. here's the count numbers from the threw slides, and if you look at those, I'm not ready to declare that those are statistically different from the counts here, and I'm kind of glossing over this 42 counts per 100 frames. I don't know what happened in that one.

As we get more samples and try and reproduce this, I'm hoping to find out whether something happened here or whether we have this kind of variability. Obviously, if we have this kind of variability I can't claim that detection limits at these numbers represent two and a half CFUs or two and a half by ten to the fourth CFUs per mL.

One of the other interesting aspects in doing these experiments was that the staph. epi. was, in our kind of novice vernacular, was not behaving very well. We were having a hard time with staining the staph. on this particular day. So we think that may be

contributing to the fact that these really are down in the noise for detection limit. Again, something further to work on.

Next slide, please.

And then the conclusions. Consistently the simple protocol was allowing about one by ten to the five CFU per mL detection. I forgot my milliliters in here. These are all in CFUs per mL, not straight CFUs.

With our protocol modifications, we think we can pretty readily do two by ten to the fourth CFUs per mL, although it remains to be truly demonstrated that we can do that.

Everything is commercially available, you know, which again, part of what I do is develop instrumentation for field use in the Department of Defense, and that's a big deal for DOD folks, and I think, you know, it's pretty important for everybody because the more development that goes into apparatus, the more expensive it's going to be.

Right now it's taking us about 45 minutes to prepare our samples and do the analysis. We think we can push that down probably by a factor of two.

The microscope slide preparation surprisingly are archival. We've put them in the drawer and come back weeks later and gotten essentially

the same kinds of counts that we got on our initial measurements.

And I think one more. These are pretty obvious. We want to work on improving the protocol. We think there's a reasonable chance to get the detection limit below ten the fourth CFUs per mL. Part of this will be sample preparation issues, and part of it will be improving our image analysis algorithms.

The post processing is readily amenable to being automated. Those issues may go away as we go to more sophisticated algorithms in real time, and I guess the additional algorithms are the ones that I'm talking about here.

And then assuming all of that works out, then you can actually start to think you don't necessarily -- since we're only using one magnification, you don't have to have a full blown laboratory microscope. There may be ways to build smaller dedicated units that would work very well for a variety of people.

Thank you.

(Applause.)

DR. FRIEDMAN: If the panel members could come up, the panel members, and those of you who have questions, go to the microphones.

On the right.

MR. TABOR: Ed Tabor from FDA.

I'd like to ask Dr. Brecher a couple of questions. You describe the ad hoc use of urine dip sticks. Do you have any idea what method is involved in the dip sticks that were used?

DR. BRECHER: It must be a low bid. Can you hear me?

In that particular study, they set their own cutoffs.

MR. TABOR: No, I don't mean cutoffs. I mean do you know what scientific method was used in the dip stick to detect bacteria.

DR. BRECHER: They used the Ames urine multi-sticks.

MR. TABOR: That's a brand name. That's not a method.

What I'm getting at is it's easy to glibly describe experiments without data and say that we should be doing things that are good rather than waiting for the perfect.

DR. BRECHER: No.

MR. TABOR: And it's easy to point to federal regulations and say, "This is what's stopping us from making progress."

The reason for this workshop is to present new data and new methods and bring it to the attention of the scientific community and to FDA, and we've heard some very exciting new methods described, some of which may be in use within the foreseeable future, but I'd like to know what federal regulations have stopped the application of any method --

DR. BRECHER: Oh, oh.

MR. TABOR: -- that you've described that is both sensitive and specific at the level that you and all the rest of us would expect for the detection of bacteria.

DR. BRECHER: Okay. I see where you're going.

It was not my intention to say that there are any regulations that have stopped the implementation, and there are people who are out there using certain commercially available or generally available methods, such as the Gram stain. In my lab, I use the dip sticks, and several other labs use the dip sticks, and the methodology is well described and published and is basically based on the use of dextrose levels and pH changes, and if you want, I've got a paper here that will tell you exactly the chemical reactions.

What I'm suggesting is that a lot of people are honestly waiting for the FDA to say one way or the other that we all should be doing a method, some method, or at least allow a methodology that would have a direct benefit to the transfusion service or the blood bank.

MR. TABOR: We've already heard data today, this morning, that glucose and pH are really not where we're going to be going.

DR. BRECHER: Well, I --

MR. TABOR: And I think what we're trying to do is identify the best new technology that can be applied to prevent a serious health problem, and the concept of taking the good without seeking the perfect sounds very nice, but I think we have to keep our eye on the scientific rationale and try to achieve something that meets 1990 standards of accuracy and reproducibility, and that will, in fact, prevent these infections.

DR. FRIEDMAN: Jim, you have some comments on this?

DR. AuBUCHON: I do not believe that the FDA is preventing us from using culturing, for example, and we are using culturing, but it's on a trial basis. The problem that we face is how to get the return on

this.

I do have to live in a real world, and I don't have increased funds available to do culturing on all of my platelets. So I will ultimately have to be able to document to the laboratory administrator that I'm not costing him more money and hopefully I'm even saving some money by doing this culturing extending the outdate.

In order to do that, I've had some preliminary discussions with FDA staff, and it's been suggested that I put in for an IND and do this under a research protocol, and I could certainly do that, but if we're looking at an event of low frequency, say, one in 3,000 or even one in 1,000, and we only transfuse at our institution about 1,200 platelet units a year, apheresis units, I'm not sure exactly what endpoint I'm going to shoot for without culturing all platelet recipients and having a very involved study.

So I'm early in my thinking about this, but I'm not exactly sure how I'm going to document the safety of the approach I'm proposing, although it's intuitively safe, but I don't know how I can document it and still get some benefit within the foreseeable future.

Any thoughts you have on that or anyone

else has, please see me afterwards.

MR. TABOR: Well, I think studying these under IND is the way it should be done. It should be done with the highest level of scientific planning and analysis, and in 1999, and we're really talking about 2000 because most of these studies are still in their early stages, we should expect tests of the highest level of technology that are hopefully rapid and inexpensive and can pick up most of these infections.

It's actually very surprising that four years after our previous workshop we still don't have a way to prevent these infections.

DR. AuBUCHON: Well, design the study that you suggest. Even if we get three or four other large medical centers to join us, design it, executive it, and show that we could extend the platelet outdating by a couple of days by culturing on day two or day three.

We would probably be to the year 2002, and I don't meant to steal the thunder of people who are going to be speaking in the next session, but I bet by about then we're going to be talking about viral inactivation and bacterial inactivation in platelet concentrates, which would render moot the issue of culturing.

So it's hard to get too much enthusiasm

because by the time we show that this will work, it will be too late. In the interval, without more people doing this there are going to be a lot of patients who are going to be succumbing to transfusion induced infection, and that's unfortunate.

DR. BRECHER: Yeah, what I'd hate to see happen -- I'd agree with you, Jim -- is that we wait another four years while we're waiting for this perfect, high tech. solution. We have several solutions out there that the data is available that get us part of the way there. To not implement them I think is a mistake, and I think, to be honest, if the media tumbles to this or if a celebrity dies from bacteria contaminated platelet and it gets out that, you know, that one in 2,000 were contaminated, I think we're going to get -- "we," the blood blanking industry and the FDA -- are going to get raked over the coals.

MR. TABOR: Well, nobody is objecting to detecting bacteria in platelets. In fact, that's the reason we're holding this workshop, and my only point was it's very easy to glibly say, "Use urine dip sticks. So-and-so tried it," but we're talking about -- - and when you say things like that and you're talking about anecdotal experience and neither the public nor the medical community will accept that as a basis.

DR. BRECHER: Well, maybe the better question is, because I think you've got a world expert panel sitting here right now, is given the technology that is available today, what can we implement rapidly that would impact on the problem of bacterial contamination of platelets.

MR. TABOR: That's a good question, and I think that's what we should be discussing.

DR. BRECHER: Okay, and so let me just give my thought on that, and then I'll introduce the panel.

I agree. I don't think the dip stick method is all that good. I don't think it's any better than the Gram stain, but it would stop a few.

However, what I think we should be doing is trying to stop as many cases as possible, and the way I see this happening is probably with the technology that is currently available is to do bacterial culture, probably using an automated system at, say, 12 hours and then again at three days. That way I think we would maximize the capture of as many units as early as possible, and leave the door open to extend the shelf life of platelets, which I think would pay for most of these cultured units.

Now, I'll open this to the panel to see if they have any other ideas using currently available

technology.

DR. YOMTOVIAN: Well, I agree. I think of all the methods that are potentially available and discussed today, culture by far and away has the sensitivity level that I believe is needed in terms of the bacterial loads that we want to detect and interdict. So I support that.

But there are other questions. Mark is suggesting a two tiered approach. That gets a little bit cumbersome, although scientifically I think it's a good idea. Where to do it? You know, should the blood centers do it routinely on day two, as in the Vox Sanguinis article by Dr. Liu, I believe, and co-workers who are now in their second phase of linking it probably to a seven day outdate.

But I think those two are inexorably -- sorry for that -- linked. You have to link an increase in storage time with the bacterial detection.

I think from my vantage point at the hospital that I'm in, I come in every day wondering if I'll have enough platelets to meet the clinical needs, and I see that only getting worse as more regulations are passed on that will reduce the blood supply, and I think this is an opportunity to really make a difference by linking detection and extending of

outdate.

I think we should do it now.

DR. FRIEDMAN: Jim, do you want to add anything?

(Applause.)

DR. WAGNER: I would like to add something. I think for the most part I agree with most of the statements that have been said here. I think right now culture is probably the only method that most people seem to have confidence in, and I agree that there is a problem with logistics, of being able to provide platelets with the current dating period.

And I think where I see the problem is, you know, I talked about false positives during my test, but I didn't talk about false negatives, and so I think what people are subconsciously struggling with is if we extend the storage time to seven days, which might appear reasonable, you know, will there be some units that wouldn't be picked up with the methodology? And is it possible to implement it, for example, under a Phase IV type of situation where there would be surveillance of the data to try to determine if this is a reasonable way of going forward?

And so I think it's a matter of how is it best handled. What's a responsible way of going

forward? And I think those are very hard issues to deal with.

DR. FRIEDMAN: Jim, before I go to Celso?

DR. AuBUCHON: I would only make the comment that after five days of storage with most bacteria you're probably looking at a concentration of at least ten to the seventh, if not ten to the ninth, organisms per mL. Multiply that by a couple of hundred mLs that you're going to infuse. That's a potentially lethal dose.

If you go from five days to seven days, those organisms are already maxed out in terms of their concentration, and it's probably not going to make much difference in terms of the bacterial load that's being infused into the patient.

DR. WAGNER: There is going to be some slow growing organisms on day six and seven that we haven't dealt with here during this discussion that are going to pop up.

We're changing the system, and there will be new things that we will learn about it, and the question, I think, before us is if we're considering this, should that change be determined before anything is implemented if we're considering it, or should it be after it's implemented with surveillance, you know, if

this sort of thing is really considered something that might be worthwhile?

DR. AuBUCHON: I vote for your proposal for a Phase IV approach because the low frequency of occurrence of contamination and the lower frequency of these very slow growing bugs means that we'll never be able to prove safety in any kind of reasonably sized study. We're going to have to do it and then see what we did.

DR. WAGNER: Right.

DR. FRIEDMAN: Celso. Thank you for being patient.

DR. BIANCO: Celso Bianco, New York Blood Center.

I wonder if the panel could give me a little bit of a sense of the other side of the equation. There was a little bit of discussion about false positives, and what, if we were to go massively to do culture in all the platelets, single donor platelets or random donor platelets that we use, what would be the rate of false positives just by accidental contamination at the time of inoculation of the bottles?

The second part of that is considering the bag configurations that we have today and the way we'll

have to sample these bags on day three or day two or 12 hours and 48 hours, what are the chances that we are going to introduce a contaminant into the bag into that process and maybe create more incidence of bacterial contamination?

DR. FRIEDMAN: Which panel member would like to respond? Steve.

DR. WAGNER: Celso, I really agree with you. I think that how we do things is incredibly important. How the bag is sampled, how sterile samples are taken. I don't think that there's really a lot of information.

We just saw four studies on false positives. What's the basis for those false positives?

And when you think about false positives from a blood bank perspective, we're not only dealing with the platelet issue because what happens, you know, when someone says there's a contaminated platelet? Well, the blood bank has to go search the red cells and the plasma and pull those out and destroy them, too, and so there are economic impacts. There are supply impacts. There's logistical impacts, and we can't just snap our fingers and do this.

And so I'm glad to see this level of

interest. I think it's really good, but I think it's also important to think very carefully about how we do it and try to do it in the most logical, reasonable, but, you know, fast way possible.

DR. FRIEDMAN: Becky.

MS. HOWLEY: I'd like to follow on to --

DR. FRIEDMAN: Becky Howley, Red Cross.

MS. HOWLEY: Becky Howley, Red Cross.

-- what you just said, Steve, about thinking through this. Approximately half of the platelets that are transfused in the United States today, maybe a little fewer than half, are platelet pools from individual platelet concentrates.

If we plan to culture every platelet concentrate made from whole blood in inventory, that is going to be a tremendously expensive and extensive job, and again, that's about half of what's given today.

Then when you find your positive culture, you're going to have to go find and throw out the red cell and the plasma. So now you've got this mounting heap of things that you're going to throw out.

The other alternative, if we have to go to a pre-cultured platelet, would be to switch to all platelet pheresis type platelets, and that is something that I don't think that the blood suppliers or the

blood supplyees have thought about or thought about financing.

And so I'd like any comments from the panel on those ideas.

DR. BRECHER: Well, Becky, the reason why we cannot pool platelets today is because of the fear of bacteric contamination of the pooling. It would seem to me that if you were going to prospectively culture platelets at times to be determined, that you could make a case to pre-pool your random platelets and then only culture that pool bag, and then that would save considerable costs.

MS. HOWLEY: Yeah, and then you could throw out all six of the red cells and the plasma.

DR. BRECHER: Well, but also --

MS. HOWLEY: The pile is getting higher.

DR. BRECHER: Well, possibly, or you could quarantine them until you could repeat the culture and maybe set it up two more times before you throw everything away, similar to the way the viral testing is done. Two out of three wins because if there was a contamination in the methodology of taking your samples, you don't want to throw that product away because it really is sterile.

MS. HOWLEY: We've cultured hundreds, maybe

thousands of co-components because when we have report of a culture positive platelet in a pool, we go back; we withdraw the red cells; we culture those. They're negative. I can't remember a time when one was positive.

I know that it's possible to happen, and you always way to do that to see if it could happen, but it just doesn't happen.

DR. BRECHER: Well, probably that reflects the storage temperatures of the products.

MS. HOWLEY: Oh, of course.

DR. FRIEDMAN: Andy.

DR. HEATON: Andrew Heaton, Blood Systems.

You know, one of the issues, as I listen to the panel talking, is those who are speaking are primarily those who are at the transfusion end of the system. Now, you are already going to face a huge bolus of cost increase as a result of NAT testing, now the deferral of NAT English donors, such as myself, and as a result of other regulatory initiatives.

In order to license or the FDA to approve some form of change, they undoubtedly would wish to see a change to the manufacturing process, not a change to the end user process.

And if you think in terms of the

implications of what you're suggesting, I presume that you're thinking in terms of having some bag of a culture medium which might be attached to a pheresis kit or a pooling kit. Then you then seek to have the manufacturer divert a portion of the product into this and then monitor it over a period of time.

That's got an enormous production implication. It would require computer tracking, computer measurements, the withdrawal of in date products. The cost implications of that would be far greater than anything else we've yet passed on, and I wonder whether the hospital environment is prepared to absorb such a major increase in manufacturing costs. From everything I see, I doubt it.

DR. AuBUCHON: Well, Andrew, I appreciate everything that our blood center does for us. That's not your blood center, but I appreciate everything that all blood centers do for their hospitals. My argument is this isn't something you can help us on; that for a blood center to try to prove sterility, bacterial sterility, is going to be incredibly expensive and inevitably low sensitivity. It's just not going to work.

This is something that's going to have to be taken up by those of us who transfuse the platelets,

who generally are holding the platelets at the time that they can be found to contain bacteria.

Now, not every hospital has an automated bacterial detection device. I understand that, but most hospitals that have a platelet inventory, that are holding platelets, that use a significant number of platelets, do have some type of automated device.

The small, little hospital that transfuses platelets once a week or once every two weeks gets its platelets from the blood center and transfuses it within a half an hour probably. That's a different kind of situation, and maybe in that circumstance the blood center could act like a transfusion service could act, the way I'm proposing the transfusion service proceed.

So I really don't look to the blood centers to solve this problem for us.

DR. BRECHER: Well, you know, if we talk about phasing things in, and I had proposed a two tiered sampling technique, but maybe the way to start is to go to a Phase IV sampling on day three or four, allowing at least a 24 hour culture, and then extending it to seven days, and I think that that would be very well received by the transfusing hospital services all over the country. It would probably be cost neutral,

if not cost saving.

DR. FRIEDMAN: Sir.

DR. KUEHNERT: Matt Kuehnert, CDC.

I just was thinking about some of the data that Roslyn was presenting, and I guess the question is primary directed towards Mark.

We're talking a lot about false positives and false negatives and using culture as the gold standard, but what would be the practical positives and negatives in that what is the level of organism in which there actually is a significant transfusion reaction in recipients? We still don't know the answer to that question, and I was just wondering in any of the studies that, Mark, you've done whether you've looked at platelets that have been released and whether they cause significant transfusion reactions.

Because I think, you know, we're sort of looking at units that have ten to the two organisms versus obviously ten to the sixth, ten to the seventh. That's a unit that's going to cause problems. We don't know with lower levels what that really means, and when we're talking about day five, extending to day seven, that may become a significant issue and already is a significant issue, but a more significant issue.

DR. BRECHER: Well, most bacteria that have

been studied will reach plateau or at least significant concentrations by day three or four. I think there's a lot of hard data that has been published over the years to show that.

What is the lowest inoculum that can cause problems? I think you have to go back to that hold NIH paper with salmonella. Back in the good, old days they were transfusing fresh platelets within a couple of hours of collection, and it is estimated that the concentration that went into the patient there was probably less than one CFU per mL.

So it's going to depend on the recipient. It's going to depend on the organism as to what is the lower limit, and it can be very low to cause disease.

However, when you're talking about storage at room temperature for multiple days, presumably every bacteria that we have been concerned about will reach detectable levels with a small sample size.

DR. KUEHNERT: So that means if there is any bacteria detected or at any level, then you would say that the unit needs to be discarded?

DR. BRECHER: I think so. That would be my opinion.

DR. AuBUCHON: If reproducible.

DR. BRECHER: Right. If reproducible.

DR. BLAJCHMAN: I just want to make a comment that it seems to me that we're going around in circles again, and the reason I say "again," it seems to me that the blood transfusion industry has been for years figuring out reasons why things should not be done.

This has accounted for delays in HIV testing and delays in other things, and it seems to me that we have an instant with bacterial contaminations and sepsis, as you heard this morning, of a serious problem, and there are other problems that need to be worked on as well, but it seems to me we have methodology.

We seem to have encountered dealing with NAT testing, despite the problems we've been implementing, other things when they've been mandated, and what is required at this point is a mandate that requires something to be done for the bacterial sepsis problem, and when that happens, we'll figure out a way of doing it.

And I think one of the things that has also happened is that nothing has been done because we haven't agreed that it needs to be done. So there aren't friendly systems. We don't even have systems. The blood packs are not readily available to do

adequate sampling. We have to take samplings from the tubes.

We've talked about this for a number of years because we haven't agreed that it needs to be done, and now we're being -- people talk about the financial impact. Well, you have a system in the United States where you haven't figured out how to put money into the system, in a DRG system, to get to pay for some of these things. So you're using that as an excuse for inaction.

And I think it's time to get over that inaction soon.

DR. FRIEDMAN: Last question.

MR. BERNARD: Bernard, Waynespol (phonetic) Corporation, directed at Jim AuBuchon.

Jim, of the many things that you're known far and wide for, quality of adjusted life years is one of them. You've been strangely mute on that point today. Would you comment?

DR. AuBUCHON: No. I think I actually did myself one better, and that is that I was able to show based on our own -- I'll be brief -- but our own experience that I don't have to calculate the cost effectiveness of culturing if I can extend the shelf life of the platelets because it's cost savings. That

means the cost effectiveness number is negative, and most decision analysis don't want to try to display that and explain it.

So if we can culture and extend the outdate, we save money in the process of making the blood supply safer. That's a win-win all around.

MR. BERNARD: So do you see this as a package?

DR. AuBUCHON: Yes, I definitely see this as a package that we can do one by doing the other and do the patient better in the process.

DR. FRIEDMAN: Thank you for attending this session.

I think the message that's getting recorded is that a group of people feel something can be done. It probably needs to be coordinated because it's not an individual. It's a multi-center activity that probably will require manufacturing input, but the tools are at hand where potentially something could be studied.

Mo Blajchman has some travel plans, and he's asked to present before the break. So I'm going to turn the program over to Steve Wagner to introduce Mo.

(Applause.)

DR. WAGNER: Len, I don't need to introduce

Mo at this point. So welcome.

DR. BLAJCHMAN: Just following protocol.

I have to catch a plane. So I need to go. So I'm going to very relatively briefly start the next session, even though I'm the third speaker, and I'm going to start the session on how we might undergo contaminant avoidance and microbial interaction.

If I can have my first slide, there are a number of potential strategies to reduce the risk of transfusion associated bacteremia and sepsis, and there are at least eight, and they're listed here and will be talked about during this session: improved donor skin disinfection; removal of first aliquot; extension of blood donor screening; limitation of component storage time; leukocyte reduction; pretransfusion detection; and lowering the temperature and pathogen inactivation.

I'm going to briefly talk about three of these that we have and review some of the literature and provide some of our own data in this regard, and these are the three approaches that I'm going to talk about over the next few minutes.

One is the data that's available about extension of blood donor screening, leukocyte filtration, and lowering the storage temperature of platelet and red cell units.

Extension of blood donor screening. Now, obviously this can only be effective in instances where there's a silent donor bacteremia, and the comment that I would make or the data that's available is that this extension of donor screening has been in ineffective, particularly in studies to reduce Yersinia enterocolitica infection.

There's a paper by Grossman, 1991, and the Austral-Asian (phonetic) group from New Zealand by Theakstan, et al., where they looked both at -- I think it's my next slide -- no, they looked both at serologic screening and donor screening, and these have been shown to be ineffective.

Now, what about leukocyte reduction? First of all, there is a fairly large body of data out there about bacterial contamination and how leukofiltration affects the level of bacteria at present. It's clear from the available information and literature that phagocytosis is clearly important in the elimination of viable bacteria that might be present in a cellular blood product unit.

Now, in spiking experiments -- and I emphasize all of the data are from spiking experiments -- the use of leukofiltration for both whole blood and for platelet units particularly experimentally has been

shown to reduce bacterial contamination. The level of bacterial contamination can be reduced by leukofiltration.

The problem with this is that not all bacteria are reduced in this result, but many are, and particularly Yersinia enterocolitica.

Now, a very important point, and I'll show you a few experiments that have been done in our laboratory, but just to summarize first that the experiments with leukofiltration indicate that the leukofiltration should be done after a hold of at least eight hours at 22 degrees, and I'll come back to that momentarily.

And I would emphasize, however, that despite the fact that leukofiltration is associated with a reduction in the number of bacteria and in some instances can eliminate the bacteria that are present, and all of these experiments, as I've emphasized, are from spiking experiments; there are no prospective studies that have been done to indicate that there's a clinical efficacy of leukofiltration in reducing the transfusion associated septic reaction rate.

Now, here's some experiments that we did. This is with staph. epi., and basically we have shown that over an eight hour incubation at four degrees --

and in this study we used 77 spiked units -- you can see the controls. The growth just keeps going on, but with an incubation at four degrees as you increase the time of incubation, you increase the growth in the bacteria, which seems to bottom out at about three to four days, but seems to start back up beyond that.

Now, if you do the same experiment in the same bags, essentially the same bags -- this is a bigger number -- with 22 degrees hold before the filtration, you can see that over an eight hour period you can actually, with the organism that was used in this experiment, you can actually show that with such a hold for eight hours, you can reduce completely the number of bacteria that are present in that unit.

This experiment, as I mentioned, was done with staph. epi. We've done similar experiments with Yersinia. This is incubation at four degrees over an eight hour period, and you can see the filtration after up to an eight hour hold had very little impact on the removal of bacteria.

However, if you did the incubation at 22 degrees Centigrade, you can see that you can reduce the bacterial load.

Again, I emphasize these are spiking experiments, but if you're going to try to reduce the

bacterial load with leukofiltration, you need to do this or it's optimal to do this at 22 degrees and eight degrees.

But I'm not suggesting for one minute that this approach be used to reduce the bacterial load in cellular blood products because there are some organisms that this works with and others that it does not, but certainly at least from spiking experiments in our lab and in other labs, it suggests that leukofiltration may be useful in reducing the bacterial load.

And, incidentally, just a word about the mechanisms. I think the mechanisms are multiple and include phagocytosis by leukocytes. It includes compleminic (phonetic) inactivation, and there is also direct effect of the filter.

We've done experiments in which we've leukoreduced units of blood so there are no white cells, and you pass these contaminated units -- platelet units we've done in most of our experiments -- through the filters, and the filters in many instances will remove the bacteria that's present, and high levels of bacteria are often reduced to very low levels.

So leukofiltration is a way of reducing the

bacterial load in both red cells and in platelets.

May I have the next slide? Not moving ahead.

It's been suggested that we lower the storage temperature of both platelet and red cell units and this would reduce the rate of bacterial growth. That's the intention, and there is some data in the literature out there.

Sorry. Go back. Back to my last slide, please.

It would be effective, particularly for platelets, to reduce the risk of transfusion associated sepsis for platelet concentrates by lowering the temperature to four degrees. However, none of the techniques that have been studied thus far to lower the platelet suspension or platelet concentrate to four degrees, if you examine the hemostatic function of such platelets that have been stored at four degrees using every method that's out there, those platelets are not hemostatically effective.

Jaroslav Vostal has reviewed this issue in a relatively recent article in Transfusion Medicine Refines, but these cold storage, despite using all sorts of inhibitors, does not protect the platelets from the platelet storage lesion that occurs during the

cold.

There's an interesting report that occurred in 1997 by Bradley, et al., and they lowered the temperature of red cells that have been deliberately contaminated with Yersinia. They lowered the temperature from four degrees to zero degrees, and that drop of four degrees seemed to have an impact on the proliferation of Yersinia enterocolitica.

There's one single report. It's interesting, but I think it needs to be duplicated. I think there's a problem with that, to store red cells at zero degrees, because the control at that temperature in terms of avoiding ice crystal formation that could do other things to the red cell is important.

I'm going to stop there and leave you at this point, and that's just a start. There will be other speakers over the next hour that will talk about other approaches to contaminant avoidance and microbial inactivation.

Thank you for your attention and giving me a chance to speak before I leave.

(Applause.)

DR. SYIN: Right now we have time to take a ten minutes break, and would you please come back at

3:05 so we will finish this session on time?

Thank you.

(Whereupon, the foregoing matter went off the record at 2:55 p.m. and went back on the record at 3:05 p.m.)

DR. SYIN: I think we're going to continue our third session.

Steve.

DR. WAGNER: Hopefully people are filtering back. I'm hoping that this slide carousel is better than the previous one that I had.

Okay. This is the third and final scientific session where data will be presented. It's entitled "Contamination avoidance and Microbiological Inactivation."

Mo Blajchman spoke of a few measures that might reduce the bacterial load, and I will speak of two others, and they involve -- I'm waiting for the slides to come, and I'm hoping that they're in a different carousel -- and the two measures that I'd like to talk about today in a real brief talk -- we'll try to keep it to about ten minutes -- is -- you're there. Good -- is diversion of the initial blood flow. This is of whole blood.

You've heard about this a bit through this

meeting, and extension of room temperature hold for whole blood, which is an old idea that I'm just bringing up for reconsideration.

I'm afraid you are going to have to advance this slide. Oh, no. We're okay.

The reduction of the bacterial load of skin associated bacteria might be achieved by diversion of initial blood flow. It won't do anything for someone who's bacteremic. It also might prevent the unnecessary destruction of whole blood.

There's a lot of cases where phlebotomists collect around 500 mLs or so of whole blood, and then they try to collect the sample tubes that are needed for viral testing, and they can't get enough blood to fill those tubes, and that whole unit has to be destroyed.

And so if you take your samples first and then collect your blood, there's a chance that you might be able to have enough blood for viral testing or infectious disease testing, and also have enough blood, which would be at least 450 mLs for transfusion.

And so these systems that have the potential for diverting blood for collection initially can potentially both reduce the bacterial load of skin organisms, as well as perhaps enable the collection of

units that might otherwise not be obtained.

There's a few papers, not very many, on phlebotomy coring. Mo Blajchman talked about this paper in the morning. There's another paper that's an abstract that appeared in last year's ISBT meeting where subcutaneous fatty tissue was found in blood bags as a result of phlebotomy.

So we know that there is tissue that is sometimes introduced into blood units, and that tissue may contain bacteria.

There's three studies that have demonstrated in somewhat different ways that skin organisms might be reduced in bacterial load by diverting the initial blood flow and collecting that into separate containers from the blood that might be given for transfusion.

There's a European study that has been talked about today. Basically they found that roughly about two percent of their units were contaminated when they collected whole blood in two satellite containers rather than the primary containers. So that was 116 out of about 3,400 units.

When they looked at the components of blood that were made from these units, in other words, red cells, platelets, and plasma, and this was all done by

culture results, they found instead of seeing 116 associated components that were contaminated, they only found seven of the components of the 116 were contaminated, and that's suggestive that some bacteria was removed by these satellite containers, and the resulting bacteria that went into the primary container contained much fewer organisms.

And these are encouraging results, but I think that further evaluation might be difficult. They had a very high culture positive rate of two percent, and typically, you know, when we look at all the data that's presented today, we've seen culture positive rates, maybe one in 3,000 or so, and so you question the high culture positive rate.

They also didn't compare the culture positive rate in whole blood pre and post diversion. Remember they're comparing whole blood to platelets or whole blood to red cells, and so in a sense that's mixing apples with oranges, and there was no quantitation to demonstrate what the reduction in bacterial load might be, and so anyone might guess what it might be.

There was another study that was done in Raleigh Carmen's lab a few years ago that was a poster that was presented at the AABB, where they introduced a

small liquid inoculum, in this case staph. aureus, inside the lumen of the needle, and they either maintained that small inoculum inside of the needle as liquid or they allowed it to dry, and then they used that needle to pierce a blood bag, a container that contained sterile plasma, and then every five -- and then they transferred the plasma into -- through the needle and collected 5 mL samples from that needle, and they did quantitative plating.

And what they found basically was that there was a 98 percent reduction of colony forming units from the first tube of blood that they collected compared to the fifth tube of blood. So there's a one to two log reduction in bacterial load in this system.

We've looked at a similar system that some work was done at the Red Cross. This is an experimental set that was constructed by Truomo (phonetic) Corporation. Jeff Meripole (phonetic) was very helpful in putting this together, and basically what it is is a needle in a diversion arm that goes to a connector which you can -- a Luer lock fitting, which you can connect to sample tubes.

This particular experimental model is not a closed system, and so it wold not be able to be introduced for blood banking use, but it's not hard to

imagine how one might devise a closed system, and this small, supplemental bag was merely to collect the air that's present in the tubing, and so initially what we did is we contaminated a sterile medication port with staph. aureus, and we basically painted it on and then allowed it to dry, and inside of this blood bad we had either whole blood or saline.

And we allowed the fluid -- the contaminated port was pierced by the needle, and we allowed the fluid to pass through and go through to the diversion arm, and we successively took six, seven mL samples of blood.

And then we closed this clamp and allowed the blood to flow into a transfer pack, and we analyzed how many bacteria were present in the sample tubes that we took, and we analyzed how many bacteria was present in the sample pack.

And basically the data, in summary, when we used saline in a system like this is that by the time you got to the third tube, about 96 percent of the bacteria were in the sample tubes and not in the system, and by the time you got to the sixth tube, about 99 percent or so percent of the bacteria were in the sample tubes and not the system.

When that experiment was repeated with

whole blood, we got similar, but not identical, results. The collection of bacteria was not quite as good in whole blood as with saline, but basically we saw about 88 percent of the contaminating organisms that were introduced were removed by the third tube, and 93 percent by the sixth tube.

So potentially taking samples in the beginning rather than later might reduce the bacterial load of skin associated bacteria.

What other measures might be considered? A number of years ago we did some studies and other people have done studies on extending the room temperature holding time of whole blood before platelets are prepared for components, and basically what you're doing is allowing the white cells, and particularly the granulocytes and the monocytes more time to interact with bacteria, and that interaction of bacteria can both kill bacteria, as well as have them sediment differently during component preparation.

And basically a group in Spain compared a six hour room temperature whole blood hold to a 16 hour whole blood room temperature hold, and this was all done with in vitro spiking experiments, and they looked at day 35, at platelets, and found in general by day 35 anyway they didn't see any bacteria in platelets.

I'll show you some more detailed information about red cells by day 35.

They did a 16 hour hold and saw fewer staph. epidermidis and few E. coli in the six hour hold than in the 16 hour hold, and they saw this at both day two and day five.

So it appears that if you incubate whole blood with organisms at room temperature for an extended period of time, there's more time for granulocytes and monocytes to interact with the bacteria, and there's a higher proportion of those bacteria when you sediment the cells during component preparation to sediment with a buffy coat, and then when you prepared red cells and platelets, there are fewer bacteria in the platelets and more bacteria in the red cells.

And we did a similar study look at ten organisms and found the same thing, that basically with respect to the platelets, there was a trend toward lower levels of bacteria in the platelets in the 24 hour held platelets -- 24 hour held whole blood from which platelets were prepared -- compared to eight hours.

Now, for red cells, it's kind of an interesting story, and I'll show you more of that in

the next slide, and basically we found very little, if nothing, in the plasma in terms of bacterial level when whole blood is spiked.

In this slide, basically red denotes when more organisms are noticed in 24 hour hold compared to eight hour hold, and these are unfiltered. And so if you don't filter red cells, but you extend the whole blood room temperature hold for 24 hours, you see more organisms for the most part in the 24 hour in red cells who have been prepared from whole blood at 24 hours than at eight hours.

Now, if you look at platelets -- oh, and if you filter these cells through a leukocyte depletion filter, a lot of these bacteria are associated with leukocytes, and so you find that you end up bringing the levels down when you filter the bacteria in this case to levels that are similar. The purple indicates similar levels, statistically insignificant differences.

Now, for platelets, if more of the bacteria are going with the buffy coat, that means fewer of the bacteria are in platelets, and bacteria normally would sediment with platelets during centrifugation based on their size and other properties, but with a 24 hour hold if white cells are associating with bacteria, then

you find out that the bacterial loads are less in the platelets if they're derived from whole blood that's been kept for 24 hours compared to eight hours.

And so both a diversion of whole blood as well as whole blood hold can have some impact on the bacterial load in components. It certainly won't prevent sepsis in all cases, but it's not clear if it will prevent sepsis in some cases.

Anyway, the next talk will be given by Mr. Carl McDonald. He's going to talk about an NBS evaluation to optimize skin disinfection. Mr. McDonald has a Master's in applied immunology from Brunell University in London. He's worked in transfusion microbiology for 18 years. He's currently the head of bacteriology in the Transfusion Microbiology Department, National Blood Service.

And so, Dr. McDonald, please.

(Applause.)

MR. McDONALD: Okay. The first slide, please.

So I'm going to talk on the National Blood Service, the NBS evaluation to optimize skin disinfection, and I'm going to start off with the objectives of our study, run through critical factors affecting donor arm disinfection, go through the

methods that were actually used to quantitate the amount of bacteria present on the donor's arm pre and post disinfection, go through methods of actually evaluating our study, go through our results, and finally finishing off with our conclusion, which we thought was the optimum method.

So the objectives of our study were to have a national, validated, best practice venipuncture procedure which would meet our MCA requirements, and the MCA, the Medicine Control Agency, are our equivalent to the FDA.

Also very importantly, we want to reduce the risk of bacterial contamination we've all heard today causes severe morbidity and morality in patients.

And also, we'd like to reduce our litigation costs, which are becoming quite substantial particularly in regard to transmissions which have occurred due to inadequate donor arm disinfection, and we feel in court we can argue that we're using the best practice procedure and hence reduce our costs.

So critical factors for donor arm disinfection. Obviously, highly important is the disinfectant or disinfectants you're actually using; the type of application device, how you're actually putting a disinfectant onto the arm, be it a sponge, a

swab, a wipe, or a gauze; the method of application, a one or a two stage process, three, four, et cetera; how many do you actually want which would be the optimum method; time of application of the disinfectant; time of drawing of the disinfectant; and the mode of application. Is it put on in a spiral manner? Is it put on in an up and down motion?

And also, we think what tends to get overlooked is the quantity of disinfectant actually put onto the arm, and actually having completed this study, we're avid believers of putting copious amounts of disinfectant onto the donor arm.

So after initial evaluation, trying out various techniques, such as contact plates, we decided that the most sensitive and practical to be used in a donor session was a direct swabbing and plating technique which performed pre and post disinfection.

This is carried out by impregnating a cotton wool swab with phosphate buffered saline, a three percent between 80. All of the materials are sterile. Swabbing a four by four centimeter area of the antecubital foca (phonetic) for 20 seconds, plating directly onto neutralizing agar plates, incubating for 48 hours at 37 degrees and then enumerating.

This slide here shows you what we found

typically looking at 100 blood donors pre disinfection counts, and these are colony forming units per plate. We had a main count of 3,099. We had no bacterias, no donors to start with had no bacteria present on their arm, and 27 percent had over 3,000 colony forming units per plate predisinfection. Another 70 percent had over 5,000.

So we've got a very, very high bacterial load before we start, and we're currently working in our laboratory on a spiral plating study to more accurately determine the content of bacteria present on the donor's arm, and we found that approximately five percent of donors have over 10,000 organisms per square centimeter present on their arm.

So we're starting off with a very, very high bioburden with a certain percentage of our donors. We're expecting a lot of our disinfection procedure to go down from very high numbers to what we obviously want to be zero in a very, very short space of time.

And I must say in our study we're put under quite a bit of pressure to come up with a technique. Not only was it excellent, but it also had to be very, very rapid, and that's pressure that would be put on us by nursing staff.

So this is what we found typically, between

ten percent of our donors predisinfection, a plate with almost constant growth of bacterium, and this is what we found ten to 20 percent of the time, showing a very, very high bioburden.

We started off with our study, the initial trial with routine blood donors evaluating three methods, and the three methods we evaluated were the North London method, which consists of impregnated 70 percent alcohol, 0.5 percent chlorohexidine wipe system. The North London method, the current method, is a one stage wipe method.

And we also tried out a two stage process to see if performing the operation twice would actually improve the procedure.

And we also carried out the method which at that time was used by the Liverpool Center, which was impregnating sterile gauze with Simpson isopropyl alcohol, hydrogen peroxide 0.125 percent, and chlorohexidine gluconate, 0.5 percent, and we're quite interested in this method to see if the hydrogen peroxide would have a sporicidal effect, which the other methods don't.

So this is the North London method. It comes in with little sachets here, patches out for a company, for Y company for us, and this is then wiped

onto the donor's arm.

And this is a deliverable methods, sterile gauze here and disinfectant mixture in this container here.

I've got to say that this is now no longer used by the Liverpool Center after an MCA inspection. After an audit they were criticized on the fact that gauze was no longer sterile after the package had been opened, and also they weren't happy about the sterility of this disinfectant once it was opened. So this has now been withdrawn from use on those grounds.

So these are our results for the initial part of the study. The three different arm cleaning procedures down here, and these are post disinfection results, coliform units per plate.

No donors after cleaning had zero bacteria on their arm, which is very, very disappointing, and over eight percent of donors had over 1,000 remaining at the end, and we had over one percent over 3,000, and for our study we on the advice of our statistician ranked all of the results on log reduction, and very, very poor log reduction was obtained by all three methods, and there is no statistical difference between all of these three methods, showing that performing the North London method twice or once didn't statistically

make any difference.

So we were quite despondent about the actual findings, particularly two of these methods, the North London method and the Liverpool method, were used by our service to routinely clean the blood donors. So very, very despondent findings about this.

So rather than going out to a full field trial for the next stage of the study, we decided to perform mini trials of nine disinfectant techniques on our own staff, (a) that we could actually use things that we possibly could not get away with with routine blood donors, and also it's much, much quicker to perform using our own staff.

So these are the nine methods we evaluated. Just running through them, we used a commercial disinfection kit, the Medi-Flex kit, which consists of a two stage process. The first stage, isopropyl alcohol, and then a second stage of two percent tincture of iodine, and these are both put onto the arm for 30 seconds and then left to dry according to the manufacturing instructions, and we've determined that the left to dry time is 30 seconds.

We also did our own adaption of this method by what we called the Medi-Flex fast method, which the only difference between that and the standard method is

we reduced the tincture of iodine application drying from 30 seconds to 15 seconds, and we did a Medi-Flex adapted method which was exactly the same as this, except the tincture of iodine, instead of being put on the arm in a spiral motion, was actually put on in an up and down, putting copious amounts onto the actual venipuncture site.

We also evaluated the Medi-Flex alcohol application devices as a two stage process for two reasons, (a) for donors who possibly could be allergic to iodine, and I should say at this point in the United Kingdom and Europe iodine is not used for phlebotomy disinfection at all. We used alcohol disinfection. So we've got no idea how many donors out there who could possibly be allergic to iodine.

And also, to see as we're using alcohol here and we're using an alcohol swab stick down here, and we also used our own wipe system, also an alcohol disinfectant, to see if the method of application of the disinfectant -- what effect that had. So we did this as a two stage process.

And then we also evaluated the Standard American Association of Blood Banks' method according to their technical bulletin, which is a two stage application of povidone iodine, 0.75 percent in the

first stage and a second stage of one percent.

Then we devised our own little method of applying iodophor in the form of povidone iodine swab sticks, 0.75 percent, and then 70 percent isopropyl alcohol.

When we did this, there were concerns expressed by nursing staff that the donors wouldn't like having their arm stained with iodine, and obviously this stage would remove it, and also we were interested to see this combination of disinfectant, how it actually worked.

So we used an impregnated commercial alcohol swab stick, and we used this as a two stage process, and we also tried out a commercial bench wipe, which was a quaternary ammonium compound as a one stage and a two stage process.

And this is the Medi-Flex commercial donor arm disinfection kit. This is a nice little package, sterile package here, which is gamma irradiated, and this one little packet serves one blood donor.

This is the alcohol application device here, and this pencil type structure here is the iodine applicator.

And this is the Medi-Flex alcohol applicator being used. It's quite an ingenious device.

You've got two sort of plastic wing type structures here with a vial of alcohol in the middle, and it works by you squeeze the two plastic wing structures together. It crushes the alcohol vial in there, and then releasing the alcohol into the sponge, and it does release a nice amount of alcohol into the sponge, and you can put a nice, copious amount of alcohol onto the arm.

Also what we liked about it, it does give a nice abrasive action, and you can give a nice, good scrub of the arm, a nice, good preclean of the arm prior to adding the tincture of iodine.

And this is the iodine applicator, pencil type structure with a little white pen tip, which is of gauze type material. This is, again, crushed between the fingers, releasing the tincture of iodine into the tip, which can then be applied with a nice, controlled motion onto the arm. We did like the way -- how controlled it actually was applying it. There wasn't any dripping of iodine around the donor or near our staff.

And this is the povidone iodine swab sticks, which used the AABB method. And I at this stage thank Dr. Mindy Goldman for supplying us with these kits and also the initial Medi-Flex donor

disinfection kits, and we were actually quite surprised that these were put into routine use because we did find them extremely messy. Once we opened the packaging, the iodine did drip everywhere, and we were quite shocked actually it could be used.

I think if we used these in the U.K., we would have a very, very large bill on donors' clothing, as well as our staff's clothing, and also more importantly, some very, very unhappy blood donors.

So this is the povidone iodine swab stick being applied to the arm with the AABB method, and we also used these sticks for our own povidone iodine first and the alcohol swab stick second.

These are the alcohol swab sticks being applied to the arm. It came as a nice, little commercial package, each individual stick being packaged individually in a nice, little sachet, rather like the swab sticks.

And these plates here show you, although I'm not going to present you the results regarding the quaternary ammonium bench wipe. For each disinfectant we evaluated, we validated the neutralizing ability of our neutralizing agar plates by putting on an impregnated cloth for the relevant disinfectant of the selectin being evaluated.

A plate with bacteria prior to putting the cloth onto the plate, and this here is the AABB one percent povidone iodine, and we've got no zone of inhibition around the plate, showing 100 percent neutralization of that disinfectant.

Here with the quaternary ammonium compound you can see this zone of inhibition, which was approximately 50 percent of that of the control, and that is why we could not neutralize the quaternary ammonium compound on this plate, and that's why we're not going to present any results regarding the quaternary ammonium bench wipe. The results were indifferent even taking this into account.

So these are the results for our mini trial, ranked again, as I said, by log reduction. Medi-Flex adapted method came in as number one. Small numbers, I should say, in our mini trials, 29, 30 donors, in that region.

We had a mean count with the Medi-Flex adapted method of three. And 79 percent of donors post disinfection had no bacteria present on their arm. This was the sort of thing we were looking for in our initial study. Ninety-three percent, less than ten; and 100 percent had less than 100 bacteria present, and a good log reduction, 2.38.

Medi-Flex fast method came second, and the Medi-Flex standard method came third, and I should say there is no statistical difference between these three sets of results, although our statistician did say that there's almost a difference, as statisticians speak. So he reckoned if we did 100 of these, we would actually have a difference between the Medi-Flex adapted and the Medi-Flex standard. There would be a difference.

And it goes to show that just applying the disinfectant, an up and down motion, copiously putting it onto the actual venipuncture site would appear -- or to the equivalent to the actual spiral method, going out in a spiral from the center outward, and the theory of that is you don't recontaminate area you've cleaned.

So as I said, the proof of the pudding is actually in the eating, and that doesn't actually appear to be true from our results. We don't think that applies.

We're also concerned with the spiral that the area you really want the disinfectant is actually where the needle is going to go in, and although you can do this nice little spiral pattern, we are concerned you may actually not put enough in that center region.

So coming to number four was the Medi-Flex alcohol application devices, again, with a good log reduction of two. I should say that no donors in this mini trial had no bacteria present on their arm, 69 percent less than ten, and 86 percent less than 100, and 90 percent less than 1,000, but we haven't got 100 percent as we have here, and there is a statistical difference between the alcohol method and the adapted method.

So the center AABB method, 39 percent of donors having zero bacteria post disinfection and not achieving 100 percent, less than 1,000, and a different log reduction and poor results attained for the last two methods.

I'll just show you, just going back, that the method of application, the application devices are very, very important. This nice Medi-Flex application device gives a nice, good scrub of the arm, and that is far superior to actually putting the alcohol, the same alcohol, on with a swab, and it's also superior to our wipe system of putting the same alcohol on to the arm.

So what you have to do from these mini trials is take out the top methods, the Medi-Flex adapted method, compare that to the North London Standard county used, North London method, to try those

out on a full field trial, and we also decided to take out the Medi-Flex alcohol method for use for donors that could be potentially allergic to iodine.

So these are the results from our final field trial, post disinfection results going Medi-Flex adapted method coming number one, 100 donors in each wing of the study. The mean count is seven. Again, results very much like the mini trial. Seven percent of donors had zero counts post disinfection. Five percent had less than 90. Ninety-eight percent less than ten; 98 percent less than 100; between 100 and 1,000, which achieved 100 percent reduction.

There was two donors who had 214, 215 counts. Excellent percentage reduction, 99.79, and log reduction, 2.67. And there is a statistical difference between each wing of this study. The Medi-Flex adapted method is superior to the Medi-Flex alcohol, which did perform well, but not in the same league as the Medi-Flex adapted method. Good log reduction, good percentage reduction, not achieving 100 percent less than 1,000, but overall not bad results.

The North London method, the one we are currently using shall I say, again, giving very, very poor results.

So summarizing, the Medi-Flex adaptive

method is the most effective method, vastly superior to the current North London method, giving a tenfold improvement in performance over that method, and the Medi-Flex alcohol times two does offer an alternative to donors allergic to iodine, and the Medi-Flex commercial disinfection system does offer considerable advantages.

And these are the disinfectant can be applied in a controlled manner, and this is particularly relevant to the tincture of iodine. Using iodine or iodophor, et cetera is not used in the U.K., and we were concerned about this, but we were happy the way the iodine could actually be put on in such a controlled manner. It didn't get all over our donors' clothing, and it wouldn't get onto our staff's clothing.

Also putting iodine onto the arm has a benefit. You can actually mark what was actually cleaned. It gives a nice stain where the needle is going to go in.

The alcohol application devices did give a nice, good, abrasive action and did put isopropyl alcohol onto the arm.

The arm cleaner's fingers do not come into contact with the donor, which stops what we have at the

moment of using these wipes, that our staff's fingers become extremely dry. It also stops cross-contamination occurring from staff to donor and donor to donor, et cetera, and very importantly, the applicators are sealed in sterile units, which obviously meets our inspection requirements.

The disadvantage of the system is the cost, which is considerably greater than our current wipe system, and also it will require increased storage capacity compared to our wipe system, which are quite small little sachets.

So in conclusion, the Medi-Flex adapted method offers the National Blood Service a national, validated and superior best practice arm disinfection procedure and we hope should contribute significantly to the reduction of the risk of bacterial transmission, which we've all heard today does cause severe morbidity and mortality in patients.

Thank you.

(Applause.)

DR. WAGNER: Thank you.

The last scientific talk today will be given by Lily Lin of Cerus Corporation. Dr. Lin has been working many years with a number of her colleagues to inactivate viruses and also bacteria in platelet

components. She's, I believe, the head of platelet biology; is that right? No -- in Cerus Corporation, and she's going to talk to us about methods -- she's going to talk to us about inactivation of bacterial in platelet concentrates by treatment with the Psoralen S-59 and UVA.

Dr. Lin.

DR. LIN: Thank you.

May I have the first slide, please?

Well, my presentation today is a summary of all the bacterial inactivation studies we have done using single donor platelet concentrate and pooled random donor platelet concentrates by treatment with the new Psoralen S-59 and UVA.

And I would like to acknowledge these key people who contributed to the work I'm presenting today. At Cerus, Aarti Savoor and Larry Corash and Dr. Peyton Metzel and Dr. Don Buchholz of the Baxter Health Care Corporation, and Dr. Folki Knutson and Professor Claes Hogman of the University Hospital in Uppsala, Sweden.

Now, Cerus in collaboration with Baxter Health Care has developed this photochemical treatment system for platelet concentrate to increase the safety of platelet transfusion, and this process involves the

addition of the new Psoralen S-59 into a unit of platelet concentrate suspended in a combination of plasma and a platelet additive solution named PAS III, then the illumination of this mixture on a UVA light device for a brief period of time, and the data I'm presenting today are generated by the staff, and the commercial system that Cerus and Baxter are developing for commercial use contains a final step by treating the illuminated platelet concentrate in an SRD to reduce the level of residual S-59, as well as the free photo products before transfusion. So that reduces the patient exposure to the S-59.

Now, just briefly, the mechanism of Psoralen is that Psoralen has a basic structure like this. It has two reactive ends of this molecule, and in the absence of light, Psoralen specifically intercalates into helical regions of both RNA and DNA, and only when UVA light is turned on, it activates the molecule, and it forms a covalent addition. This is a 2+2 cyclobutane addition to pyridine bases on the nucleic acid, and because it has two reactive ends, both a mono addition of the compound, as well as the cross-link can occur if there is another pyridine base on the opposite strain of the nucleic acid.

Modified nucleic acid can no longer

replicate, and pathogens whose genomes have been modified by S-59, no longer infectious, and it is this mechanism that works for platelet concentrate because platelets are terminally differentiated cells. They do not contain nuclei, and the ex vivo storage do not require nucleic acid.

So the photochemical treatment process uses 150 micromolar of S-59 with a three Joules per centimeter square of UVA light, and the UVA illumination device developed by Baxter is capable of delivering this dose of UVA light in a brief three minutes.

Now, the system is developed to accommodate the whole unit of platelet concentrate in the blood bank and also the treatment is using the platelet storage containers. The plasma and platelet additive solution -- the use of platelet additive solution increases the pathogen inactivation efficiency.

And the other reason of using a combination plasma and platelet additive solution is that it's now compatible with buffy coat platelet concentrate. As you know, buffy coat is made by a different random donor procedure, and some European countries already are using a platelet additive solution in their preparation.

So I'm going to describe two types of experiments that we have done to demonstrate the bacterial inactivation efficiency of this system. In one group experiment, we have measured the bacterial inactivation kinetics. In another group of experiments, we have measured the whole unit bacterial inactivation with five days of storage post photochemical treatment. For the kinetic study, only single donor platelet concentrates were used, and for the whole unit inactivation we have used both the single donor platelet concentrate, as well as the buffy coat derived platelet concentrate.

The method for the kinetic inactivation briefly described here, each unit of platelet concentrate was inoculated with ten to the five to ten to the six colony forming units or CFU of bacteria per mL of platelet concentrate, and the inoculated platelet concentrates were then treated with 150 micromolar S-59 and UVA light.

And after one, two, and three Joules of UV light illumination, samples were withdrawn for analysis of viable bacteria, and the methods for bacteria detection are the standard microbiological plate assays. So that allows us to quantify the residual viable bacteria.

So the results are summarized here for six strands of Gram positive bacteria, including the most common contaminants, staph. epidermidis, and the inoculum level, as shown here. We target somewhere between five to six, and we did get 5.3 up to 6.2 log CFUs per mL, and after illumination with one, two, and three Joules of UVA light, the levels were reduced drastically, and most of the samples were below the detection limit.

And I will come back and show you the inactivation kinetic curves in a minute, and I just want to point out that the total log reduction after three Joules of illumination is greater than the input level that's each -- for each strain of bacteria we demonstrate greater than five, up to greater than 6.8 logs of inactivation.

And similarly, in this slide I have summarized the results for four strains of Gram negative bacteria, including the klebsiella, salmonella, Yersinia, and enterobacter strains, and the inoculum was as expected. We have achieved five, 4.9 to 6.3 logs of inoculum level per mL of platelet concentrate, and after illumination the levels were reduced, and the total log reduction achieved were between 5.5 and greater than 6.7 logs.

And here, this slide, the inactivation log reduction is plotted as a function of the UVA dose, and as you can see, for the six Gram positive strains of bacterial, they're very sensitive to this photochemical treatment. After one Joule of illumination between greater than four to greater than 5 logs inactivation were achieved, and with additional illumination of UVA light we further reduced levels of viable bacteria. Most of them were below the detection limit that is indicated by the arrow here.

And the inactivation dose response for the Gram negative strains appear to separate into two categories. One group of Gram negative bacteria shows a sensitivity similar to the Gram positive strains, and a couple of Gram negative strains show slight or more resistance to this treatment.

However, after three Joules of treatment, we have achieved a greater than five logs of inactivation.

Now, the second group of experiments involve the whole unit inactivation. The methods are briefly described here. For each unit of in this case it's apheresis platelet concentrate, was inoculated with ten to the three to ten to the four CFU of bacteria. This is the inoculation per unit. We

attempt to model the real level in the platelet concentrate units immediately post collection.

Then each of these units was treated with 150 micromolars of S-59 and three Joules of UVA light. After treatment, the units are stored for a total of five days, and after the five days of storage, the treated platelet concentrates were then cultured for viable bacteria.

And the results are show here for five of the Gram positive bacteria that we looked at. I forgot to mention early on that the number in the parentheses indicate the number of replicates we have done for that strain of bacteria using, for example, here is a four independent platelet concentrate units.

So the inoculum we have achieved for this set of experiments was between 3.6 logs for bacillus up to 6.8 logs at the high end for staphylococcus pyogenes.

So each unit after the treatment and the five days of storage, we cultured the platelet concentrate, and no viable bacteria were detected.

Similar results were obtained for Gram negative strains of bacteria. Four of them are shown here. Each were done in four replicates, and the inoculum level was between 4.8 to 6.7 logs. Now, keep

in mind these are per unit.

And after treatment and the five days' storage, no viable bacteria were cultured. So this shows that these bacteria are sensitive for photochemical treatment and also under the conditions we used, no bacteria escaped the photochemical treatment, since if they did after the five days of storage they would come up.

And this system now is so robust that we use it to test the systems being developed for commercialization to look for any possible nooks and crannies that might shield the bacteria from photochemical treatment.

So our results obtained from the apheresis platelet concentrate have been extended to buffy coat derived platelet concentrates, and experiments we've done here. Each buffy coat derived platelet concentrate was prepared from a pool of five random donor buffy coats, and they were made in a combination of 35 percent plasma and 65 percent platelet additive solution.

And two of these units were then pooled and inoculated with ten to the three to ten to the six CFU of bacteria into the pool, and the pool is redivided into two identical units. One unit was not treated,

and the other unit was treated with 150 micromolar S-59 and the three Joules of UVA light and then stored for up to seven days.

And during the storage at day one, day five, and day seven samples were taken from both the control and the treated units and cultured for any viable bacteria.

And the methods for culturing the bacteria in the buffy coat experiment is different. We used the BacT/Alert automated system. So the output is, instead of in colony forming units, the outcome of this assay is given in the time from the start of the culture to a positive reaction. So for the Gram positive bacteria results with five different Gram positive strains we have achieved the targeted level of inoculum, somewhere between three to ten to the six -- three to six logs CFU per unit.

And the results for the control units are shown here. The numbers indicate the number of hours from the time the four mL of platelet concentrate was inoculated into the BacT bottle, and as you can see, the untreated samples all had bacterial growth, mostly on day one, and certainly day five and day seven. Some are slow growers, that it was test negative on day one, but came up on day five, and certainly on day seven.

Now, in contrast, the paired photochemically treated units showed no bacterial growth in all of the samples, with the exception of one of the two units inoculated with the bacillus.

The results for three of the Gram negative bacteria strains are shown here, and again, we did achieve the three to six logs of inoculation, and the control untreated units showed bacterial growth on day one, day five, and day seven. In contrast, the paired treated units showed no viable bacteria, indicating complete inactivation of these bacterial strains.

So in conclusion, our data so far demonstrate that the photochemical treatment system with S-59 and UVA is effective in inactivating a wide spectrum of bacteria, with high efficacy in platelet concentrates, and this treatment system is robust and is compatible with either single donor or pooled buffy coat platelet concentrates.

And I'd offer just a last slide, a little bit of additional information. That is, this photochemical treatment system has also been shown to inactivate a wide range of viruses, and the condition used for the bacterial and viral inactivation retain in vitro platelet function, and clinical trials with health volunteers have demonstrated acceptable

viability of the platelets after five days of storage.

And this process is currently in Phase III clinical transfusion studies both in Europe and in the U.S.

So thank you very much for staying for my last presentation of the day.

(Applause.)

DR. WAGNER: Thanks, Lily.

Before you leave, I'd like to invite you and the other speakers, I guess, excluding myself because it's difficult to moderate and to be moderated at the same time in front for a panel discussion.

Yes. Please identify yourself.

PARTICIPANT: While applying the skin prep., you have shown the picture that they're holding under the arm and stretching the skin. Is that the standard practice? The preparation, applying the antecubital foca, the picture shows that holding the arm and possibly stretching the skin.

MR. McDONALD: That was just for the sake of the photograph.

PARTICIPANT: That's not the standard technical?

MR. McDONALD: No, that was just for the photograph, yeah.

DR. WAGNER: Please use the microphone.

MR. McDONALD: That was for the purpose of the photography. That was for photographic purposes. It's not used routinely.

DR. WAGNER: I have a question for Lily.

Does Psoralen -- are spore forming bacteria less resistant to Psoralen mediated inactivation?

DR. LIN: Well, that's a good question. In fact, I think, preformed spores are resistant to the photochemical treatment, and this, I think, explains why one of the experiments with bacillus failed.

DR. WAGNER: So it's a very, very broad inactivation method, but as we see always in biology, nothing is perfect.

DR. LIN: Nothing is prefect, correct.

DR. WAGNER: Yes, Mark.

DR. BRECHER: I was just speculating maybe that because the bacillus was the bug you had trouble with, it's such a large organism compared to the other bacteria, that the size of the organism may have some way or something to do with it. It may need longer UVA radiation to penetrate the bacteria.

DR. LIN: Well, we have actually in the early -- the very first slide that's showing the inactivation kinetics, we used the non-small forming

bacilli. In fact, we can kill five logs and six logs easily. So once they germinate, they're very susceptible to inactivation, but it's the spores that during illumination they might escape the inactivation, and during storage they germinate, and you find bacillus.

DR. BRECHER: I do have one question. At the ASH meeting a couple of years ago, the survival data was presented on this, and while the in vitro recovery was acceptable, the in vivo survival was acceptable. As I recall, there was about a 20 percent decrease in in vivo recovery, and a 20 percent decrease in in vivo survival.

So does that mean you would need -- let's see, 20 plus 20 is 40 -- 40 percent more platelets to get the same kick out of a bag of platelets?

DR. LIN: Well, I think the best person to answer this question really would be Dr. Larry Corash, but I will try.

I mean, I would not calculate it the way you did. I mean if we did see 20 percent, I think the recovery and survival goes together. If you have a reduced recovery, it would translate into reduced survival in the meantime.

DR. BRECHER: Okay. Well, at least you

need 20 percent more platelets.

DR. LIN: But then really our Phase III clinical trial would gather enough data to show if that would be necessary.

PARTICIPANT: We did some of those. I don't know why you add 20 and 20. There was a decrease, but the decrease was the same as you would see in the control with plasma storage, about a 42 percent survival and about a 120 hour recovery, 40 percent recovery and about 120 hour survival, which is what you would get in non-PAS (phonetic) stored platelets, if you stored it in plasma. If you stored it in PAS, you got the higher level.

So when you Psoralen treat in PAS, you got back to where you currently are getting something in plasma. So there was a quid pro quo for the viral inactivation, which is basically what you're seeing today. The results with Psoralen treatment and PAS are similar to what we're getting as we speak today.

DR. WAGNER: If you have a comment, please go to the microphone, please.

Mark.

DR. BRECHER: I'm sorry. My comment was that just may imply that we need to store all of our platelets in PAS to get better recovery and survival.

DR. WAGNER: Yes, please.

DR. KUEHNERT: Matt Kuehnert, CDC.

I just had another question about your talk. It looked like a promising method, but one thing that confused me was you had a slide that had the amount of bacteria inoculated, and then after treatment you had some negative log values.

DR. LIN: Yes.

DR. KUEHNERT: And I wondered if you could explain how they could become negative.

DR. LIN: Yeah. That's just a function of how large of a volume you assay. If, for example, you only assay one mL and you did not find anything so your titre is less than one per mL and log is zero, but then when you assay more than one mL, for example, three mLs, and you did not find any bacteria, so your titre is less than one in three and the log of one-third is a negative value.

DR. KUEHNERT: So you're assuming that you have the same number of bacteria or that your sample from one mL is the same for, say, the three mLs. You're making that assumption?

DR. LIN: No, we cultured the three mLs.

DR. WAGNER: They used more than one plate.

DR. KUEHNERT: Oh, I see. Okay. Thanks.

DR. WAGNER: Yes, please. Chris Boles.

DR. BOLES: Perhaps this has already been published, but could you speak to how your processed platelets score in assays of mutagenicity and carcinogenicity, like the Ames test or something similar to that?

DR. LIN: No. The data of our toxicology study has not been published or disclosed as of today.

DR. WAGNER: Yes, Ros.

DR. YOMTOVIAN: Roslyn Yomtovian, Cleveland.

May I ask you, Steve, a question? Two speakers today, yourself and I think Mo Blajchman, talked about -- maybe there were other speakers, too -- about the reduction in levels of growth when you divert varying amounts of samples. You know, to create a bag with that configuration, I mean, what is the barrier to that?

I mean if that really does reduce the incidence of contamination between 75 and maybe 90 percent possibly, I mean, why aren't we seeing that as one of the sort of first line approaches to this problem, keeping with the concept that what harm will it do? I mean, how much more will it cost to, you know, reengineer bag designs to do that?

DR. WAGNER: Yeah, I think that's a good point. My own sense is that we're going to be seeing a number of these systems being submitted in the near future, and I'm not sure how they'll be dealt with.

Obviously you have to be concerned that when blood passes through this diversion arm, it's not anticoagulated, and so it potentially can clot, and so I think you have to show that your device actually works the way you hope it to work.

But, you know, these are issues that companies can deal with, and I expect in the future that we may see more of this. I think it's a reasonable idea to think about.

DR. BARBARA: To extend that logically, and I fully agree with the point, you probably ought to think about how well you're cleaning your arm, and maybe there needs to be some form of a continual brief assessment or monitoring as the efficacy of arm cleansing.

I don't know around the world. I don't think people have really addressed the question. We've always cleaned arms, you know. We assume that it works, and I'm not sure that we really have the data that it does work.

And looking at today, we've been looking at

this total package of approaches to reduce the problem, starting to think about the same level of systematic effort that we quite readily employ for the viruses. You know, here we've got systems that will apply to bacteria in general. For viruses, we're quite happy to add on millions of dollars worth virus by virus.

So it's no great effort, I think, to start thinking of all of these stages systematically, and as I say, I'd start with some form of monitoring for the effectiveness and build in a requirement to demonstrate effectiveness, and then think about the diversion, as well.

DR. WAGNER: Yeah, I think this area, particularly of arm cleansing, is one that has been generally ignored for the most part, with the exception of some work that Mindy Goldman has done, for a number of years, and I think it's incredibly important, but just has not really been systematically studied.

We are just, I think, beginning to learn more and more about it, and I certainly would encourage cooperation between countries and investigators and promote studies of these type to try to see what the best arm cleansing technique is.

This idea of diversion is, again, another one that is simple and may not be particularly

expensive, and I would promote companies into investigating whether or not these sorts of devices might be considered for submission.

Any other questions?

(No response.)

DR. WAGNER: If not, I'd like to thank the speakers for their very nice talks,a nd I think we move on to the closing session, which is the tough one. It's future direction and potential impact, and the first speaker is Dr. Edward Snyder.

And Dr. Snyder is from Yale University.

DR. SNYDER: Hello. I will be mercifully brief.

First of all, I could not prepare slides in advance because I didn't know what was going to be said.

Secondly, my handwriting is, for those of you who know medicine, DNR. It's do not resuscitate. So I will try to walk you through these. I wrote these as we were listening to the various talks.

What I decided to do was to go through the list that Jay Epstein mentioned in the beginning, which is what Merlin Sayers had said five years ago about where things were. I learned many years ago not to go against Merlin Sayers ever.

So the first thing, what he said was that the concept of transfusion associated sepsis is not a new concept. Where are we in 1999 based on that? And what I heard today, it's still not a new concept, but there are now new approaches, new hopes, new enthusiasm, less inertia, and new dollars.

And I think the new dollars is the important aspect. People have listened. I remember Mo Blajchman railing at the ceiling, the sky, and anyone who would listen to him, as well as others who were less histrionic, but still had the same sense of enthusiasm like Ros Yomtovian and so forth, talking about this as if they were talking to wall, and now I think the wall is finally moving.

We have heard you. We now believe that this is a problem, and we will be moving forward. So I think we have made progress in this area.

Next slide or next acetate. My handwriting gets worse as they go along because I got tired.

Then he said in 1991 the impetus for all of this was a mini Yersinia epidemic that occurred. In 1999 the impetus is there, but it's changed. It's now the impetus to achieve a zero risk blood supply.

Immanuel Kant came up with a categorical imperative which I remember vaguely from my college

days. The categorical imperative was act so that your act should be universalized. That is, if you help an elderly man across the street, that's a good thing to do.

The categorical imperative for the new millennium is act so that you can explain it to Ted Koppel on "Night Line," and not have him think that you're wrong.

The promotion of the public health in a zero risk blood supply is the impetus for getting rid of bacteria. Somebody mentioned to me, tongue in cheek, that there are more people injured in vacuum cleaner accidents than are injured, than are killed by bacterial contamination of the blood. Why are we spending this much time on it? This was mentioned by an unnamed person outside.

The fact is that we are trying. We deal with one in 700,000, one in a million risks of HIV as intolerable, and when you have bacterial contamination, although the numbers are low, the risk is there. The population has very little trust in the blood supply internationally, and we are trying to retain that, and you see this everywhere with new variant CJD and so forth.

So the impetus today is still there, but

it's no longer a mini epidemic of Yersinia. It's to achieve zero risk, which is asymptotic. I don't think we'll ever get there. It's to promote public health, promote public trust and confidence, and it's become a worldwide effort, and that, I think, is extremely rewarding and exciting, that there can be all the efforts from the various countries involved.

And also as was pointed out very appropriately, autologous blood donation is more at risk probably for sepsis than allogeneic because often these autologous donors come in with surgery that may involve some septic process that they may not even be aware about it. Hip replacement, it's a problem and they may actually have an occult septic hip and so forth.

So these are issues that affect all blood donors, including autologous, as opposed to the viral issues.

Number three, the field was taken to task for an absence of data. In 1999, we have BaCon with AABB, FDA, CDC, and the Red Cross. We have SHOT, hemovigilance, hemosurveillance, the FDA. The AABB has the National Blood Data Resource Center; the Heidelberg Symposium. It's a worldwide effort.

Data is being collected as we speak, and

it's our job to try to coordinate all of this, not only within the United States, but nationally because we're all members of the globe, and it's becoming a worldwide effort, and I don't think we can anymore look to Dr. George Nemo and the NHLBI for funding or CDC. I think we have to look possibly to some of the manufacturers, to other sources of funding in order for us to pull all of this together, and I think a meeting like this may provide some impetus because at least it shows we're all reading from the same page in the "missalette," as they say.

Next slide. I don't say that, but other people do.

(Laughter.)

DR. SNYDER: In 1994, we were taken to task for under reporting. In 1999, it's still a problem, but less so. The gap is wide, but narrowing, and questions were raised. Do we need to achieve 100 percent reporting?

Dr. Roth, I believe, mentioned something about this, that the gap is narrowing; the question of whether you look at number of units transfused or the number of total units given out.

The question is whether we're going to achieve this by education and regulation, and if I have

to say something that may be a little surprising to myself, it has to be through regulation. You are not going to convince the hospitals and the third party payers in this world that you need to screen units of blood or do blood cultures on all these units because it's good medical care or because it's appropriate or because Ted Koppel would like you to do it.

It has to be done by the gentleness of our next speaker telling us, "I think it's a really good idea if you try to get rid of bacterial contamination of blood."

In 1994, there was the request for the need to increase the ability to recognize transfusion reaction better, which meant clinician education. In 1999, BaCon is attempting that. The AABB, ASCP, state and regional conferences, all are aimed at education. It's much stronger.

Canada has the transfusion safety officers, which appear to be more educational than regulatory from what I can find out.

The need that we still have is to increase the BaCon education efforts, get the slides and so forth out to more centers.

FDA regulation versus hoping, which is the appropriate way? And I think it's regulation. Nothing

says "I care" like a page of 483s.

(Laughter.)

DR. SNYDER: The JCAHO awareness would help. If the JCAHO and our friends from the FDA state that it's appropriate to do bacterial cultures and so forth, this frees up hospital enthusiasm. Nothing else, I tell you standing here from someone who is in the middle of a hospital that has had its third budget reduction and we now are down to a four unit platelet pool at Yale, down from 12; some of this was good medical care, excellent Red Cross efficiency. Some of it was budget reduction.

Eventually we're going to go to virtual platelet transfusions where we just show a picture of a platelet and ask them to think thrombopoietic thoughts and then bill for my time.

(Laughter.)

DR. SNYDER: Unless there's a mandate from the government -- and I know Dr. Tabor had strong feelings about the FDA being the fall person for this -- I think the federal government needs to step in and do something and say, "This is appropriate," even if it's a strong suggestion. We'll see what Dr. Vostal has to say after I leave.

And maybe increasing public awareness would

help because then they would call their Congress person and the Congress persons would then beat up on the FDA, and then it would happen that way.

So either way, I think it has to come from a mandate, either federally or from other countries, and so forth. Like leukoreduction is sort of pushing the U.S. into this.

Next slide please. I have to keep you awake. It's late in the day.

In 1995 -- it used to be 1994. I got tired -- more investigation --

(Laughter.)

MR. SNYDER: -- into techniques, referring specifically to the loss of the chemiluminescence that Mark Brecher talked about.

Well, what subjects do we have here? We have leukoreduction filtration, automated blood cultures, Gram staining, dip sticks, diverting blood bags, swirling, platelet or pathogen inactivation by S-59, solvent detergent, Inactine (phonetic), some technologies that weren't mentioned here, autoepifluorescence. All of these technologies are here. People are trying very hard to get all of this to the field.

What Jay Epstein said a while ago to me was

that he wants promising technology which is standardizable, and that's what is needed for the agency to be able to evaluate this.

And some of you say, "Well, what about S-59? What effect is that going to have on bacterial testing, dip sticks and so forth, or some of the other assays?"

And I'm reminded of that scene in "Raiders of the Lost Ark" with Indiana Jones where he's standing there trying to get past, and there's a man with a scimitar with this big, huge sword swirling around, and he says, "Aw, heck," and pulls out a gun and shoots him.

And I think may be what happens. This may be the gun, and SD or Inactine or other technologies that may just shoot the technologies that are being developed by some companies that may be eliminated. That is a possibility as the field and free enterprise does its thing.

So that's something that all of the corporate people have to consider, that new technologies coming out may totally obviate, obsolete, if you will -- I just made that word up -- their technology, and that's the way things are in the field today.

Next one. I think there's one or two more, and then I'm done.

Judicious use of blood products was the task in 1995, and it's still a task in 1999. What the physician does not know about blood transfusion risk, the plaintiff's attorney will, and that is still true today.

And I tell that to our residents and the house staff, that if you don't know what kind of work you're doing in blood products, your patient's attorney will, and that's still the challenge, and that still relates to the appropriate use of blood.

Future needs? Well, risk factor research, as we've heard about, whether it's arm prep. or coring of needles; sentinel BaCon sites I think is a superb suggestion. I had considered calling them BaCon inactivation transfusion sites or BaCon BITS, which I thought was very clever. It was easier to come up with an acronym for that than Salad Shooters. So I chose that.

That, however, I think is an excellent idea, not to negate national evaluation, but I think if you have sites like there were TMAA sites, transfusion medicine sites that the NIH had, if you could set up sites that really would look at every unit because

we're not going to culture every unit. I'm not going to get a call, four in the morning, and fly in in a costume, Ninja gear costume, to evaluate a patient who had a chill or a fever.

I think the criteria for the BaCon study are very good, but it's a nine-to-five type study when you've got two nurses who can run off and do it, and the floors are not going to do this, and I think that's why there's so much under reporting. It's a very difficult study.

Maintain international reporting I think is critical. Fast tracking, new pathogen inactivation technology. Jay said they wouldn't stand in the way, but it has to be appropriate technology that's standardizable, and the agency, I believe, would fast track. Perhaps Dr. Vostal will comment.

We need increased regulation. I'll beat that drum one more time. Increased education, increased public awareness, increased research funding, NIH and possibly through the SBIR program, which is now giving even more money for development of these types of commercial efforts.

And the last acetate is in '95 and '99, there's no one strategy that works best, and that was true then, and it's true now. When all else fails, do

something. That was said by Dr. AuBuchon. Dr. Brecher said similar things when he talked about his "farfigneuton" (phonetic) or that German thing that he was talking about versus Dr. Tabor who said, "Only do good science." He didn't say, "When all else fails do something." You can do something, but he wanted good science, and Mark obviously meant the same thing, as did Jim.

But what Mo Blajchman said essentially was when all else fails, regulate, which is what I've been saying. What Mo really said was, "Give us a mandate, and we'll do the rest." That's what we did with PCR testing and NAT testing, and I agree with him.

I have finally come 180 degrees, that I believe the way this field will move forward is not by the good efforts of the voluntary organizations, but I think the FDA needs to assume a role of leadership and to gently push us into some type of bacterial testing, keeping all of these things going while we're developing inactivation technologies.

Thank you very much.

(Applause.)

DR. SYIN: Thank you, Dr. Snyder. What a summary.

And next one, we're going to have Dr.

Vostal from Division of Hematology in Office of Blood to present closing remarks, and currently he is Medical Officer in Division of Hematology, and he is current Section Chief for the Platelet Lab, right?

Thank you.

DR. VOSTAL: Thank you very much.

Dr. Snyder is sure a difficult act to follow.

It's my pleasure to be able to present some comments from the FDA and the closing remarks for this workshop. I actually foolishly did make some slides ahead of time. So could I have the first one, please?

Well, this morning we started out with a couple of general objectives. The main objective was to get the current information on bacterial contamination of platelets. I think we've been very successful in obtaining this information and in reaching this goal.

This workshop has been very helpful to us in telling us exactly where we stand and where we've some from since the last conference in 1995.

The second objective is to encourage the future research and development efforts to minimize risk of platelet transfusion associated bacteremia and septicemia. We hope that this will take place. I

mean, we certainly hear that there are areas of interest and concern in the transfusion community, and we hope that something will certainly come out of this workshop.

Now, this is the general FDA perspective, and I put this together before having the benefit of listening to the discussion at this workshop. So some of these comments are rather general, but I did job down some more specific ones I'll go into later.

The primary goal of FDA, of course, is the safety and efficacy of blood products for transfusion. You're certainly concerned with the high rate of platelet transfusion associated morbidity and mortality, and this gets driven home to us every time we see one of the mortality reports that comes across our desk.

I think it's very sad that people are dying from contaminated platelet transfusions, and we certainly want to do something about that.

We commend the efforts expended by the CDC and collaborating medical centers to determine the extent of the problem. This is a very important effort, which we hope will give us the underlying rate that we can then work on to decrease together.

We certainly encourage research and

development of bacterial detection and decontamination methodology. There have been some exciting developments since the last workshop, and I hope that some of these will be able to reach practical use.

Of course, we're always willing to discuss novel approaches to achieve the goals of decreasing bacterial contaminations. We like to think of ourselves as a user friendly agency. So please give us a call or arrange a meeting with us, and we'd be happy to discuss any idea that you have and help in any way we can to bring it to market.

Now, some of the thoughts I had while I was listening to the discussion here I wrote down, and I'd like to share those with you.

I certainly heard that there are some strong opinions in the audience that the FDA should do something now and not wait for the ultimate test or ultimate solution to the problem. I think having this workshop here is a first step towards doing something because we certainly need to find out what the contaminating rate is and know where we're starting from.

I think it's fortunate that we're dealing with a familiar foe, and that's bacteria. This is in contrast to the issue with CJD where we're not familiar

with the pathogen. We have difficulty detecting it and inactivating it.

Here we're dealing with bacteria. We're familiar with bacteria. We know what to do about them. So I think we're way ahead of the game in that problem.

It appears to me that there actually could be too many choices in terms of addressing this problem, and the difficulty is in picking out the right choice for a solution.

I've heard some interesting ideas today. I think especially of interest to me are those things that could be done very simply, such as prevention, for example, the diversion of the first 15 cc's from collection; novel skin decontamination; or increasing the whole blood hold. I think these are simple things we could do, and since the rate is so high, I suspect that anything we do might have a beneficial effect.

Now, we heard about bacterial culture for all of the units. I think this is a viable option. I think there are issues we have to work through about when to culture and whether we're going to be able to get the benefit of platelet storage extension for bacterial culture. And we're willing to talk to the community about this and work through this problem.

Okay. So what are we going to do about this? Well, as the FDA, we certainly will go back and look at the data that was presented here at the workshop. There was so much presented that we really have to go over it again and see what we could use and what could be useful in designing any studies or solutions in the future.

We're hoping that you will be able to do the same, and we're looking forward to working together with you in dealing with this problem.

Okay. I was going to go through this, but I think we covered this very well during the workshop today. So let me just move on to thanking the workshop planning committee that did a wonderful job in arranging this workshop in a very short period of time.

Dr. Chiang Syin was the chairman. He was the tireless driving force behind getting this organized.

Dr. Mo Blajchman, Paul Aebersold, William Jarvis, Roger Dodd, George Nemo, Kay Gregory, David Stroncek, Paul McCurdy, Stephen Wagner, Kia Sen, John Finlayson, and Joe Wilczek, all of these individuals contributed greatly to being able to put this workshop together.

So that concludes my comments. I would

like to thank the speakers that participated today and the discussants and also for the people in the audience who have stayed around to listen to the end of the workshop.

I look forward to working with you in the future, and have a good trip home.

Thank you.

(Whereupon, at 4:32 p.m., the workshop was concluded.)