DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

UPDATE ON: LEUKOCYTE REDUCTION OF BLOOD AND BLOOD COMPONENTS PUBLIC WORKSHOP

Wednesday, July 20, 2005

8:00 a.m.

National Institutes of Health
Lister Hill Center Auditorium
Building 38A
8600 Rockville Pike
Rockville, Maryland 20852

C O N T E N T S

C O N T E N T S (Continued)

P R O C E E D I N G S

Welcome and Introduction

DR. EPSTEIN: Good morning, everyone. I know people are still taking their seats, but we have a very busy program, so it is important for us to try to start on time and then keep on time. If I could ask everyone, please, to take their seats, so that we can get started promptly, I would appreciate it.

My name is Jay Epstein and I am the head of the Office of Blood Research and Review at CBER, FDA, and on behalf of the Food and Drug Administration, which is one of several cosponsors, I would like to welcome you all this morning, and note that we are sharing the organizations' workshop with support from the National Heart, Lung and Blood Institute, and also from the Department of Health and Human Services, Office of the Secretary/Office of Public Health Science.

It is very gratifying that we have had over 150 registrants, suggesting that this remains an important topic in transfusion medicine, and

although Dr. Williams will outline the agenda in more detail, I just want to highlight the three major themes.

We will be reviewing studies regarding the clinical benefit of leukocyte reduction particularly in the non-targeted population and information that has emerged since the most recent public discussion about four years ago.

We will then talk about the experience with leukocyte reduction, about FDA's current considerations regarding product standards and quality control, and then some updates on the stage of development of removal of prions by leukocyte filtration.

Now, the issue of universal leukocyte reduction, that is to say, non-targeted leukocyte reduction for blood recipients, has been contentious for a number of years.

The FDA's consistent point of view has been to encourage universal use of leukocyte reduction, and the reason is because of, first of all, the known benefits in targeted groups

including preventing febrile non-hemolytic transfusion reactions, reducing risk of cytomegalovirus transmission, and reducing alloimmunization, but there, the concept is, well, you don't always identify the patient who needs leukocyte-reduced product, so is it not a better precautionary measure just to do it for all, and then there are these potential benefits which are not as well established, such as reducing other cell-associated infections, both known and unknown, the issue of potential benefit in clearance of prions, and then some even more controversial issues reducing postoperative infections, tumor recurrence, et cetera.

Now, we brought this issue to the Blood Products Advisory Committee meeting in 1998, it seems like a long time ago, where the general concept was endorsed, and we followed that a few years later with a draft guidance which encouraged leukocyte reduction as a general safety measure, but we were not able to establish a requirement.

Our legal counsel advised us that that

would require rulemaking because of the fact that non-leukocyte reduced products are already identified in the CFR. Of course, the issue of cost versus benefit became very central to many people's thinking and received a lot of attention in the blood industry.

For that reason, the issue was also brought to the Department's Advisory Committee on Blood Safety and Availability, which, in April 2001, essentially endorsed an FDA strategy to develop a rulemaking that would establish leukocyte reduction as a requirement.

However, in the interim, we have recognized that there has been an evolving debate in the scientific literature, some studies showing general benefit, some not, and consequently, we have been more in an expectant mode seeking to identify the evidence base that might underpin any ultimate policy.

At the same time, we have been moving forward with clarifying the expectations for product standard and quality control, since any

product labeled as leukocyte reduced ought to meet a well-defined and a meaningful standard.

So, this is where we are and we are hoping that this workshop will shed some light on the current scientific state of the art on these issues.

Now, I would like to, before we launch, thank the staff who have been most involved in developing this workshop. Alan Williams has been the chief organizer and will be our moderator with strong support from Sharyn Orton who is his deputy at the Division of Blood Applications in my office, and administrative support from Rhonda Dawson, Marty Edwards, and very active participation and support, as I have said, from persons at NHLBI and DHHS.

Many of you are aware of the report of the recent demise of Tibor Greenwalt, who passed away at the age of 91 this last Sunday, July 17th. I think as the beneficiaries of the Titanic legacy that he left in transfusion medicine, it would be fitting to say a few words and perhaps observe a

moment of silence.

Dr. Greenwalt was born in Hungary on January 23rd, 1914. He immigrated to the United States in 1920 at the age of 6. Earned an Undergraduate Degree and a Medical Degree from New York University and studied hematology at New England Medical Center.

His interest in blood diseases remained lifelong. He began his research career in the U.S. Army in India, and after that, he became medical director of what became the Blood Center of Wisconsin, which has been a lead research institution in this field.

Dr. Greenwalt served as the Vice President of the American Association of Blood Banks, of which he was a founding member, and probably the first person on record to conceive of the idea, and he served as the National Director of the American Red Cross Blood Program where he was credited with establishing the Rare-Donor Registry both for the Red Cross and the AABB.

In his laboratories at the Red Cross in

Washington, D.C., he directed research in hepatitis and the storage of red blood cells. He developed the first filter for white blood cells. He did landmark research on blood grouping reagents, and his lifelong work on red cells continued at Hoxworth where he continued his research really up until his terminal hospitalization, and where he was active in developing new storage solutions.

He was also the founding editor of Transfusion, which is recognized as the foremost publication in the world for new information regarding transfusion medicine. He contributed to over 200 major books and research papers in the scientific literature.

He was elected in 1984 to the Institute of Medicine of the National Academy of Sciences in recognition of his role and his contributions, and in early 2005, Dr. Greenwalt was awarded the most prestigious honor in transfusion medicine, the Karl Landsteiner Memorial Award for lifetime achievement in blood and transfusion sciences. We all could aspire to such accomplishments and probably few of

us will ever achieve that.

Let me just ask everyone to take a moment in silence in memoriam.

[A moment of silence observed.]

DR. EPSTEIN: Thank you very much.

I will now give the podium over to Dr. Williams who will explain what we are doing today.

Workshop Overview

U.S. Regulatory Considerations and International Policies Regarding Leukocyte Reduction

DR. WILLIAMS: Thanks, Jay.

What I wanted to do just firstoff is briefly summarize what the goals of the workshop are because there are just a couple themes that are going to permeate the day and are the areas where I think FDA really wants to update itself, as well as provide data to update you as the audience.

The first one, as Jay mentioned, is to review any new evidence regarding leukocyte reduction for non-targeted recipient populations. There have been a lot of discussions previously and we are just going to go ahead and assume that there

is an accepted medical value of leukocyte reduction for targeted recipient populations as Jay mentioned.

We are interested in updating the current data on leukoreduction failures and adverse events related to the leukoreduction procedure. These can include incomplete filtration, incomplete white cell removal, recipient adverse events, and blood establishment experiences overall. There will be several talks targeted specifically to that area.

Finally, an area that poses a bit of a dilemma for process control is the fact that patient populations or subpopulations that need a leukoreduced product for safety reasons need to have a product that really is leukoreduced, and the label needs to reflect the content of the component.

On the other hand, for a broader scale or universal product that is being produced, you need the capability of high throughput and efficient quality control, so how to balance those two strategies within one production laboratory is a

key issue, and we hope to have some sound discussion on that.

So, just to walk through the agenda, I am going to, following the agenda itself, just give a few of the prior regulatory considerations just in terms of stage setting for what FDA's considerations have been in the past, and then later today, we will give current considerations, which then, in turn, might be modified by today's discussions.

Our first data review talk with be by Rob Davenport from the University of Michigan addressing recent studies on the value of pre-storage leukoreduction for non-targeted recipients.

Following that, Dr. Ed Snyder, a long-term colleague, will be updating us on the Yale-New Haven Hospital Program of leukocyte reduction for all its transfusion recipients that they have several years of experience with this now, and he addresses it both from a patient welfare and I think a cost-benefit relationship.

We will also have a talk from Dr. Avery Nathens, who is going to present a currently unpublished study on the impact of pre-storage leukoreduction among transfused trauma patients.

Finally, to end this morning's session, an overview talk on adverse events associated with leukoreduction and manufacturing failures, and some data developed at the NIH Department of Transfusion Medicine on sickle cell hemoglobin and several other factors leading to filter failures.

Then, we will follow with questions for morning speakers and the open public hearing.

In the afternoon, right after lunch, Tim Malone from Florida Blood Services is going to follow up a really very excellent talk given by Herman Leparc at one of our Blood Product Advisory Committee meetings just on the practical aspects of leukoreduction in a blood center and quality control within the production and component laboratories, and also go through some of the data that their laboratory has been doing in evaluating potential use of pooled ABO matched component

samples for residual white cell counting.

We also, in the interest of presenting current experiences, contrived a survey together with America's Blood Centers primarily and also the American Red Cross, regarding what the current leukoreduction proportion of units are that are being distributed today, what the experiences are with quality control, how white cell counting is done, what rate of filtration failures are occurring, with what products, and so forth.

These data will be shared with us by Dan Waxman, representing America's Blood Centers, and Dr. Fred Walker, representing the American Red Cross.

Dr. Orton and I will then spend a short time going over FDA's current considerations regarding leukoreduction, including process validation, quality assurance and monitoring, processing, testing, and the licensure process.

That will be followed by another open public hearing, which will then go into a panel discussion with a total of seven luminaries that we

were able to recruit to give their opinions on a couple of the key major issues that are the theme for the day, namely, universal leukoreduction and quality control strategies.

Then, the last session, related but a little bit distinct from those two main themes, Dr. Luisa Gregori from the University of Maryland, working with Dr. Bob Rohwer, is going to give both an overview on some of their latest data on prior reduction by filtration.

This will be followed by Dr. Jerry Ortolano from Pall Corporation sharing data with respect to the Pall Leukotrap Affinity Filtration System.

So, it is a full day and we are going to move it right along, but there should be a lot of information.

We will start sessions on time. If you are five minutes late coming back from lunch, you are going to miss five minutes of content. Sorry, but it's a full day and we really have to do that.

We have a five-star cafeteria downstairs

here, so you ought to be able to get through the lunch process pretty quickly.

I am going to just show a few slides on some of the regulatory history with respect to leukoreduction just in terms of stage setting and a little bit about some of the international practices.

The current FDA recommendations, although there have been some draft recommendations in the interim, the current in-force recommendations go back to the 1996 FDA Memo. In terms of quality assurance monitoring, the recommendation is for evaluation of 1 percent of representative products or at least N equals 4 per month.

For residual white cells, that memo references a standard of less than 5 x 10 6 residual white cells per collection or component, and 85 percent retention of original product.

For single donor platelets, that value is proportional, so that in a pool of 6, one ends up with the same standard for residual white cells.

All evaluated products must meet

specifications, and if a failure is observed, the label needs to be revised and the process investigated, and details, methods available for counting at that time, which included the Nageotte manual method, flow cytometry, and other validated methods.

As Jay mentioned, we issued draft guidance on the topic of pre-storage leukoreduction in January of 2001, and there were a few changes in the proposed standards and quality assurance monitoring scheme at that time.

In harmony with the current European standard, we recommend that a product specification of 1 x 10 6 residual white cells, and introduced a statistically-based quality assurance scheme, and this was based on a binomial consideration, and we put into the draft guidance a candidate specification that 95 percent of products should meet the 1 million specification for residual white cells with 95 percent confidence.

This could be achieved by running cycling quality assurance every three months, which would

involve 5 for the week, 20 per month, or 60 per quarter, and if one had zero failures within those 60 counts, one would achieve that 95-95 criterion.

That guidance also recommended considering the testing of all donors for hemoglobin S, because it was known that that was one of the donor factors that contributed to clogged filters, and made a recommendation for 100 percent quality control of components that were destined to be used for CMV-susceptible recipients and particularly in lieu of CMV antibody-negative units.

There were a lot of comments received to that draft guidance and I am just going to summarize them here. Although the European standard is 1 x 10
6 residual white cells, and FDA certainly supports removing as many of the white cells as possible, there is concern that although current leukoreduction filters are easily capable of removing white cells to that level and far below, whether or not counting methods currently in use for residual white cells could be validated to count at the 1 million level.

The comments coming in included that consideration, as well as the fact that when a filter failed, it generally failed big time, and there were a lot of white cell contaminations, so perhaps the difference between 1 million and 5 million was not likely to be medically meaningful for patients who needed a leukoreduced product.

There was a lot of discussion and a BPAC public discussion on the feasibility of sickle cell hemoglobin screening and a BPAC vote that, in fact, this was probably not feasible on a universal basis for blood establishments, but certainly could be worked into a quality assurance process. If it was feasible, it could help to identify donors whose collection should not be put through the filtration process.

The recommendation for 100 percent quality control of units destined for CMV-susceptible patients was soundly criticized as simply not being practical, because a component laboratory at a blood establishment simply wouldn't know what units were going to that patient population, so it would

involve maintaining a dual inventory probably generally not a easy or practical thing to do.

So, there was considerable comment about that recommendation and whether or not that was practical despite the fact that, as I mentioned, for quality control for targeted recipients who really need a leukoreduced product, there is potential value certainly in looking at all of those units.

There was a lot of discussion about the statistically based quality control paradigm including a BPAC discussion.

The general sense of the committee and, in fact, a vote was that 100 percent quality control for all leukoreduction procedures was simply infeasible, and although the statistical-based process control appeared to have merit, there was some concern as to whether even with that cycle of 60 counts, that would, in fact, potentially put a blood establishment into an endless loop or they found one had to requalify, had to do the counts again, and would spend a lot of time doing quality

control and trying to recover from failures given the fact that the failure rates at that time were really a very broad range.

So, the sense of the committee was we are willing to consider this as a recommendation, but we do have some concerns and it needs some additional thought.

As reflected in the summary of the BPAC discussion here, the first one shows the sickle cell vote which was unanimous against that recommendation.

There was also discussion at that committee meeting about the filters themselves and whether sufficient parameters regarding their use and performance were elucidated in the product labeling and whether or not labeling needed to be revised to more carefully specify units that were eligible for filtration with the conditions of filtration such as time and temperature to the filtration process should be.

At that session, as well, there were a lot of abstentions simply because they felt there was

not a lot of data to address that issue, and a mixed vote with 2 voting yes and 4 voting no in terms of label revisions.

The committee did recommend better investigations of leukoreduction failures and their causes, so that some of the information could be refined in this area, and did mention specifically, based on the Canadian experience reported at this meeting, that mixing, particularly mechanical mixing with a validated process appeared to really reduce the clogging factor that occurred during collection.

That session did not address process control strategies, but a supplement one did. Now, a lot of thought has gone into this quality assurance monitoring factor and I think it has been educational for us, as well as the public discussions I think have really helped to sort of flesh out some of the parameters involved, and as we start thinking about statistical quality control.

Defining process failure really depends

upon appropriate and distinct control points. It is not usually helpful to lump everything together and consider it a failure and base all of your quality monitoring around that, and candidates here would be that incomplete filtration should be considered independently from white cell contamination, which should be considered independently from therapeutic content of the final product.

In 2001, when the last public discussion was held, leukoreduction processes had relatively frequent reported failures, and some of these were poorly understood. Probably because they weren't all investigated, not all of them were process failures, but until it is investigated, you often don't know that.

At the time of that Blood Products Advisory Committee discussion, a survey was done among users, and we got a range of 0.3 percent to 13 percent total observed failures of the process, a very broad range. This was in contrast to when we polled the filter manufacturers, they reported a

failure rate in their laboratories of 3 per million, so really, quite a broad range in terms of failure of the process.

It was known at that time that when a filtration was slower than anticipated, that was often correlated with poor white cell removal.

It was known that among donors with sickle cell trait, about 50 percent led to clogging the filter before the process was complete, and of the remaining 50 percent, the unit appeared to filter, but left a lot of white cells in the ensuing product, so that an individual with sickle cell trait, you generally had a successful leukoreduction about 25 percent of the time.

It was also recognized that there are other poorly understood donor factors. Dr. Stroncek is going to give some really interesting data on what they have found in terms of their overall failure rates.

It is known that donor failure rates appear to be inherent to the donor at least for the most part, that if a donor fails on one occasion,

that they are likely to fail in subsequent collections, so there is a message there.

It was also recognized that some of the failures are lot specific to the filters being used, and some of the high rates that were observed simply were due to the fact that during that time period of the survey, they had a bad lot of filters that added to their failure rates.

As mentioned, Canada implemented universal leukoreduction some time ago and presented data publicly at one of our BPAC sessions, and it showed pretty clearly that mixing particularly using validated procedures reduced clogging the filter and loss of product.

What are the implications of failure? This is important in defining a quality assurance monitoring plan. In terms of safety, if a product is labeled as leukocytes reduced, and has high levels of contaminating white cells, patients who need the product can be harmed, for instance, those who really are susceptible to CMV infection and its terrible consequences for an immunocompromised

individual.

On the other side, there is an efficacy consideration, that if there is undue loss of a therapeutic product, it reduces the potency of the product on one side, but if the filtration is actually incomplete, it results in loss of product and wasted blood resources.

So, one simply wants to put all the parameters in place to keep this an efficient and safe process.

Some considerations about process control. What I am going to do is just give a couple, a little bit of a structure to it, and Dr. Orton in the afternoon is going to give a little more detail about some of the specific mechanisms that can be used for process control.

One needs to consider whether or not it is important to do 100 percent product qualification versus a sampling scheme, and whether or not that should be a statistically based stamped sampling scheme.

That really depends on how critical is the

final product specification, particularly from a safety aspect. One needs to consider the appropriate distributions for the statistics being used, whether or not the outcome can be dichotomous or needs to be a continuous outcome.

For instance, in the use of the binomial, one doesn't necessarily need to quantitate the residual white cells that are there. One could basically make use of that statistic by using a pass/failure scheme if that would make it technically more feasible.

Whether or not the distributions need to be log normal and converted to fit into a statistical plan is also a possibility.

Use of one-tailed versus two-tailed statistics. I forget where it originated, but one of the examples given is an automobile piston versus a white cell count. If you build an automobile piston and it has to go into the cylinder, it is not going to work if it's too large, but it is also not going to work if it's too small, so you need a two-tail assessment of the

quality of that piston.

Whereas, for a while cell count, you really don't care if it's too low. What you are interested in is the one-tail as far as whether it exceeds a certain medically important value.

The frequency of the QC cycle is important - how long can an out-of-control process be tolerated if your process is out of control, you know, how important is it that you stop distributing units that are out of control before your mechanisms for quality assurance pick it up.

I just created a couple of points. Contrasting at 100 percent product qualification versus a statistically based plan. Considering 100 percent product qualification for residual white cells, and this is not unheard of. There are some component laboratories in the country that have been doing this.

Whether or not it would be feasible on a large scale for all leukoreduced products is certainly something that would get a lot of discussion, but the advantages are it would produce

100 percent label leukoreduced product which would meet the product standard.

It would reduce inappropriate white cell exposure to a susceptible patient subpopulation, such as CMV susceptible, and it would potentially stimulate new technologies that would facilitate cost effective white cell counting.

What are the disadvantages? A large part of the country still counts white cells manually, and even current processes are, in the blood establishment's view, very labor intensive.

Although it is improving a little bit, there still is a limited selection of automated counting devices which can be used, and blood centers, because of the overhead for the manual counting and the expense related to dedicated staff, blood centers may ultimately choose to provide leukoreduced products, which I think is contraindicated in terms of FDA's overall encouragement of the use of leukoreduced products for the reasons Dr. Epstein mentioned.

The use of the binomial was detailed in

the January 2001 guidance. If one defines a specification that 95 percent of product needs to meet defined specs with 95 percent confidence, this results in a product that has no 95 percent conformance and is, by definition, a safe and pure product, and 95 percent as a confidence interval is a standard accepted scientific norm.

This translates to the probability of less than 5 in 100 that chance nonconformance will exceed 5 percent. The way one reaches that, as I mentioned briefly before, is met by zero counts observed, zero observed failures out of a total of 60 counts, or one can predefine that one is going to count a few more. If you count 93, you can have 1 failure and still be within the tolerance range.

This is based on an exact binomial distribution, single tail, it doesn't require log normal distribution, and the white cell counts can be pass/fail.

The recommendations of the January guidance did give us an example that 60 consecutive white cell counts would be a suitable scheme for

quality assurance monitoring, that ongoing QC could entail 1 percent of the total production, but not less than the random 60 counts per quarter, that QC failure would require a consecutive count of the next 60 units, and that if it had zero failures, the establishment could resume normal QC, but if one experienced 1 or more failures in that consecutive, one would need to define the process as being under control and initiate an investigation and corrective action.

FDA, I think in any of its recommendations, will encourage alternate equivalent procedures which would meet the goals of its recommendations, and there are other published schemes specific to quality assurance procedures for leukoreduction, and these could be submitted to the agency if the agency has a license supplement for prior approval and would be carefully considered.

The binomial approach assures that 95 percent of products labeled as leukocytes reduced will meet the product standard with 95 percent

confidence. The quality control workload at the collection centers, while probably higher than it is today with current existing schemes, would still be considerably less than would be needed to count all products, and it would help to ensure that leukocyte reduced products would more readily available.

What are the disadvantages? Again looking at the CMV-susceptible patients, leukoreduced products are currently commonly substituted for CMV seronegative products, and because many of the transfusionists supporting these patients, in fact, don't have a complete degree of confidence in a leukoreduced product, they often call for products that are both CMV seronegative and leukoreduced just to be protective.

At the 95 percent range, occasional products with levels of residual white cells that exceed the product standard may unknowingly be transfused to susceptible patients. I think as we found in the course of the Blood Products Advisory Committee discussion, the quality control strategy

proposed may be too complex for training and ongoing implementation by blood center staff, and if that happens, that would contribute to reduced compliance, which is always a concern.

There was a BPAC discussion December 13, 2001, and the Committee voted on the question, does the Committee recommend Option 1, i.e., that FDA should recommend to industry that all products labeled as "leukocytes reduced" meet the defined standard as demonstrated by evaluating all such products for residual white cell content.

The Committee voted a unanimous No to that question. Keep in mind that the question as phrased would encompass both manual and automated procedures for producing the product.

Question 2. If No to Question 1, does the Committee concur with the modified statistical quality control strategy, the binomial, as outlined? There was general support for this with considerable discussion as I mentioned earlier as far as its practical use within an ongoing component laboratory.

I wanted to close by just discussing some of the standards and procedures in place elsewhere in the world. I think some of the best available data is part of the report issued by the Council of Europe with their annual survey. They do a survey for products produced and the characteristics of these products.

In general, in Europe, the processes for leukoreduction are a combination of buffy coat removal and subsequent filtration using fully validated procedures, and while not absolute, they do recommend pre-storage filtration within 48 hours.

Council of Europe Standards are for a residual white cell count of less than 1 million, minimum hemoglobin content of 40 grams, and a hemolysis of the units less than 0.8 percent of the original red cell mass, and this is monitored by looking at 4 units per month.

Process control. Their quality control assessment is currently 1 percent of all units collected with a minimum of 4 units per month, and

the standard is met if 90 percent of the units tested fall within indicated values.

The Council of Europe, as well, has had discussions and is moving in the general direction of statistical process control. I think you will see some evidence of this in subsequent versions of the Guide to the Preparation, Use, and Quality Assurance of Blood Components.

So, I think as well as being considered here in the United States, the same considerations are underway in Europe.

In terms of proportion of blood components that are leukodepleted, which is the term used in Europe, there are currently according to the survey 34 percent of countries that are doing 100 percent leukodepletion of red cells.

This is based on the survey of which 45 countries are eligible, 29 completed the survey, and 10 reported 100 percent red cell removal. These are Austria, Finland, France, Germany, Ireland, Luxembourg, Netherlands, Norway, Switzerland, and the UK.

I also added in here that Canada has also been doing this for some time and we are very fortunate to have Dana Devine here who heads the R&D program for Canadian Blood Services, and she is going to in the course of the panel, and hopefully, also give some of the Canadian perspective on leukoreduction.

As far as 100 percent leukoreduction or leukodepletion of platelets, a slightly higher value, 46 percent of European respondents, and the list I mentioned before, Belgium, Latvia, and Iceland also leukoreduced their platelet components.

That is the end of my introductory discussion. I want to emphasize again, and I am ending a little earlier to set the stage, we have a lot of material being presented. I know our speakers have some good material and a lot of slides. I encourage healthy discussion, but we do want to stay within the time limits, so we can get everything presented.

At this point, I will introduce Dr. Rob

Davenport, who is head of the Transfusion Medicine Unit at the University of Michigan. Rob has kindly agreed to present an overview talk on leukocyte reduction in non-targeted populations.

Recent Studies Addressing the Value of Pre-Storage Leukoreduction for Non-Targeted Recipients

DR. DAVENPORT: Thank you. I appreciate the invitation to speak here today.

I think it is important to start out with a couple of minutes of sort of what the background is, how we got to where we are right now in particular with respect to what would be the generally more agreed-upon indications for leukocyte reduction, that being reduction of CMV transmission, reduction of HLA alloimmunization in reduction of febrile transfusion reactions.

In terms of CMV transmission, the landmark study is the Bowden study, which was a randomized clinical trial in the setting of stem cell transplantation in Seattle, that randomized subjects to receive either CMV seronegative components or components that were leukocyte

reduced at bedside.

These are familiar to you, so I am not going to spend a lot of time on it. The main outcome measure was CMV infection within 100 days from transplantation. This study reported equivalent rates of CMV infection between the two arms, however, there was a higher rate of CMV disease in the group that received the bedside leukocyte reduced. This has been a very contentious issue.

With respect to alloimmunization, again, there is a landmark study, which is the TRAP study, which you are probably very familiar with. This is a randomized clinical trial in the setting of acute myelogenous leukemia that randomized subjects to receive four different platelet products, all the red cell products were leukocyte reduced, and the platelets were either unmodified random donor platelet concentrates, filtered random donor concentrates, UVB-irradiated random donor concentrates, or filtered single donors.

The outcome measures were alloimmunization

monitored by lymphocytotoxic antibody testing and platelet refractoriness. There was a significant difference between the control group receiving the non-leukocytoreduced products in terms of both development of HLA antibodies and refractoriness to platelet transfusion, however, the three study arms were really equivalent, and there was no additional benefit seen in the single donor platelets.

The greatest benefit was seen in the subjects who had not been previously exposed to HLA antigens either through pregnancy or through transfusion. For those who had been pregnant previously, there was minimal benefit.

In terms of febrile non-hemolytic reactions, there are a multiplicity of studies out there, a number of observational studies, and some prospective case-controlled studies that all used a variety of methods of leukocyte reduction, but all pretty much reached a similar conclusion of the potential benefit.

That is where we stand in terms of where the basic data come from for what would be

considered the targeted populations. While we could probably argue all day exactly what the targeted groups are, I think this pretty well summarizes the individuals who are at significant risk of some adverse outcome from CMV transmission or HLA immunization or are at higher risk of recurrent febrile reactions. So, presumably, non-targeted is everybody else.

What I want to try to do is to review clinical studies, I am not going to focus on laboratory studies, particularly in the last four years of the impact of leukocyte reduction in terms of CMV transmission, in terms of alloimmunization, in terms of febrile transfusion reactions, and in terms of clinical outcomes.

There is a variety of data sources and I will be the first to admit I did not do an absolutely exhaustive review, but I believe that what I have here to talk about today is truly representative.

Some of these are randomized clinical trials, some are cohort "before and after" studies,

and a couple of meta-analyses of both randomized trials and "before and after" studies.

If you are interested, the references are all at the end of the presentation, and the numbers in square brackets refer to those references.

So, to begin with, CMV transmission, since the Bowden study, I am not aware of a randomized clinical trial of sufficient size that would really be equivalent to the Bowden study. There was, however, a follow-up to that study from the same institution, which was a prospective cohort study that looked at two groups of individuals.

All of these were CMV seronegative donor recipient pairs who were undergoing stem cell transplantation, and the two periods that they looked at were differentiated by the kinds of compounds that they received.

During the first period, all of the red cells were leukocyte reduced pre-storage filtration, and random donor platelet concentrates and single donor concentrates were also leukocyte reduced by filtration by post-storage methods.

During the second part, the only difference was that the single-donor concentrates were leukocyte reduced by process, so were not filtered.

The main outcome measure was CMV antigenemia by day 100 from transplantation. The patients were monitored weekly for CMV antigen.

They gave data on the quality in terms of number of leukocyte reduction failures. Many studies do not provide these data. These were sampling data, but they reflect a very low rate. I think it is, though, important to recognize that some of the single-donor platelet concentrates had really quite high levels, and these would not be considered leukocyte reduced.

In addition, I think it is important to recognize that there was a very low rate of CMV seropositive donors in this. About 8 percent of the single-donor concentrates were, but just over 1 percent of donors of either random donor platelet concentrates or red cells were seen of the seropositive. So, there clearly is some

pre-selection going on in this population unless Seattle is incredibly clean combined to Ann Arbor. It must be all the coffee.

So, the incidence of CMV antigenemia was significantly higher during the second period as compared to the first. It was about 4 percent of individuals in the second period. It was about 1.7 percent cumulative in the first period, and it appeared to be somewhat earlier in that that there was a steeper initial part of the curve here.

In univariate analysis of the 24 individuals who developed CMV antigen compared to those who did not, the significantly different factors were the total number of units that the patients received, the total number of those that were from CMV-positive donors, and within that, it was red cells from CMV-positive donors and single-donor platelet concentrates from CMV-positive donors. Random donor concentrates were not significantly different.

Out of the individuals who received at least one component from a CMV seropositive donor,

14 out of 235 developed CMV infection compared to 4 out of 194 individuals who received only CMV seronegative components.

Now, to my arithmetic, those don't add up to all of the patients, so I had a little bit of trouble interpreting these, but it looks like out of those who only received CMV seropositive components, there was about a 2 percent baseline rate.

That pretty well agrees with the Bowden study and with other experiences. That seems to be the basic underlying rate of development of CMV.

Out of those who only received CMV seronegative components, there were more during the first period than during the second period. In fact, about one-quarter as many individuals during the second period received only CMV seronegative components as compared to the first period.

The outcome of these individuals, overall there was a 3 percent rate of development of CMV antigenemia. Most of those individuals received preemptive antiviral therapy. There were some who

were autologous transplants with low levels that elected not to. No individual developed CMV disease.

So, on the basis of this, these authors suggested that it may be not prudent to abandon CMV seronegative for certain populations, particularly those who would be at risk of disease and would not be monitored and given the opportunity to have viral therapy.

However, this is an association, it is not a causal relationship. It doesn't directly compare CMV seronegative components versus leukocyte reduced, and in multivariate analysis, the one factor which appeared to be important, that fell out statistically between those who got CMV infection and those who didn't was the number of CMV seropositive red cells that the individual received, not platelets.

This, I have a little trouble interpreting given that presumably the same process is being used in both of these populations, so I can't quite interpret what that means.

Then, I don't know whether these can be generalized populations where the donors have a much higher incidence of CMV seropositivity. This is clearly a pre-selected population, and with most of us dealing with donor populations, the 50 percent or more that are CMV seropositive, presumably, one would expect to see a higher rate of CMV transmission, but we really don't know.

To touch briefly on the VAT study of viral activation in HIV, this was a double-blind, randomized study which enrolled individuals who were HIV-positive and had concomitant CMV infection, the issue being would transfusion of a non-leukocyte reduced component predispose to activation of either viral infection.

So, the individuals were randomized to receive either unmodified red cells or leukocyte reduced by filtration red cells. The outcome measures reported were levels of HIV RNA, peripheral blood levels of CMV DNA, and overall survival.

There was no difference in the baseline

characteristics between the two groups or in terms of transfusion treatment. There was also no difference in overall outcome in terms of survival between the two groups.

Looking at levels of viral nucleic acid following the first transfusion, there was no difference in terms of peripheral blood HIV RNA, and when broken out to patients who were not receiving any kind of antiretroviral therapy, there similarly was no difference between the two study groups, and there was no difference in the two study groups in terms of the amount of CMV DNA in peripheral blood following the initial transfusion of red blood cells.

So, this study pretty well lays to rest the issue of whether transfused leukocytes in the setting of HIV infection might be resulting in either HIV or CMV reactivation. It does not, of course, address individuals who do not have HIV.

Turning to alloimmunization, this is a large retrospective study reported out of British Columbia that looked at individuals undergoing stem

cell transplantation for acute leukemia.

This was a "before and after" study, the first period being prior to the introduction of universal leukocyte reduction, the second being afterwards.

Importantly, between these two study periods, there was a significant change in practice in that the platelet transfusion threshold for prophylactic transfusions was reduced. The outcome measures were lymphocytotoxic antibody production and clinical platelet refractoriness.

The patients were monitored weekly for HLA antibody and clinical refractoriness was defined as the occurrence of a corrected count index of less than 5 on two subsequent transfusions.

There were some significant differences between the two groups, particularly in terms of the number of platelet transfusions that were received in the second group and the number of donor exposures that were in the second group, and this reflects primarily that they had become much more stringent in their threshold for prophylactic

transfusions.

As a surrogate marker and associated marker of refractoriness, individuals who received any HLA-matched single-donor platelets product were less in the second group, and the total number of such products that were transmitted were less in the second group.

The overall rate of alloimmunization was approximately one-half of what was seen in the pre-leukocyte reduction period.

The most significant impact was in individuals whose previous exposure had only been through transfusion. We don't know exactly what the type of product that they were transfused with. Presumably, it was non-leukocyte reduced although we really don't know.

There was also a reduction in individuals who had been neither pregnant nor transfused, but in individuals who had been previously pregnant, there was not really a significant reduction, and that is similar to what was seen previously with the TRAP study.

In terms of platelet refractoriness, there was an overall decrease to about one-half of the number of patients who became clinically refractory. Out of those, a smaller proportion were defined as alloimmune refractory, and that being the finding of both a poor corrected count index and development of positive or presence of positive tests for lymphocytotoxic antibodies within two weeks one way or the other of the transfusion event.

Again, this is most significant in those who had had prior exposure only through transfusion, not very significant in terms of those who had been previously pregnant.

A randomized study reported out of Europe from the Netherlands of alloimmunization in the setting of cardiac surgery found some slightly different data. These were randomized individuals who were undergoing cardiac surgery, receiving both intra-operative and post-operative transfusions, so they were randomized prior to the first transfusion.

They received either buffy-coat depleted red cells, pre-storage filtered red cells, or post-storage filtered red cells. The clinical outcome measures were lymphocytotoxic antibodies developing at two intervals that they looked at 3 to 10 weeks following operation and 20 to 30 weeks.

There was little difference between the three groups in terms of their transfusion requirements and prior exposure. About 40 percent of individuals had had prior exposure, and that is not surprising in a cardiac surgery population which tends to be older.

All individuals in all arms received at least 2 transfusions. The mean was 4 with a range being 2 to 6, the range here shown in parentheses. Less than 10 percent of individuals received platelet transfusions. They don't specifically state whether or not those platelet transfusions were leukocyte reduced.

They divided the outcome in terms of several groups. The first is those who were negative on lymphocytotoxic antibody testing before

and then were negative afterwards, and that constituted around 65 to 70 percent of the groups, and there were overall no differences between the control and the two study arms.

Those who were negative at the time of randomization and then became positive constituted about 10 percent, again no difference in terms of the groups. Those who were positive initially and then became stronger were about 6 to 7 percent.

Those who were positive initially and then became negative were about 8 to 9 percent, and those who were positive initially and remained positive at approximately the same strength were again about 6 to 7 percent.

So, they did not observe any statistically significant differences among these groups. So, this suggests that at least in the setting of a relatively small- and short-term exposure in cardiac surgery, that there may not be a very large effect in terms of development of alloimmunization.

Turning to the data on febrile reactions, these are somewhat difficult to interpret because

of both the way that the data are reported and how do you know that a patient has a febrile reaction. It is a diagnosis of exclusion, and even when very carefully performed, there are certainly more than a few instances where it is difficult to tell.

As one who regularly signs out these reactions, I have to admit there are times when I am virtually flipping a coin trying to decide if this individual, who has been previously febrile, but isn't febrile today, has neutropenia, is on antibiotics, and cultures are negative, what kind of reaction he is having.

In addition, the way the data are reported is convenient for blood bankers, but not necessarily convenient for really data analysis. They are in terms of numbers of reactions reported per total number of units transfused. Few studies actually report the number of patients transfused.

Few studies report the number of patients transfused who didn't have reactions, and all of these rely on some kind of a passive reporting system. For all of us in blood bank, we know that

these reactions are under-reported. So, those make it somewhat difficult to interpret.

I extracted data from 6 retrospective cohort studies and 1 randomized controlled study where I could get these reactions in terms of total units transfused. I calculated confidence intervals based on those published data, and these are what I found.

Out of these studies, all of them had lower rates following the implementation of leukocyte reduction, however, two of them what would here be labeled as 3 and 6 did not find statistically significant differences, the other ones did.

So, this is certainly supportive of decreased rate of febrile reactions, but it is a little hard to interpret given the difficulties with making a diagnosis and the reporting data.

The TRAP study in the initial publication has one line that says that there was no difference in terms of reaction rates between the groups, however, more recently that has been re-analyzed

and from the reported reactions in the more recent analysis, I extracted those that were either febrile or showed rigor reactions, and pooled those together.

Just comparing the group that received the pooled random donor unmodified concentrates versus the filtered random donor concentrates as the most comparable groups, the reaction rate was significantly reduced in those who had received the filtered concentrate, about 1.6 percent compared to 2.5 percent in the control group. In parentheses there is given the confidence interval.

The reactions were most associated with components that were greater than 5 x 10 6 total white cells and storage period that was longer than two days. These are not surprising data. So, it does appear that leukocyte reduction decreases the rate of febrile reactions, but it certainly doesn't completely eliminate them.

In terms of outcome studies, a randomized, controlled trial in the general hospital population, which is the Dzik trial, has received a

great deal of attention.

This specifically excluded individuals who had an indication for leukocyte reduction, so it was looking at presumably the non-targeted populations. The patients were randomized to receive unmodified red cells and pooled random donor platelet concentrates versus pre-storage reduced red cells and process-reduced single-donor platelet concentrates.

The primary outcome measures were in hospital mortality and post-transfusion length of stay. They also did some cost analysis, but I am not going to be discussing that today.

The patient characteristics between the two were relatively well balanced. About 60 percent were surgical and 40 percent non-surgical. Within the surgical group, the largest were cardiothoracic and orthopedic. So, this reflects a large tertiary care population.

The primary outcome measures in terms of both mortality and length of stay, they demonstrated no difference.

In terms of subgroup analysis, looking specifically at patients who underwent cardiac surgery, patients who underwent colorectal surgery, as two populations where there is suggestion that leukocyte reduction might reduce mortality or length of stay, they found again no statistically significant difference. That also was true for other surgical cases, as well as non-surgical cases.

A number of concerns have been raised about this study, one being the age of the red cells that were used in the two arms, as that they were older in the leukocyte reduced group, however, these are still comparatively relatively fresh.

Certainly compared to what we commonly have on the shelf at the University of Michigan, these would be considered pretty much equivalent.

The source of the platelets in the two groups was different. There are pools of 6 random donor platelet concentrates in the control group, whereas, it was process reduced single donor concentrates in the leukocyte reduced group.

Thus, there were more donor exposures in the control group, and potentially, there could have been a higher content of white cells in single donor platelet concentrates that would not have met leukocyte reduction criteria.

There was an exclusion of individuals who had leukocyte reduced indications, and there was a fair number of protocol violations occurring in both groups. When the data were analyzed, rather than on the intention-to-treat basis, but in terms of the actual transfusion groups, again, they found no difference between these two populations.

Much of the data come out of European studies, there have been relatively few within the United States. This is a randomized, controlled study out of Europe and the Netherlands, which looked at patients undergoing aortic aneurysm surgery, both emergent and elective, and patients undergoing gastrointestinal surgery.

They were randomized to receive either buffy coat depleted red blood cells or filtered leukocyte reduced red blood cells. This was a

double-blinded study. The principal outcome measures were mortality and ICU stay, and the secondary outcome measures being incidence of multi-organ failure, infection, and length of hospital stay.

Whenever evaluating studies that look at infection as an outcome, there is always a problem with how those are defined. These investigators used CDC criteria for post-operative infection, which include nonculture-positive infection, such as pneumonia can be diagnosed on the basis of chest x-ray findings and clinical findings in the absence of a positive culture.

So, there always is a question of whether or not a bias could be introduced when you are looking at outcomes that are not strictly based on objective criteria, such as a positive culture.

On the other hand, undoubtedly, cultures are not always positive on individuals who are truly infected for a variety of reasons including that they often receive antibiotics prior to the time that those cultures are taken.

So, this study randomized individuals at entry, so that about half of the randomized subjects actually got transfused. This means that on the intention-to-treat analysis, you are looking at a population where about half of them do not undergo the intervention, and when you do the analysis according to transfusion, you are eliminating about half of the individuals in both populations who were, in fact, randomized.

So, looking at the data in terms of intention to treat, there was a significant difference in terms of length of stay in hospital, which favored leukocyte reduction, mortality within the group of individuals undergoing gastrointestinal surgery also was statistically better, favoring the group receiving leukocyte reduction in the other two groups, and overall, that did not reach statistical significance.

In terms of multi-organ failure, while the total population was significantly better, favoring leukocyte reduction, the subpopulations did not achieve statistical significance.

Then this was analyzed in terms of the subgroups who actually received transfusion, it was about, as I said, 50 percent of the individuals, and the overall mortality rate was not significantly different, and in particular, that group who had undergone gastrointestinal surgery where, in the intention-to-treat analysis, there was a significant difference, this no longer was, which suggests that individuals who are not transfused did better.

There was a difference that remained in terms of lower length of stay. Overall, that favored leukocyte reduction.

Another randomized, controlled trial coming out of the UK looked at cardiac surgery patients who underwent coronary artery bypass grafting, aortic valve replacement, mitral valve replacement, or a combination of those.

They were randomized into three groups, one that received plasma-depleted red blood cells, one that received buffy coat depleted red blood cells, and one that received filtered leukocyte

reduced red blood cells. The main outcome measures were hospital-acquired infections and length of stay and development of fever.

This was not a blinded study, but the treating clinicians were unaware, so it's a semi-blinded study, similar to the Dzik trial, where no specific effort was made to either blind or inform the treating physicians, so it was in a sense a semi-blinded study.

Again, the criteria for infection were similar in terms of they are about the same as the CDC criteria, again, for individuals in particular with pneumonia, there is a possibility for a clinical diagnosis.

The number of percent of patients who actually were transfused within these two groups is presumably 100 percent although it is not specifically reported as the randomization occurred at the time of the order for the first transfusion.

In terms of overall infection rates between the filtered group, the buffy coat depleted group, and the plasma reduced group, there was no

significant difference, however, when they analyzed in terms of patients who actually were transfused according to protocol, they did find a difference in that the filtered and buffy coat depleted groups appeared to have a lower rate compared to the plasma reduced group although the infection rate in the plasma reduced group is relatively high, being about 20 percent.

In terms of length of stay, they found no difference, being a median of 6 to 7 days, the range of 3 to 5 days.

In terms of patients who had fever, there was a difference in that there was a lower rate of fever among those who received either the filtered or buffy coat depleted groups compared to the plasma reduced group.

The number of patient days with fever compared to the number of potential days at risk were similar in the two groups. Fever is, of course, a surrogate marker of infection, but can mean other things including febrile non-hemolytic reactions, so it is a little bit difficult to

interpret this.

Another randomized trial coming out of Europe focused on again cardiac surgery in the setting of aortic or mitral valve replacement with or without concomitant coronary artery bypass grafting.

This was a double-blinded study. Patients were randomized at the time of the first transfusion. About 90 percent of patients in both arms actually received transfusions, about 10 percent did not. Randomized again to a control arm, which was buffy coat depleted red cells versus those who, in the intervention arm, received filtered leukocyte reduced red blood cells.

The main outcome measures were mortality at 90 days. Secondary outcome measures were in-hospital mortality, length of stay, and infections. Again, infections defined basically according to the CDC criteria.

So, analyzed on the basis of intention to treat, so this includes individuals who were not transfused, there was no difference in terms of

90-day mortality, however, there was a difference in in-hospital mortality favoring the leukocyte reduced group. There was also a lower rate of documented infections in the leukocyte reduced group. There was no difference in terms of multi-organ dysfunction scores.

Analyzing the patients who received transfusions, so this is excluding about 10 percent of the randomized individuals, again, there was no difference in the 90-day mortality, in-hospital mortality remained statistically significant, and a higher difference was seen in those individuals who received more units. So, those who received four or more units had an odds ratio that was favoring leukocyte reduction.

There was also a difference seen in terms of total infections between the two groups, favoring leukocyte reduction.

In a retrospective cohort study coming out of France that looked at individuals who underwent abdominal aortic surgery in two-year time frames, one prior to the implementation of universal

leukocyte reduction and one after universal leukocyte reduction. The prior group received either unmodified or buffy coat depleted red cells, and the latter group received filtered leukocyte reduced red blood cells.

Again, randomization occurred at the time of the first transfusion, so the transfusion rate in this study was 100 percent. This study also reported individuals who received autologous transfusions, however, I am not including that in this particular presentation, so I don't believe that it is particularly germane.

The main outcome measures were 30-day mortality and again infections, similar criteria for diagnosis of infection as with the other studies, so that there is some clinical criteria.

In terms of the two groups, in the leukocyte reduced group, there was a higher incidence of hypertension and the use of diuretics, so these might favor or predispose to worse outcome.

On the other hand, there were a good deal

less patients who had a diagnosis of coronary artery disease, had prior intervention for coronary artery disease, either angioplasty or bypass grafting, and a lower rate of patients with respiratory insufficiency, so those would favor better outcomes presumably within the second group, the leukocyte reduced group.

So, there were some significant differences in baseline characteristics between the two groups.

In terms of the major outcomes, there was not a statistically significant difference in short-term mortality between the two groups and in terms of documented infections between the two groups although there did appear to be a trend toward lower rates within the leukocyte reduced group.

Given the differences between these two populations, it is a little hard to make a causal relationship there.

A large retrospective cohort study of pre- and post-leukocyte reduction was reported out of

Canada. This involved individuals who had received red cell transfusions for cardiac surgery, for hip arthroplasty, or for admission to a surgical ICU including patients who had trauma.

It evaluated two years, one year that was prior to the implementation of leukocyte reduction, one year post implementation, with a period of time in between allowing for conversion, so the two groups were really quite distinct.

They received either unmodified red blood cells during the first period or filtered leukocyte reduced red cells during the second period. The number of patients who received platelet transfusions is not explicitly stated. About 10 percent of individuals in both groups appeared to have had previous transfusions, so it is a little bit difficult to tell from the way that the data are presented.

It was analyzed in terms of in-hospital mortality and nosocomial infections, and the criteria for diagnosis of infection were really quite tight and were well described within the

paper.

There were a few significant differences between the study populations in that there was a lower incidence of severe lung disease although that was a relatively small group.

There was higher use of beta blockers, aspirin, and ACE inhibitors in the second group, the post-URL group, which I believe is important because the breakdown of the patients was about 65 percent of them were cardiac surgery, 25 percent were general surgical ICU patients, 10 percent were hip replacement, and also there was a slightly lower rate of transfusion within the post-URL group.

Looking in terms of mortality in the unadjusted group, achieved statistically significance. In the adjusted group, as reported here was not different, however, I have a little bit of trouble understanding this given that as stated in the paper, when adjusted for the use of cardiac medications including aspirin, beta blockers, angiotensin- converting enzyme

inhibitors, mortality shifted from significant to non-significant association.

So, this figure does not quite seem to correspond with the text of the paper. There was not a significant association with infection, but there was a lower rate of fever and antibiotic use both in the adjusted and non-adjusted groups.

Since fever is, of course, only an indirect marker of infection and antibiotic use tends to be driven by fever, these are somewhat indirect.

A couple of meta-analyses of randomized clinical trials comparing allogeneic leukocyte reduced and allogeneic non-leukocyte reduced transfusions have been reported. They have reported somewhat different results.

This was a Canadian group that included in their analysis 10 surgical studies that were divided among cardiac surgery, colorectal surgery, GI surgery, and a couple that included mixed surgical populations.

There were large differences between these

in terms of the number of patients who were transfused ranging from 2 percent not transfused to 73 percent transfused. Only one of these studies were the physicians blinded to the intervention, and a variety of leukocyte reduction methods were used.

The authors of this study did not address the issues of homogeneity of studies. They did not do statistical analysis for that point of view, so it is unknown from this analysis whether or not non-homogeneity issues may have been introduced.

They analyzed the outcome both in terms of intention to treat, that is, all patients randomized and a subgroup analysis of those who were only transfused.

So, overall, in the intention-to-treat analysis, there was no statistically significant difference in mortality, however, within the group that was cardiac surgery, that did achieve statistical significance favoring leukocyte reduction.

Within the subgroup analysis, looking at

only patients who received transfusion, there was overall reduction in--I am sorry, I am looking at infection rates, I am getting ahead of myself--these are infection rates, overall, was not different, was lower in the subgroup analysis of individuals who were only transfused. Again, this held true for those who underwent gastrointestinal surgery.

In terms of mortality rate, overall, did not achieve statistical significance, but for those who underwent cardiac surgery, it did lower rate favoring leukocyte reduction, and when this was broken down in terms of only patients transfused, while there still was not a statistically significant difference overall, it did appear that individuals who underwent cardiac surgery had a lower rate of mortality.

There is some controversy about how one should analyze such groups, whether you should do intention to treat or subgroup analysis, and how to interpret these. When you do intention to treat, you are directly comparing the randomized

population, so it is the cleanest analysis, whereas, that is not necessarily true when you are doing the subgroup analysis.

When you do see positive effects in the trial and intention to treat, you can be quite confident that that is a real effect and that is why it is, for instance, in drug studies, is the most desirable way to do an analysis, because the confounders, such as individuals who are not treated, not transfused, would tend to dilute the power of the study, but if you see a positive effect, you can still believe in it.

On the other hand, if you see a negative effect, the dilutional effect of having untreated individuals in both arms then becomes a problem. Subgroups may or may not be representative of the randomized population.

If it is a blinded study, then, you can be relatively confident that the subgroups were not influenced by the treatment choice or treatment allocation. On the other hand, unblinded studies may be particular susceptible to the introduction

of such bias.

So, there are reasons to stick with intention-to-treat analysis, there are reasons not to.

A second meta-analysis of randomized clinical trials that compared, on the intervention arm, allogeneic leukocyte reduced transfusions, but also included autologous red cell transfusions as in the intervention arm, compared to non-leukocyte reduced either whole blood or red cell transfusions in the control arm came up with slightly different types of studies and slightly different conclusions.

This also included studies that were addressing cardiac surgery, colorectal surgery, and others including the Dzik study of mixed general hospital populations. Four of these studies were, in fact, studies of autologous blood rather than leukocyte reduced blood, so it would need to be looked at differently. The author of this study only looked at intention-to-treat analysis.

Overall, combining these, there was not a

statistically significant difference although a trend toward favoring leukocyte reduction. In particular, breaking these out in terms of cardiac surgery, though, there was a difference in short-term mortality that favored leukocyte reduction.

Ignoring the studies which looked at autologous transfusion and picking out only those that used pre-storage leukocyte reduction in the intervention arm, the summary odds ratio just barely failed to achieve statistical significance although there was a trend that favored leukocyte reduction.

The same author did a subsequent meta-analysis of "before and after" studies of universal leukocyte reduction, so these were cohort studies that reported either post-operative infection rate or mortality prior to or after the implementation of universal leukocyte reduction. A couple of these studies are ones that we have just gone over.

These included two that were cardiac

surgery, one aortic surgery, one orthopedic and cardiac surgery, which were broken down separately. Also included was a mixed surgical population and one that addressed the neonatal population.

Again, in terms of looking at all of these and in terms of summary odds ratio just failed to achieve statistical significance although a trend appeared to favor leukocyte reduction.

Of those that reported adjusted post-operative infection rates, presumably adjusting for other variables, again, it did not achieve statistical significance in terms of the overall odds ratio although a trend that appeared to favor leukocyte reduction.

In terms of unadjusted mortality, similarly, it is notable that one study, which was the Williamson study out of the UK, which separately reported orthopedic surgery and cardiac surgery, the cardiac group did report a higher rate of unadjusted mortality in those who received leukocyte reduced than those who received the control group, and to my knowledge, this is the

only such study which has suggested possibly adverse effect of leukocyte reduction.

The authors attributed this to mortality at one particular center, so there may be a center effect. This study has reported meeting proceedings, but to my knowledge, has not been published yet in a peer-reviewed journal. So, those things make it a little bit more difficult to interpret.

Of the studies that reported adjusted mortality rates, presumably adjusting for other factors, again, summary odds ratio did not achieve statistical significance, but did appear to favor leukocyte reduction.

In terms of the use of leukocyte reduction in the neonatal population, there is very little data. There is one systematic review of clinical trials of leukocyte reduction in the neonatal setting. The authors, however, were unable to do a formal meta-analysis due to basically lack of data.

They were able to identify two randomized controlled trials, one "before and after" study,

and one non-randomized controlled trial. Two of these studies looked at CMV infection and two of them looked at alloimmunization.

When these data were pooled, neither CMV infection nor alloimmunization achieved statistical significance although these was a general trend that appeared to favor leukocyte reduction. These are, as you can see, very small numbers of patients, so it is a little bit hard to interpret these. Basically, I don't think that we have enough data to really make a conclusion.

So, based on all this, what do I take away from what I believe is the state of the current data? CMV transmission by leukocyte reduced components is low. Whether or not it is exactly equivalent to seronegative still I believe is not entirely answered.

I believe the best data available remain the Bowden study as being the largest study that has directly compared those two populations. I don't believe that since then we have data that significantly contradicts that.

I think it is clear that in the setting of HIV infection, leukocyte reduction doesn't alter the course or result in reactivation of viral infection.

In terms of HLA alloimmunization and platelet refractoriness, they are both reduced by leukocyte reduction, they are not eliminated, and the power of leukocyte reduction appears to be greatest in the population that is heavily transfused and has not been previously exposed particularly through pregnancy, which is not surprising to us.

So, whether the effect occurs or how significant it is if it does occur in the very brief transfusion episodes, such as isolated surgical transfusions remains to be shown.

Leukocyte reduction does reduce, but it doesn't eliminate febrile transfusion reactions. I find it rather difficult to interpret the reports due to the subjective nature of the diagnosis due to the reliance on passive reporting, so it is hard to say what exactly that rate is.

I have noticed, though, that what we are not seeing is the bed-shaking or rigor reactions to platelet transfusions that used to be pretty common in the Hem/Onc population. I can't attribute that directly to leukocyte reduction because I don't have the data, but it certainly appears to be pretty striking to me.

Short-term mortality in cardiac surgery does appear to be reduced by leukocyte reduction. The effect is relatively modest, but does appear to be real. Whether or not long-term mortality is reduced is still controversial and remains to be addressed. Obviously, in cardiac surgery, there are a lot of other issues that are affecting long-term mortality.

I don't think that it has been shown that leukocyte reduction has a significant effect on post-operative infections, however, interpreting these studies is somewhat difficult due to varying definitions of infection, which is very hard to do within the clinical setting.

The beneficial effect of leukocyte

reduction in the general hospital population has not yet been demonstrated, however, I think it is notable that no one has, with the possible exception of the Williamson study, shown an adverse effect of leukocyte reduction in the non-targeted populations.

So, it does appear to be quite a safe procedure, so whether or not it has additional benefit in non-targeted populations outside of cardiac surgery, I don't believe has yet been demonstrated.

The references are there for those who are interested and I think I have stayed on time. Thank you very much for your attention.

[Applause.]

DR. WILLIAMS: We have a little time, so if anyone has questions on this excellent summary.

DR. EPSTEIN: Thank you for this very comprehensive overview. I guess it continues to trouble me that study after study, especially meta-analysis after meta-analysis shows a trend to favor leukocyte reduction for a number of

parameters including infection and mortality.

It has always raised in my mind the question of whether any single study is large enough to show statistical significance for a modest but real effect.

I am just wondering, you know, I think you drew the correct conclusion where there is lack of statistical significance, but does it mean anything to you that you have seen so many different approaches show essentially the same trend with essentially the same magnitude.

DR. DAVENPORT: Yes. My personal take on it is I do believe that there is a modest effect, but that it is going to require such an enormous study as to be virtually impossible to perform to absolutely prove it, and so we are left with the practical question of given that it appears to be a pretty safe procedure and the trend certainly looks like it is favorable, should we simply go ahead and implement it, and I guess that is exactly the problem that you are facing, and I defer that to the FDA.

AUDIENCE PARTICIPANT: Rob, do we know what the risk is for the acquisition of CMV infection in a hospitalized immunocompromised non-transfused patient?

DR. DAVENPORT: No, we don't, and that is an excellent question. From both the Nichols retrospective study and the Bowden prospective study, the rate of CMV infection in those who received CMV seronegative components, were CMV-negative to begin with, received transplants that were CMV-negative, so they are about as clean as we know how to get, is about 2 percent.

So, that appears to be what our floor is, about 2 percent of individuals who, as far as we know, are not getting clear exposures through things that we are doing, are developing CMV infection.

AUDIENCE PARTICIPANT: On your slides, I think it is the slide that says, "Concerns raised about the study," I don't know whether it is referring to the TRAP study or not.

DR. DAVENPORT: I am sorry, I am referring

to the Dzik study of the randomized controlled study in the general hospital population.

AUDIENCE PARTICIPANT: So, the age of the red cells there, you said that there was concern about the difference between the control and the leukocyte reduced, 11.5 days and 18 days. When were the red cells leukoreduced, were they leukoreduced upfront or were they leukoreduced at day 18?

DR. DAVENPORT: They were pre-storage leukocyte reduced, so presumably within 24 hours. So, if there is an effect, then, it is due to the storage of a leukocyte reduced component.

DR. KLEIN: Thank you for a very nice overview and your extensive review. Did you find any data on other cell-associated viruses, such as Epstein-Barr virus, HHV-8, HHV-6? Also, while we all know that you don't eliminate graft-versus-host disease, did you see any evidence of any effect on graft-versus-host disease in any of your reading by reducing the number of leukocytes transfused?

DR. DAVENPORT: I didn't see any clinical studies addressing this. There is a number of laboratory studies that indicate that other cell-associated viruses, particularly HTLV-I, EBV, are reduced by leukocyte filtration. I didn't see any clinical studies, but I am open to correction if you know of such.

In terms of GVHD, we certainly have case reports of GVHD occurring in individuals who have received filtered products. We don't have, to my knowledge, data from either before or after studies or any other randomized controlled studies on the incidence of GVHD or the severity of GVHD, whether or not it is impacted by leukocyte reduction.

I think that would be a terribly difficult study to do given how complex GVHD is, although I think it is a very worthwhile one to try to address.

If anyone wishes to correct me? Okay.

AUDIENCE PARTICIPANT: Rob, the European studies generally use as their control arm, buffy coat depleted cells, which we know have somewhat

fewer white cells than what we use as non-leukocyte reduced in this country.

Do you think that the degree of leukocyte reduction in buffy coat depleted cells sort of prejudices those studies against a significant outcome?

DR. DAVENPORT: As far as any data I can speak to, I can only say that it appears that buffy coat depletion reduces the rate of febrile reactions, but I am not aware of any data that specifically address either outcomes, CMV infection, or alloimmunization.

I believe that the number of residual white cells in a buffy coat depleted unit is too high to really make any difference, but yes, it is a potential confounder as we use a different method of routinely preparing our red cells than the Europeans do.

DR. BIANCO: Besides the CMV studies, that is, where you were looking at an infection, before any of the other things that we expect from leukoreduction, all the studies that you have shown

and that I have read, are population studies, that is, comparing one population to another population.

Do you know of any longitudinal study of patients that will show that after a certain period of time of being exposed to a non-leukoreduced product, they will develop more fever, more alloimmunization, but on individual patients as we can do with an infectious disease?

DR. DAVENPORT: No, I am not aware of any such study. The CMV studies have looked at out to 100 days, and they have only looked in the specific transplant populations. I am not aware of any study that has any systematically followed out individuals.

MR. ENGEL: Rob, by the way, I think this was an outstanding review of a lot of the studies out there. The one study that raised a flag for me was Baron's study where you have the differences in the study groups between the hypertension, the diuretics. It appears that there is a significant difference between the control and the leukoreduction groups there.

Is there any explanation for that?

DR. DAVENPORT: I agree that that is a major confounder in that particular study. The authors did not address it. In doing any of these "before and after" studies, obviously, medicine marches on, and fortunately, outcomes are getting much better.

AUDIENCE PARTICIPANT: Seeing that overall trends it seems favoring leukoreduction, wouldn't you say that the standard of care would favor giving a leukoreduced product versus not?

DR. DAVENPORT: That is for the audience in this room to decide. I would suggest that there are some good reasons to move in that direction, and there doesn't appear to be a really compelling clinical argument against it.

AUDIENCE PARTICIPANT: I want to re-emphasize something that you alluded to, and that is the studies that use CDC or NNIS definitions of infections invariably come up with infection rates that any hospital epidemiologist would close the unit for.

DR. DAVENPORT: Yes, they appear to be alarmingly high.

AUDIENCE PARTICIPANT: They are awful and they are not I am pretty sure from a clinical standpoint to be taken at face value. So, I think it is critical as you look at these studies to look at the hard endpoints, length of stay, and those sorts of issues.

I would close any unit that had some of those infection rates at the hospital I am responsible for, and I think it is critically important not to rely upon the infection data to make a decision, which is different than saying which side of the fence or which top of the fence I am sitting on at this point.

DR. DAVENPORT: Good point.

CDR GILSTAD: Colleen Gilstad from the United States Navy. I have a question. If there is a loss of 15 percent of the potency of a red cell unit or a platelet unit via leukocyte reduction, why in the studies did they not show an increased number of unit usage in the two arms?

DR. DAVENPORT: Presumably, because the way that we prescribe red cells is by units rather than by the actual content. Now, one of my other soapboxes is that we really ought to be labeling our units for what is in them and prescribing them on the basis of what is in them.

I think it is a matter of the way our practice has evolved to simply give two units rather than to actually target it for a therapeutic dose. I think it is a failure on our part to practice transfusion medicine as scientifically as we could.

Thank you very much.

[Applause.]

DR. ORTON: Thank you again, Dr. Davenport.

Before I introduce our next speaker, I would ask that everybody please turn off your cell phones for those of you who haven't or put it on vibrate. Thank you.

Our next speaker is Dr. Ed Snyder, who is Professor of Laboratory Medicine at Yale University

School of Medicine. He is going to describe the Yale-New Haven Hospital Universal Leukocyte Reduction Program for us.

The Yale-New Haven Hospital Universal Leukocyte Reduction Program

DR. SNYDER: Thank you very much. It is a privilege to be here. I would like to just comment that we all have had our experiences with Tibi, and one of the poignant moments that I had with him that I often remember is when he told me--I asked him how he was doing, and he said fine. He said, "You know, Ed, there is three phases of a man's life. There is childhood, adulthood, and you are looking good."

Three people today told me I was looking good, so thank you, Tibi, very much, and I am a firm believer that when you speak of someone and you talk about them, that they are alive at least in your heart. So, I think Tibi will live in our hearts for many, many, many years to come.

I am here to talk about the leukoreduction process at Yale, what we have been doing, and I am

going to rely heavily on the data that I have generated.

First, my conflict of interest statement. I have to say this. I was told not to spend more than 10 minutes on it, so I will try not to, but I am a member of the Pall Board of Directors. I have been that for about four or five years.

I do clinical trials for companies that are involved with leukocyte reduction, and those are the ones in white, plus we do studies for everybody because that is what I do for a living. However, I do have no corporate equity, I do not get paid by any of these companies.

Any monies that come in for the fee for services go to Yale University, which pays my salary, and I have no--other than the Pall Board, being on that, and again I have no stock in any of the companies as shown in the proxy statement. So, let me just say that upfront. Hopefully, the data that I show you will justify my view of leukoreduction.

At Yale-New Haven Hospital, just to give

you a brief review, the total patients transfused are about 7,400 patients a year, so we transfuse about 620 or so patients a month, and that has been reasonably stable for a while.

The total products transfused have dropped. There was a peak somewhere in the mid-nineties, which was our Liver Transplant Program, which I am pleased to announce has started again as of a week ago. So, we will hopefully see how that goes, but our total product usage has dropped, so we are about 48,000 components a year.

It is about 6.7 products per patient transfused over the years. Our red cell usage is about 20,000 units per year. Our sole source supplier is the Connecticut Red Cross. Our random donor platelet usage has dropped dramatically.

We use pooled random donor platelets. We had used originally about 12 when I first came to Yale a while ago and then we dropped it for a variety of medical and budgetary reasons, and are now using a 4-unit pool. We are using about 15,000 units a year. Again, they are 4-unit pools. So,

we use about 5,000 pools a year roughly.

Our single donor product use has spiked in 2004, because we realized that we get less loss of money by using single donor platelets in the outpatient because of the CMS reimbursement, so you lose less, that is income. So, we use about 1,000 units a year in the outpatient area only, and the inpatient, which is still under a DRG, gets the random donors.

With that as a little bit of a background, the accepted benefits of leukoreduction, there are only three that I consider. I don't think we are ever going to see decreased length of stay. I don't think we are going to cure cancer, and I don't think we are going to end poverty by using leukoreduction filters.

All we need to do from my perspective is decrease febrile reactions. I think that, in and of itself, is lovely. I spend more money giving factor VIIa into the sewer system at Yale-New Haven Hospital for patients who don't need it than I ever will spend on leukoreduced blood filters, and I

will have a little bit of data to show you that.

I think decreased CMV transmission occurs. We have not done a CMV test at Yale since the year 2000--knock on pressboard, no problems--and HLA alloimmunization, I also agree with the TRAP study, and I think Rob Davenport did an absolutely superb job of going through a huge amount of data in a very coherent way, and I thank you very much for that effort.

Decreased alloimmunization is very difficult to test at Yale. We give very few HLA matched platelets anymore. For those patients who need them, we use cross-matched compatible, which is not an HLA typing process.

We have published a paper in Transfusion, which is what I am going to talk about, and then I am going to update the paper. This was a paper that was listed as Paglino in Rob's review, his references, Reduction of Febrile but not Allergic Reactions.

I will point out that 0.3 of an FTE Yale research nurse was funded by the Pall Corporation.

We needed some support for a research nurse as part of the study. Pall provided the funds to the university that went to supplement this nurse's salary.

So, again, there was no money other than just for that, but I do mention that in terms of full disclosure, and Pall had nothing to do with the writing of the paper, the data, or any of that.

So, what the study consisted of was we had used selective leukoreduction for a whole period of time since I have been at Yale, and we had about a 30 percent level of leukoreduction. We looked at starting in January of '05 and then we considered that through October 1998 to be the period of about 30 percent, 25 to 30 percent. That was decided by the physician as to whether to use that for selective leukoreduction.

Then, we went through a transition phase when we converted to 100 percent leukoreduction, when I decided that was what I wanted to do for the university and the medical center, and then we went for a universal leukoreduction phase, which started

January 2000 through December 2002, at the time the paper was written.

So, we had a pre-universal leukocyte reduction phase, which was about 30 percent, we had a transition period, and then we had a period where we were 100 percent or 99.9 percent leukoreduced starting about January 2000.

We did that so I didn't have to divide the transition phase into, you know half went this way, half went that way, just make it its own unit or just ignore it for most of the calculations, so I could compare pre- versus post.

So, this is not zero leukoreduction, it actually starts at 30 percent, so based on that, any improvement or loss of improvement could be for that 70 percent of non-targeted people. Now, the non-targeted people, you could say, I mean that is a euphemism. They are all targeted, it's a question of when.

They are either targeted when you are responsible for the hospital budget or when your successor will be responsible for the hospital

budget, but it is like we only put seat belts in cars where kids are bad drivers, because they are the ones that are likely to have the accident, you put it in so everybody can benefit from it.

That's my philosophy. That's why I give you my conflict of interest way upfront, so you will know I am not a talking head for the corporation. I really believe that leukoreduction is helpful for patients, and I don't have to cure their cancer, but I may be able to make them feel better and prevent some CMV transmission along the way.

So, these were the number of units that were evaluated, 85,000 units of red cells in the selective phase, 60,000 units in the universal pre-storage leukoreduction phase.

For platelets, there were 100,000 units that we evaluated, and in the post-, there were 37,000 units, and I listed them as pools also for purposes of the evaluation. What we were looking for was febrile reactions which were really defined as febrile reactions that were reported to the

blood bank.

Again, it is passive, as Rob pointed out. So, evaluations of the reactions per month for the pre-storage period was about 6.3, and then dropped to 2.94 in the universal phase. This had a p-value of 0.001, 53 percent reduction as reactions per month. These are for red cells alone for febrile reactions.

When done as reactions over the total, the rate dropped from 0.34 to 0.18, and as you can see, during the transition, there was a drop in the middle, 6.3 reactions went to 5, then, to 2.9, and the rate was 0.34, 0.29, 0.18, was about 47.1, which you would expect there to be comparability.

For allergic reactions, there was really no change at all, as expressed the p-value, and you wouldn't expect there to be a change, but the purpose of looking at allergic reactions was to see if there was a problem with reporting of the reactions from the floor.

So, that was for red cells. For platelets, we went from 7.6 reactions per month

down to 0.43, 94 percent drop, similar with the pooled. That was with the pooled, and this was the reactions, the rate was 0.44 down to 0.04, and it was a 90.9 percent drop in the rate.

Allergic reactions, 37 percent, 28 percent decrease. Some of that was due to the fact we had decreased the pool size during this time period from about 6 units to 4, so my thought was there were fewer reactions because there was less platelets being given, so there was less allergens being given. The rate was 0.1 to 0.09, so we didn't see a change really in the rate, there was just a drop that was not considered statistically significant in allergic reactions.

Looking at it as a bar graph, this is the selective period of time. This is the transition. This is universal on the upper lefthand corner here with this slowly dying green thing, and as you can see the rate dropping.

For the analysis, we did not look at the transition period when we did the analysis.

For red cell, for allergic, there was no

difference in the upper righthand corner. For platelets, there was a very marked drop. For febrile reactions, for allergic reactions, there really wasn't much change at all.

So, then, the odds ratios bore this out, I am going to skip this. So, our conclusions were that--I am sorry, not conclusions--what we decided to do was there was always the concern that maybe our nurses were just doing a bad job, they were off doing their nails or he was looking out the window when the patient was rigoring their merry way, off the bed, onto the floor, and rolled out the door, so nobody caught it.

So, we evaluated 500 patient charts, undertaken to see if the decline was due to under-reporting by the nursing staff. This is sort of like, you know, a container comes in, in San Diego, and you pick one bag a week to seek if there is cocaine in there, and you randomize that to is there cocaine anywhere in the shipment.

We looked at 500 even though there were thousands of people being transfused because I

couldn't devote an entire nurse to this process. So, we randomly selected 300 patients who received red cells and 200 who received platelets, all without a report of a reaction over the period of time.

We reviewed the charts, and it says 100 patients were interviewed, but that should be 300, 300 patients were interviewed, 200 red cell out of the 300, and 100 platelet recipients out of the 200, to see if they had symptoms. The bottom line, as we detail in the paper, is that 1 patient had a fever of 100.2 that was not reported, and there was no change in nursing protocols, there was no change in our report of a transfusion reaction.

We didn't start putting Tylenol or acetaminophen in the water supply. Everything had been the same, and I had been at Yale that entire period of time, so that nothing really had changed much in the way that we approach febrile reactions. So, my thought was this was not due to under-reporting.

The conclusions were that there was a

significant decrease in the rate of febrile reactions, the pre-storage universal leukoreduction decreased to platelets and to red cells. There was no change in the allergic reaction post-universal leukoreduction. The decrease was not attributable to nursing inattention, and the universal leukoreduction decreased febrile reactions and provided patient care.

Our administration agreed that the improvement in patient care was acceptable as a reason to spend some dollars, so now let's update for you, and let me go through this because all the ensuing 8 graphs will express it similarly.

The ordinate on the left is allergic reactions or febrile reactions. This is listed as the number of reactions, so that is the individual number per month, or it will be the rate.

On the right ordinate is percent of leukoreduction. So, the ordinate on the right, the leukoreduction is seen in blue. It starts off about 20, 25 to 30 percent starting with 1995, and then moving to about the year 2000 when we slowly

converted to 100 percent over several months, and then this period is the period of universal leukoreduction.

Each of these black markers on the bottom abscissa is a year, and the red dots are reactions per month, so there are actually 12 red dots for each year period. There is a best fit curve that is put in by the program.

So, what you see is for allergic reactions, we have some very banner months with very high allergic reactions up to about 7 or 8 reactions, and then others where we had relatively little, and as we went to full leukoreduction, I think you can see there was really not much change in the number of reactions, for allergic reactions to red cells.

Then, the rate of reactions to red cells, the same data only plotted, the left ordinate is rate, and not number, and it should be pretty much identical, and as you can see, the curve is actually going up a little.

For some reason, we have had an increase

in allergic reactions. We haven't increased the number of red cells. It is that people are getting itchy, I guess, for whatever reason.

For febrile reactions to red cells, we did see a change, and as you can see here, there is a drop. This drop was the drop of about 30 percent, that was statistically significant, and, yes, we still see febrile reactions to red cells, and I don't really understand why we see these many.

There is something in red cells that is a cytokine that is in an inflammatory cytokine. I am no sure what it is. There is not much else in red cells besides hemoglobin and some RNA and some other things, but it is there, whatever it is, I believe.

Actually, if you look, it is sort of a bimodal type of a--you could draw a line through here. This is the pre-storage. Then, you could draw a line through here. So, there is a drop of 30 percent.

Have I cured cancer, have I saved some child who can now walk again? No, but we have

decreased, I think, this is five years of data, so this is not like yesterday afternoon, and the total number of patients is 7,200 x 5, is about 35,000 patients.

I think the data does speak for itself, that there has been a decrease in incidence of febrile reaction by about 30 percent. Again, we started off here with about 25 to 30 percent leukoreduction, so these are the non-targeted people supposedly.

Then, the same thing for the febrile reaction, the rate is shown over here, as well.

So, allergic reactions to platelets, now, again, we see a slight increase in the allergic reactions to platelets. The number, it is hard to explain exactly what is happening.

Dr. Dumont had a paper talking about sap running in the areas and its relation to CMV and other things. There are lots of allergens that occur during times of the year, both in Colorado, and I think he also did this with I think Dr. Elfath and looking at things in Virginia, and there

are lots of things that go on in the allergen world that we don't understand very well, and it may be reflected in the patient population.

Then, the allergic reactions for the rate again has been pretty minimal, and you could draw a line across it here, and the rate has been the same.

For platelets, this is our data, and it is hard to believe that it had that much of a drop, 94 percent, but it has been sustained. During 2003, we had 1 or 2 reports for the entire year.

Now, you could say, well, the nurses are missing something, but they are not missing 500 reactions. I mean no one is that nasty that they just ignore that many number of patients.

The increase over here was the onset of the bone marrow transplant program and the transplant program in general, and this was the transition period, so I got on the blue line, the blue line to 100 just in time from my perspective, and most of these bumps are actually the same person who has had more than one reaction, because

this is actual numbers.

So, this is 1 reaction, this may be 2 over here. This is probably 3 or 4. That is basically one patient that was repeatedly transfused, who responded to a very low level of white cells. Then, if you look at the rate, it is the same.

So, I am convinced. When I said there is something in red cells, why are we seeing such a good reduction in platelets, but not in red cells, I don't know, and that has basically been reported by others.

So, the cost of leukoreduction, when we started, it was $300,000. It didn't cost us anything to leukoreduce, and these are pooled leukoreduced units prepared by the Red Cross. The cost was zero because of cost shifting, and the cost shifting and we also used reallocation and decreased outdating, which is well known to be voodoo economics, and that is perfectly lovely.

Cost shifting and internal reallocation are the same thing, I just used two different words, so it would sound like they were two

different things, but what we did was we stopped doing CMV testing, and if we CMV test--she is standing up, that's not a good sign. He had an hour and 15 minutes, I am going just two more minutes, I am sorry.

It's 20 to $25 for a CMV unit, and if we did only a third of the units that we transfuse, which is 16,000 units for CMV, that would be $400,000. So, whether you pay me now or pay me later, you are spending the money. It is just a question of what you are spending it for.

In 2004 dollars, it went up to about $420,000, again, platelets would be zero because we have shifted it all onto the red cells. So, yes, it does cost money. We have an $8 million budget. I do spend 0.5 percent for leukoreduction, and what am I getting? What is your saving in febrile reaction workups?

Now, here is an area we could talk about for weeks, but we are not, just for mere seconds. I put it in, in terms of direct costs and incremental. So, you can look at all of this. We

have blood bank, you know, the serology and the labor time and the microbiology, yada yada.

The direct of $109 was for those of you who think, yes, we spend a lot of time working it up. For those of you who say give me a break, the tech is there, she is doing the same thing, he has got the same stuff, so I will give you 23 bucks, and if you want to throw in a replaced unit, because they had to send the unit back because of the fever, you can add a couple hundred dollars for the red cells or the platelets.

So, I gave it to you both ways. You can have transfusion workup light, or you can have transfusion workup full boat. So, I view, over five years, I prevented 132 reactions a year. I prevented four reactions a month for red cells and seven reactions a month for platelets.

I honestly think that we did that. That is 660 patients over five years, 132 a year. Even if it's 100 bucks, it's 13,000 and there is 66,000 I saved over five years, not to mention the number of patients that I think I helped a little bit.

I can't think of a way that I could spend a few hundred thousand dollars that would benefit patients as well, when there are people who are spending that much in factor VIIa for people who don't need it, et cetera, et cetera.

So, I think this is justification enough plus the fact that we have cost shifted the CMV, and it is essentially cost neutral because that would have--I can't worry about the people who are off 8 West or 8 Onc, who have oncology concerns, who were supposed to get CMV-negative leukoreduced, but I don't know about it, because the house staff is still looking for colonic polyps, their own colonic polyps for the first two months, and don't know what they are doing. We don't have to worry about that. That, to me, is an important issue--I am out of control here, Alan.

[Laughter.]

Two more slides. CMV testing. We have done 200 allotransplants at Yale over the past five years--I am not talking about autologous where we do about 100 or 200 a year, 150 a year--200 allos.

Ten percent of them are CMV seronegative for both donor and recipient. None of them have seroconverted, and these people are monitored with a PP65 weekly for seroconversion. Twenty people is a very small number. Charlie Shiffer would think it is an insignificant number. He has taken blood bankers to task for having small n's in some of their studies.

But the point is that they do weekly surveillance and we have had no seroconversion. We do not do CMV testing, and there has been no reported transfusion-transmitted CMV seroconversion of any patient.

There was one seroconversion that occurred. It was a breastfeeding mother, and she was CMV-positive. So, I don't believe that we are doing our patients a disservice, even the allotransplantations, and this is in full conjunction with the oncology and the neonatologists.

So, the conclusion is at Yale, decisions, as everywhere, as all hospitals, decisions are only

made for medical reasons, to improve patient care, and at Yale, I believe universal leukoreduction is an improvement in patient care.

I think we have the data. This is ongoing, I may come back every four or five years and update you, to your great dismay. Whether universal leukoreduction is the best thing since sliced bread, I don't know. We think it works at Yale, and I am willing to justify it financially to patients and to Ted Koppel on Nightline if I need to.

Thank you very much.

[Applause.]

DR. ORTON: Thanks a lot, Ed. That's why we invited you.

Our next speaker is Dr. Avery Nathens from the Department of Surgery at the University of Washington. He is going to talk about the Impact of Pre-storage Leukoreduction Among Transfused Trauma Patients.

The Impact of Pre-Storage Leukoreduction Among Transfused Trauma Patients

DR. NATHENS: I am Avery Nathens. I am a trauma surgeon at Harborview Medical Center at Seattle. I am a little out of my league in terms of background here, but it is a privilege to be here and a privilege to present these data.

This is a randomized controlled trial that we just completed at the end of 2004. We have just finished data cleaning and data analysis, so these results are preliminary and we haven't quite submitted this for publication yet.

In the trauma population and in critically ill surgical patients, there is a very clear association between the transfusion of allogeneic red blood cells and a high likelihood of infection and multiple organ failure, and this holds true whether it's trauma, elective major surgery, cardiac surgery, et cetera.

The mechanism is thought to be related to transfusion-related immunomodulation. I won't go through the background here, but the sense is that the passenger leukocytes that contaminate every unit of red cells play a role in the induction of

energy, as well, these cells play an important pro-inflammatory role in a number of clinical and experimental studies suggesting that there is this relationship.

It is pretty clear and we have heard a lot of evidence supporting this that the transfusion of leukoreduced blood is associated with fewer febrile transfusion reactions, lower rates of platelet alloimmunization, and lower rates of CMV infection.

The effects on infection risk and multiple organ failure is really inconsistent across studies depending on whether it is a North American study, a European study, the types of analysis, the risk of transfusion, as well as the baseline rates of infection. These all seem to impact on the likelihood of there being a positive effect.

Our goal in this study was to evaluate the effect of pre-storage leukoreduction compared to standard allogeneic transfusion in patients with hemorrhage due to trauma. The reason we chose this population is (a) I am a trauma surgeon, and (b) these patients really are at very high risk for

infection and multiple organ failure.

The typical baseline rate of infection in a transfused trauma patient is around 30 percent. These patients also have large transfusion requirements.

So, this was a double-blind, randomized controlled trial. We managed to get emergency waiver of consent, so patients would come in, they would be immediately cross-matched, and at the time of cross-match, undergo randomization. So, again, this was an emergency waiver of consent.

These patients were randomized at the time of type and cross-matched to either standard packed cells or leukoreduced transfusions. These are the inclusion and exclusion criteria. We limited it to enter patients who were over the age of 17 as per IRB.

We limited it to those who were transfused within 24 hours of injury. The baseline risk of infection drops dramatically for people who are later transfused, which is why we focused on this to get a very high baseline rate of infection to

minimize our sample size.

We excluded patients who had active infections, prisoners, an IRB requirement, those expected to die very quickly, those who received blood products at a transferring institution.

Also, because of inventory concerns, we were required to exclude patients who were proven to be AB-negative or B negative, those with a positive antibody screen, and those who our regional blood center knew required irradiated, leukoreduced, or CMV seronegative products. So, those patients were unfortunately excluded post hoc.

We randomized patients using a stratified scheme based on age and mechanism of injury. This is solely to ensure that there is equal numbers of patients with these risk factors in each group.

The randomization was performed by a hospital-based transfusion support service once they received the request for a type and cross. Also, in patients who required uncrossed-matched blood, the transfusion support service would

automatically randomize the patient as they brought study blood refrigerators to the emergency room with uncrossed-matched blood, either leukoreduced or regular.

We would never know what the next randomization was going to be. That is, this was concealed allocation. So, again, there was no way to guess what the next patient was going to be, and the units were blinded and labeled for research purposes only. So, it was completely double-blinded.

The subjects received study blood products for the shorter of the duration of this hospital admission of 28 days. The study products were again pre-storage leukoreduced within 24 hours of collection by a Pall filter, and quality control suggested, but 90 percent of the units were actually less than 1 x 10^6 with the remainder between a and 5 x 10^6.

I was concerned that the leukoreduced blood, because Puget Sound Blood Center doesn't routinely administer leukoreduced blood, that there

would be a differential in the age of the transfused blood, so we actually controlled the inventory, so that all patients received blood that was younger than 25 days.

Anybody who required platelets received pre-storage leukoreduced apheresis platelets.

The study endpoint is as follows. It is any infectious complication within 28 days of randomization. We actually followed these patients up for 28 days even if they were discharged.

Based on some preliminary data, we have performed a sample size estimate with a baseline risk of infection of 30 percent, which is pretty typical for these patients. We expected a relative risk of infection in the leukoreduced group of 0.4. This is based on a study of colorectal surgery patients.

It resulted in an absolute risk of 12 percent in the leukoreduced group with a power of 90 percent, an alpha of 0.5. We assessed we need about 117 patients per arm. Because this is a waiver of consent study, we anticipated that some

patients would be randomized and would deny us consent, so we inflated the sample size calculation appropriately.

We had a variety of secondary endpoints that we were interested in. One was the rates of multiple organ failure as assessed by the Marshall score, which is really a composite score of failure in 6 different organ systems, mortality, ventilator days, ICU and hospital length of stay.

So, we randomized a lot of patients, and this addresses the issue of what do you do with patients who are never transfused. A lot of patients were randomized, a lot of patients were excluded post-randomization, and I have the breakdown on the next slide.

324 patients were actually randomized who met the inclusion criteria. These are patients who had no exclusion criteria and received blood within 24 hours; 56 refused consent. We have mortality data only on these patients. The IRB did not allow us to actually pursue obtaining primary endpoint information on these patients.

So, using a modified intent-to-treat analysis, we have 268 subjects that form the meat of the rest of the presentation.

Why were patients excluded? The vast majority of patients were excluded because they were not transfused within 24 hours, and there was a variety of other exclusions, as well, some because they were too young, a few patients were randomized that were not trauma patients.

Prisoners were randomized because we did not know they were prisoners at the time. Some patients had prior transfusions for this injury event, and so on. The majority of patients were excluded because they were not transfused within the first 24 hours, but you will see proportions across both groups are the same.

Again, this is blinded, so biases probably are not introduced here.

These are the baseline characteristics in the modified intent-to-treat population. This is the average trauma population, typically male, typically in the high 30s, low 40s, equal racial

distribution across both groups. The majority of patients were young with limited comorbidities, and almost 20 percent of patients had penetrating injuries.

These patients are thought to be at higher risk because of perforated viscus and intra-abdominal problems. So, again, very similar baseline characteristics across the two groups. Injury severity was also similar across groups. Injury severity score is a simple anatomic way of describing how severe the injuries are.

Anybody with an ISS of more than 15 is supposed to be severely injured, so severely injured population. Maximum IS score looks at the worst injury in all body regions, 6 being fatal, 5 being fairly severe. Again, equal distributions of severe injuries across both groups.

Just over 50 percent of patients were in shock. Looking at some measure of shock, the Emergency Department lactate level was high, so these were sick patients, and the lowest ED hematocrit is demonstrated here, again equal

between both groups, so a very nice randomization.

We looked at their blood requirements. Their transfusion requirements across the entire hospitalization were similar with almost 6 units of blood. The transfusion requirements in the first 48 hours was also similar across both groups, and no differences in rates of transfusion of FFP, platelets or cryoprecipitate.

We did a fairly good job of inventory control. The age of transfused blood across both groups was 16.8 days and 16.9 days, so no difference here.

Now, this is the important slide. This looks at infectious complications which, as you recall, was our primary endpoint. If you look at pneumonia, rates were no different. We limited it to patients with an invasive diagnosis of pneumonia only.

These were diagnosed as per either bronchoalveolar lavage or protected specimen brushings because of the difficulty in identifying patients with pneumonia. No difference. The

confidence intervals here all include 1.

Blood human infections no difference. Surgical site infections no difference. UTI is no difference, and the rate of any infection was about 12 percent lower in leukoreduced patients versus other patients, but this was not significant.

Now, importantly, we used CDC and NNIS definitions here, but the only infection that did not require culture positivity were superficial surgical site infections. This is as per the CDC. Every other infection here required a positive culture.

We then looked at whether there was interaction between study arm and severity of illness. We looked at patients with shock or without shock. Again, no difference in outcome. There is a trend here with patients in shock doing worse, but not significant. We looked at whether there was a dose response, people who are massively transfused versus those who weren't. Again, not much of a difference.

We looked at patients with evidence of

physiologic shock, looking at ED lactates. Again, no difference. Injury severity score no difference across high ISS versus low ISS. So, no matter how you look at these results, there appears to be no difference in rates of infectious complications in this patient population.

What about second endpoints? We understand that infectious complications are difficult readout. Mortality was no different, whether it is hospital mortality or 28-day mortality. The median length of stay was similar across groups, 13 days versus 12 1/2 days. An ICU length of stay was similar.

Ventilator days was also similar. We looked at Marshall Multiple Organ Failure Scores, and both groups received a score of approximately 5, so difference in the degree of organ dysfunction.

There were some patients who either died early, that is, died before they can get an infection, and about 16 patients out of 268 were lost to post-discharge follow-up.

So, we did a time-to-event analysis with the event being infection, and this is Kaplan-Meier analysis here, and you will see that the leukoreduced group, in red, has an identical curve to patients receiving standard blood here, in white. So, again, no difference in the time-to-event analysis either.

This deals with the censoring that is inherent and people who die early are lost to follow-up.

So, just to summarize, there is really no measurable effect in this high-risk population requiring transfusion whether you look at infectious complications, multiple organ failure, or resource utilization.

There is lots of differences across studies, and Dr. Davenport has focused on this particular aspect. It is possible there is differences in the baseline risk of infections in the patient population with regard to their baseline immuno-inflammatory state. Study design and sample size also plays a role here.

Given our baseline rates of infection, to achieve significance here, we would need about 996 patients per group. If we truly performed an intent-to-treat analysis where the rates of infection would be lower, because we would actually include patients who weren't infected, we probably need several thousand patients per group.

So, to actually do this study in a high-risk population, it is very, very difficult.

With that, I thank you for your time and I can answer questions now or subsequently.

Thank you.

[Applause.]

DR. ORTON: Thank you, Dr. Nathens.

I have a couple of announcements and then we are going to have a coffee break. We will come back and Dr. Stroncek will give his presentation. Then, the morning speakers will be available for questions.

Would all attendees verify their information and walk-ins provide information at the registration desk, if you haven't done so. There

is to be no food or drink including water in the auditorium. That is all I have.

Please be back promptly at 11 o'clock. We will start then. Thank you.

[Break.]

DR. ORTON: Can I ask everybody to get seated, so we can start, please.

Our next speaker is Dr. David Stroncek. He is Chief of the Laboratory Services Section, Department of Transfusion Medicine at NIH. He is going to discuss Adverse Events and Manufacturing Failures Associated with Leukoreduction.

Adverse Events and Manufacturing Failures Associated with Leukoreduction

DR. STRONCEK: Thank you.

We have heard Dr. Davenport talk this morning, presented a lot of data in an area where it is difficult to draw many conclusions. I am going to talk about an area where I think everybody thinks they know the answer, but there is not a lot of data in the published literature.

I am going to go over first some common

adverse reactions with filters just very quickly, hypotensive reactions, activation of complement by filters, coagulation activation, and hemolysis, and then focus most of my talk on manufacturing failures, slow filtration, inadequate removal of white cells, and the occlusion of filters.

Hypotensive reactions associated with filters are well known to all practitioners of transfusion medicine. Typically, they occur within the first 10 minutes of transfusion, and in one study, the mean was within 4 minutes of the start of a transfusion.

These pressure drops are actually quite severe. In the first published report of this in Vox Sang in 1998, they reported an average fall in systolic blood pressure of 53 millimeters of mercury and a diastolic blood pressure of 25 millimeters, so they are quite significant.

These were first reported with the transfusion of platelets, but they have also been reported with red cells. They occur with bedside filtration exclusively, and clinically, they are

associated with patients receiving angiotensin-converting enzyme inhibitor therapy for hypertension or ACE inhibitors.

The mechanism of these reactions has been fairly well worked out. They occur just with filters that have negatively charged filter material. These negatively charged filters activate a number of factors in the contact system of coagulation, and factor XII is produced, prekallikrein is produced, and high molecular weight kininogens are produced.

One of those is bradykinin which can cause hypotension. The problem is that the ACE enzymes rapidly degrades these kinins and when patients are on ACE inhibitors, their bradykinin doesn't break down very quickly and patients experience hypotension.

Again, because these bradykinins eventually break down over a matter of less than an hour, these are only problems with blood that is filtered at the bedside. It is not a problem with in-lab filtered units or pre-storage leukocyte

reduced units.

Again, there have been a number of studies, of paper published on the activation of complement and coagulation factors by filter. As far as complement, again it is filter-dependent complement activation. It is hard to predict which one, but some filters will activate complement and some won't.

The issue with complement activation is that the theory is that when it--well, we know when complement is activated, neutrophils are stimulated, and these neutrophils then release proteolytic enzymes and generate toxic oxygen species, but again, these neutrophils are removed immediately by the filter, so it is not an issue, a clinical issue anyway with filtered blood.

There have been a number of studies that have looked at coagulation activation by filters, and there is a small amount of activation of complement or complement factors. Differences are significant before and after filtration, but now clinically relevant. The falls are really quite

small.

This could be an issue for some filters if plasma is being filtered, but in this country, people aren't using, at least for the most part, aren't filtering fresh frozen plasma.

There have been some reports of hemolysis with filters, and when units are filtered, there is a slight increase in plasma hemoglobin. There have been studies that show that this increase in hemoglobin increases with age of the unit, so with pre-storage leukocyte reduction, this usually isn't a problem, but if units are filtered in the lab, and as the red cells get older, tends to be more hemolysis, there again, there is variability in the degree of hemolysis with filter, and in some cases, there have been clusters of clinical problems reported with actually a significant amount of hemolysis with some filters, but these problems tend to come and go.

There has been one nice study looking at three different filters and the effect of age on hemolysis, and this was published in 2000 by Gammon

et all. They looked at three different filters and they looked at red cells. Again, with age, this is the plasma hemoglobin before and after filtration.

As red cells went from 1 week old to 6 weeks old, the difference in the pre- and post-plasma hemoglobin increased. Again, the second filter had quite a bit more hemolysis than the first, and the third was less than either of the two. So, again, it showed there is variation between filters, and it is affected by age.

I would like to change directions a little bit and talk about, which is probably most problematic for at least pre-storage leukocyte filtration, and that is what we have lumped as manufacturing failures.

Typically, these can occur as occluded filters or filters that the blood filters, but it takes several hours instead of several minutes to filter, or units that filter normally or appear to filter normally, but they have high residual white counts.

At least in some cases, at least with

sickle cell trait, these are probably all related, it is probably not a separate thing.

Most of the attention about this problem is focused on blood donors with sickle cell trait. As you know, donors with sickle cell trait carry--they are heterozygous for hemoglobin S or sickle hemoglobin. They typically have normal hemoglobins. They are healthy donors otherwise, and they do donate blood, and they make good blood donors. In fact, when we are looking for unusual blood types for patients with sickle cell anemia, they make good donors.

It has been known for quite a while that blood from sickle cell trait donors doesn't pass through filters well. In 1970, it was reported that sickle trait red cells don't pass through millipore filters. These aren't clinical filters, these are just laboratory filters, and people were studying the pathophysiology of sickle cell disease and sickled red cells, but anyway, it was shown that in addition to red cells from patients with sickle cell disease, red cells from patients with

sickle cell trait did not pass through filters.

It wasn't until 1974 when Hipp, et al., found that red cells stored in CP2D, red cells collected in anticoagulant used for blood didn't pass through these millipore filters, and then in 1989, about the time we first started using filters for leukocyte reduction, it was reported that sickle trait red cells didn't pass through Sepacell bedside filters, and in some cases, post-filtration blood counts were high. They reported this in about 40 percent of units from patients with sickle cell trait.

Then, in 1994, there was another report of this time multiple clinical blood transfusion filters were tested, and they found 50 percent of sickle trait donors' red cells filtered slowly, and 75 percent had high residual white counts. So, really, it has been known for over 10 years that there is an issue with filtering blood from patients with sickle cell trait.

In about 1997, the literature is filled with several cases, several reports of problems

with filtering blood from donors with sickle cell trait, and these reports occurred all different types of filters, so this is not unique to any one filter. This really occurs with filters by all manufacturers and of all types.

So, how often does this occur? How big a problem is it? Again, there isn't much in the peer-reviewed literature on this, but at BPAC, in 2001, there were a couple of nice presentations concerning this.

Walker et al., presented the data from the Canadian Transfusion Service, and what they reported, looking at data collected over a year, is that the number of units that didn't filter, that were blocked, were really very small, less than one-tenth of a percent or 0.8 percent, and most of these units, they thought most of the incidents of not filtering were due to clots in the blood bags or about 65 percent.

They thought these clots in the blood bags and the filters were associated with prolonged collection times or greater than 20 minutes. As

was mentioned earlier, they do use uniformly in Canada, mixers or rockers when they are collecting their blood, so they didn't think it was a problem with inadequate mixing of the blood.

What about sickle cell trait? Well, as part of that same presentation, they presented data from the Canadian Blood Service's Toronto Center, and this time they presented data from a 5-month period, and over that time, they filtered about 60,000 units of blood, and at this particular center, the failure rate was 0.07 percent, so again a very small incidence of filter failures.

They tested these donors for sickle cell trait using a Sickledex test, and they found that 30 percent of the failed units were due to sickle cell trait. They looked at this and 7 of the people they found that had sickle cell trait had donated previously, and 5 of those 7 previous donations failed to filter, so again, this supports what we have heard earlier, that patients, if they have sickle cell trait and their blood doesn't filter once, it is probably not going to filter a

second time.

Again, as part of that other study, well, how many units fail manufacturing due to not passing the minimal leukoreduction standard, and here is the data from Canada. This is over a 4-year period. They actually tested quite a few units, 3,000 red cells, and very few really failed. If you use the current AABB standard, it is less than a tenth of a percent. For whole blood, it's about 1,600 units, and again, less than a tenth of a percent, and platelets, it was 7,500 units, and it was less than 0.7 percent.

So, it seems to be failing, the residual white cell count seems to be less of a problem than occluding filters.

There was a presentation at the same meeting by Rebecca Haley of the Red Cross, and she presented data from the Red Cross, and the Red Cross had a little higher rate of filter failures. Instead of being 0.08 percent, it was 10 times higher, it was 0.8 percent.

Their data wasn't quite as good, but they

did have some data breakdown on what the cause of filter failure was, and the most common thing they noted was clots. They also noted user errors, cold agglutinins, and then some patients with sickle cell trait, but they really thought that was a very small percent of the people of the whole units. They had some with high residual white counts, some with low red cell recovery, and a lot of them, they really didn't know the cause or didn't have data.

We have looked at similar data at the NIH Department of Transfusion Medicine, and we looked over a two-year period. We collected about 11,400 units of red cells at that time, and we had 37 filter failures, of filters that were occluded, and the percent, that represents 0.3 percent, so our rate was between the Canadian experience and the American Red Cross experience. Of ours, like the Canadian experience, about a third of them or actually 30 percent of those failures, we attributed to sickle cell trait.

We looked at the donors who the filters failed, and if they were African-American or if

their units failed more than once, we contacted those donors and many of them knew they had sickle cell trait. Others we had, we came in and tested them, and then we asked them to be research donors and used them for some of our other studies.

We still, though, had a number of units that failed in patients that didn't have sickle cell trait. We do use rockers on all of our blood collection, so we don't think we have a problem really with lots of clots, so it is really we don't know what the cause of those failures were.

So, I want to focus a little bit on some of our studies on addressing the issue and why doesn't sickle cell trait donor red cells filter. Again, red cells from patients with sickle cell trait have 30 to 40 percent hemoglobin S. There is a typing error in the slide.

Patients that have sickle cell disease, 92 to 100 of their hemoglobin is hemoglobin S. So, at normal physiologic conditions, even in venous blood, there is not enough hemoglobin S in sickle trait red cells to sickle. People are wondering

why do they occlude filters.

One hypothesis is that the red cells are sticky, and this is based on the fact that in patients that have sickle cell disease, their red cells are continually sickling in venous blood than unsickling, and over thousands of cycles of sickling and unsickling, they will have membrane changes and they will have irreversibly sickled cells, and even before then, they will have problems with adhesion of the red cells, and the red cells are stickier to venous endothelium.

So, some people thought that this might be an issue with sickle trait donor blood. We consulted our experts including Alan Schechter at the NIH, and because red cells from sickle trait donors don't sickle in those patients, he thought that was not true, that there had been a number of studies that had shown that sickle trait red cells aren't sticky, but when we talked to him about how we collect blood, when we told him that the anticoagulant had a low pH of 5.4 and was hyperosmotic, he immediately thought that the red

cells probably sickle, because a low pH and high osmolality favor sickling.

So our hypothesis is that sickle trait blood is sickling, and its sickled red cells are stiffer and more viscous, and those are occluding filters.

This shows you some data slides on the issues about sickling of blood from patients with sickle cell trait. This shows impaired filtering. It is not working now, but anyway, hemoglobin saturations of 80 to 100 percent, there is no impairment of filtering of sickle trait blood. Again, this is laboratory filters, this is not blood filters.

It is not until saturations reach about 60 percent where red cells sickle or hemoglobin S polymerizes in sickle trait blood, and filtration is impaired.

Now, if you go back and look at the hemoglobin saturation curve, you will notice that in the lungs, partial oxygen pressure is about 100 and hemoglobin is 100 percent saturated. In

tissues, the partial pressure of oxygen is 40 and hemoglobin saturation is about 70 percent.

So, in typical conditions, hemoglobin S isn't polymerized in sickle trait donor blood.

To show our theory that we think that the blood sickle trait red cells do have polymerization, we used the standard technique used by people investigating sickle cell anemia. What we did is we added carbon monoxide to blood, and carbon monoxide bind hemoglobin tightly. It will displace oxygen and it fixes hemoglobin into this oxygenated configuration.

It doesn't really release for a couple hours, so when this carbon monoxide is bound to hemoglobin, then, hemoglobin S won't polymerize. So, our hypothesis is if we put carbon monoxide into sickle trait blood, it should filter.

So, we collected a unit of blood from a patient with sickle cell trait. We split it in two, and half we left untreated and filtered with an RCM1 filter, and half we prepared red cells incubated with carbon monoxide and then filtered

the red cells.

What we found out was when we treated these units, we treated 4 units, and here is the percent of hemoglobin S. It ranged from 35 to 40 percent. The untreated ones, 3 out of 4 occluded filters, and the carbon monoxide treated, all 4 filtered. The red cell recovery actually was quite low on 2 of these, and the other 2, they partially filtered. The red cell recoveries were quite nice on the other ones. They were a little less than you would expect because we used regular filters and a half unit of blood.

Anyway, the carbon monoxide units filtered very quickly showing that hemoglobin S polymerization is causing the occlusion. To show that the anticoagulation was in part a problem, we then collected a unit, but half a unit we collected in CP2D and half in heparin, and then we filtered with it with RCM1 filters.

Again, we found out that when we collected blood in CP2D, here, we had 6 units, and 5 out of the 6 occluded the units after partial filtration.

One filtered completely, but when we collected in heparin, all the unit filtered completely, again showing that the low pH and hyperosmolality of CP2D is a problem.

We hypothesized that if citrate is a problem, well, if we collect red cells by apheresis, this should really help filtration. When red cells are collected by apheresis, we use a Haemonetics machine, and less citrate totally is in the blood unit, because with whole blood, when we collect blood by phlebotomy, the ratio of citrate to red cells is 1 to 8, but by apheresis, it is 1 to 16.

In addition, when we collect blood by phlebotomy, all that citrate is sitting in the bottom of the bag, so the first few red cells are really sitting in very concentrated anticoagulant, where with apheresis, the citrates is metered in, so the red cells just aren't exposed to that much citrate at any one time.

Sure enough, the red cells filtered better, the sickle trait red cells filtered better

when we collected them by apheresis. This time we collected, what was it, 7 units here. We still had 2 that occluded the filters, but the other 5 filtered normally. The red cell recovery was fine, and the residual white cells were fine, too.

What we did note, though, why didn't these 2 filter, and actually, we compared 4 that filtered quickly and 3 that either didn't filter at all or filtered slowly, and the potassium--well, there are a number of measures were the same including pH and osmolality, but the oxygen levels were lower in the units that didn't filter.

So, again, we think besides being the anticoagulant, oxygen tension makes a difference. Sickling is reversible, so we hypothesize that if we can increase the oxygen tension in a unit of blood before it is filtered, they should filter fine. I don't have time to show the data, but we collected units of blood from patients with sickle cell trait, we split them, stored them in a normal bag or stored them in a highly oxygen-permeable bag, teflon, and with larger surface area, we are

able to increase the oxygen levels and oxygen saturation, and those units filtered normally.

Here are some aggregate data. This shows a number of units that we filtered, and it shows the relationship of oxygen tension in these units of sickle cell trait blood and filtration rate in milliliters per minute, so it is really the rate of the red cells going through the filter.

Zero means units were occluded, 12 ml a minute is about as fast as the units went, and as you can see, the units that had a saturation less than 60 didn't filter at all, but as we got above 60, the units filtered fine.

So, this is almost the same point I showed you before, where at the 60 percent saturation is where hemoglobin S polymerization starts to occur.

I would kind of like to sum up now and, in summary, at least for pre-storage leukoreduction, it is found that about 0.1 to 0.01 percent manufacturing losses occur. These failures or losses are due to clots, sickle cell trait, cold agglutinins, user error, and probably manufacturer

error, since in some cases, manufacturing problems with the filters.

With sterile-dockable filters, units are filtered in lab after they have been stores for a while, hemolysis can be a problem, and if bedside filters are used, hypotension can be a problem.

Modifications can be made to bags and collection sets to reduce at least some of these problems, particularly we think we can reduce or eliminate the problem with sickle trait donor blood. Unfortunately, we don't quite have the means available now.

Apheresis of red cells is a partial answer. The problem, though, with that is that the apheresis device we have at our institution is a double red cell collection. There is many people that don't like to donate two red cells at once. Also, the red cell apheresis devices do have a weight limitation. Smaller donors just can't donate by red cell apheresis.

So, I would like to thank my collaborators at the NIH including Dr. Susan Leitman, Karen

Byrne, Dr. Bianchi, Dr. Harvey Klein, and Alan Schechter and Connie Noguchi, who were really helpful and were collaborators on these studies at the NIDDK.

Thank you.

[Applause.]

DR. ORTON: Before we start our questions for our morning speakers, is there anybody who wanted to speak in the open public hearing?

[No response.]

DR. ORTON: If not, I would ask that our morning speakers come to the table.

Let the questions begin.

Questions for Morning Speakers

DR. BIANCO: Dave, you did not mention in your list of bad things that can happen with filters, the previous story of backaches and some reactions that some patients had. But ultimately, what you said is that there are very few bad things that happen with blood that has been filtered.

I think that is an important consideration in the things that we are doing, but regarding the

sickle cell, not infrequently you have a sickle cell trait donor that has a rare blood type that will be very appropriate for a relative of these patients, would you transfuse it without filtering, do you think this is a clinical problem? Will you avoid it?

DR. STRONCEK: That is not that simple of a question. We do use universal leukocyte reduction, so we do have a fairly large group of patients with sickle cell disease we transfuse, and I think we would probably, if we had a relative that needed a donation of a specific phenotype that was hard to find, we would collect the unit and then transfuse it specifically to that patient.

But I think it needs to be said, though, that nobody really-- I think the data is pretty good now that these red cells are sickling and they probably stay sickled for a while. We don't know how well they are going to store, so I guess if we use it within a week or two, we are fine, but I think we really even need to go back now and say even blood from sickle trait donors that is not

filtered, can you really store it 42 days.

DR. ORTON: Can I ask that you identify yourself and your affiliation, please.

Dr. Sayers.

DR. SAYERS: Merlyn Sayers, Carter Bloodcare, Dallas, Fort Worth.

I have actually got a question for Dr. Nathens and a question for Dr. Snyder.

I don't want this to sound like a confession, but in a previous life I spent a long time at the blood center in Seattle, certainly long enough to appreciate that the program there has some very innovative ways of making components.

I am just wondering, Dr. Nathens, if you have the residual white cell counts in those red cells that were not leukofiltered.

DR. NATHENS: My impression was this was in the range of 5 x 10
9, but Dr. Strong is here from the blood center, and he might be able to provide some additional input, or was here.

MR. STRONG: Merlyn, you know that we don't routinely do white counts on our blood

donors, but one would have to assume that they are in the normal range.

DR. SAYERS: No, I was thinking, Mike, of certainly not the blood donors, but of the transfused non-leukofiltered red cell product that was being transfused to the patient. You have got quality control, the white cell counts and the leukofiltered red cells.

MR. STRONG: All of our leukofiltered red cells would be quality controlled, so we do know the data on that. It would be the non-leukoreduced units that we don't have white counts on.

DR. SAYERS: Okay. Ed, the selective leukoreduction period was that period when the liver transplant program was up and running. I am just wondering were those liver transplant patients getting leukofiltered or non-leukofiltered products? Were they in the 30 percent?

DR. SNYDER: It was probably some overlap, but the nature of our liver transplant program was such that if they got something that was red and cold, it was good. They used so much blood, so

there was no way we could have.

Now, since all the blood is leukoreduced, they would get that regardless, because that is all we have.

DR. ORTON: Dr. Klein.

DR. KLEIN: Harvey Klein, NIH. I have a question for Dr. Nathens.

I noticed that you use a lot of plasma in trauma, and I think it was actually about 1 unit for every 2 units of red cells. So, I have a two-part question.

The first is, was that equally distributed across the patients, or was that a small number of patients that got a ton of plasma?

Secondly, do you know what the residual leukocyte count of your fresh frozen plasma was?

DR. NATHENS: Actually, I don't know the residual white cell count of the fresh frozen plasma. I can't answer that question. As far as how the FFP was distributed, about half of patients actually got some FFP, so what you are looking at is the reflection of patients who received a lot of

FFP, but there were a small number of patients. So, about half received none, half received some.

DR. ORTON: Larry Dumont.

DR. DUMONT: For Dr. Snyder. Some would say, maybe may would say it's a fever, so what, and why not just treat with antipyretics, and I was wondering if you might comment on the clinical picture, really, if you are the patient in the bed, how that feels, and also the total impact on house staff, the people that are dealing with these day-in and day-out.

DR. SNYDER: Right, those are good questions.

I think it is very cavalier of people to accept pain and suffering for someone else, so the many times we have had these meetings, and people have said, well, it is just a febrile reaction, these are people who criticize patients for complaining because they don't feel well, yet those very same individuals, be they house staff or nurses or whomever, will take three days off if they get a cold, and assume it's their right

because they have PT or whatever.

So, I have never been impressed with the idea that it's just a fever. I think, yes, having a small fever is no big deal, but if we have the ability to have a positive impact on patient care, and it is reasonable to do it, plus there are other benefits, I think it is a reasonable attempt to try to incorporate that into medical practice.

So, I decided that at Yale, since I had not a lot of control over a lot of things, but I had control over some things, that I move forward and take the bold step and say we are going to universally leukoreduce because I didn't want to have to worry about patients boarded off the Oncology floor, or people that were transplanted, but we didn't know about it.

I didn't want to have to worry about not giving CMV to people who they thought they needed it, plus--and Harvey Klein mentioned other types of non-CMV viruses that are also presumably removed. There are some data that they are removed by filters, as well, leukoreduction filters.

So, I thought there were multiple benefits. Certainly for children, you know, having febrile reactions I think is unnecessary. Can I convince administrators who are looking at the bottom line? Harvey also had made the comment that if filters were free, we would all be using them. So, somewhere between is it cost effective and what is the benefit to patients.

The superb study that Dr. Nathens did, which was a huge amount of work, looking at really hard outcomes, I am not surprised filters didn't really show much of a benefit. I am not looking for a huge amount of things, I am not looking for a filter to lift the space shuttle into orbit in place of the solid rocket booster, it doesn't do that. It is not going to serve to solve a lot of the ills of mankind, but I think it does have a useful role in transfusion medicine therapy.

I think it improves the quality of patient care, and I don't think we should assume, because someone is having a chill and temperatures only up to 100.2, that they had handle it and be a man or

be a woman and take it. I think that's absurd philosophy from my perspective.

DR. ORTON: Dr. Klein.

DR. KLEIN: I have a question for any of the people who run transfusion services. Untargeted patients are frequently targeted patients who are undiagnosed. Those of us in tertiary care centers recognize that people get transfused before they enter our center.

Do you have any idea of any data preferably, but ideas are okay, about how many, what percent of those patients appear at your institution, have already been transfused, and you would give them leukoreduced blood had you known in advance?

I am talking about patients with aplastic anemia or acute myelocytic leukemia who have not been diagnosed at their primary care center before they get to you.

DR. SNYDER: The only way you might get a handle on who has been transfused before they got to us would be looking at the red cell

alloimmunization for people who hadn't been at Yale in the computer, who come in with an allo antibody. It is more difficult in women who obviously could have had children, but I don't know what the numbers are.

I am sure that there are a bunch. The thing that impressed me several times are people who were transplanted in Boston and came in to Yale, and the only reason we knew they got transplanted was that their blood type had changed from what we had had when they first came to Yale, before they were transplanted elsewhere.

So, it was just enough of the left hand not knowing what the right hand is doing. The blood bank would be held accountable for a whole series of missteps if you didn't irradiate blood for somebody or whatever.

You try to control what you can. I don't think we should irradiate the entire blood supply because of the concerns about shortening the outdates, but I think we can, by leukoreducing, I have that much less I have to worry about, the

institution not doing the right thing or not tracking patients properly, but I wouldn't know what percentage of people.

DR. DAVENPORT: I would like to add just one comment on the febrile reaction, and that is, if you look at patients who are having true rigors, there is some evidence that their metabolic rate doubles or triples, and somebody who had got a compromised cardiorespiratory system is not going to tolerate that for very long.

I don't know of any specific case reports or data showing that people who have rigor reactions are having a higher rate of myocardial infarctions or something, but there is a group of patients who are having severe febrile reactions, and it is physiologically important, and those are being mitigated to a large extent by leukocyte reduction.

DR. NATHENS: I have another comment that addresses the febrile reactions, as well. For the most part, the most important trigger for searching for an infection is the presence of a fever. The

presence of a fever leads to excess antimicrobial use and probably leads to more frequent diagnosis of infection, some of which might be clinically significant and some of which might be not.

So, clearly, having fevers leads to more antimicrobial use, and in the clinical trials, leukoreduced versus regular blood, you are going to have more febrile fever days, if you want to call them, more days with fever in the non-leukoreduced group.

The clinicians are going to be searching harder for a source of infection in those patients. So, the small trends that we are seeing with the lowering of infection rates might represent some detection biases in the leukoreduced group.

DR. BIANCO: Just a follow-up to these comments. I am Celso Bianco, America's Blood Centers.

In a once-in-a-lifetime transfusion, that is correct, you would prevent those just by having the entire inventory leukoreduced and irradiated like Dr. Snyder mentioned, but if you were

transfusing a patient regularly, and the patient has some febrile reactions, I think that the practice there is to place these individuals in the targeted group, isn't that correct, even in hospitals that don't normally transfuse 100 percent leukoreduced products? So, it doesn't keep repeating itself.

DR. DAVENPORT: Yes, I think that is true. Our previous policy was if a patient had two febrile reactions, then, they would go on bedside leukoreduced.

DR. ORTON: Dr. Sayers.

DR. SAYERS: A question for Dr. Stroncek. That information that Rebecca Haley presented at BPAC some years ago, unknown causes of filter failures was something like 50 percent of Red Cross's experience.

Now, were they genuinely unknown, or had the cold agglutinins and the sickle hemoglobin been ruled out?

DR. STRONCEK: The data was presented rather quickly, but with the Red Cross system being

so many different centers, I suspect it was generally was unknown and it wasn't investigated.

DR. ORTON: Tim.

MR. MALONE: Tim Malone, Florida Blood Services.

A question for Alan Williams. In your proposed guidelines, the 1695 rule, are we still referring to per device manufacturer and per collection site, or are we getting away from that possible?

DR. WILLIAMS: I hesitate to be quite that specific because that was a 2001 draft guidance and we are now looking at potential new recommendations which will be introduced this afternoon.

What I think it needs to refer to, and I may have to defer to Sharyn on this, is per manufacturer, per device type, per operating procedure, i.e., method, and anything else that would significantly impact the procedure, but those I think would be the two main categories.

DR. ORTON: I can clarify. Even the 1996 guidance were QC for platelet pheresis is by

machine type, by product type, by site. The 1996 guidance talks about 1 percent of your collections. So, as Alan indicated, 2001, and those QC parameters, that was draft. We are going to be talking about an alternative this afternoon for QC.

It again looks at your entire set of collections, but the leukoreduction QC, using the 1996 guidance, is not by collection, site, et cetera, so if you have more questions about that, Tim, we can talk about that later, but you will see we are moving very much in kind of the collection arena for QC in general.

DR. BIANCO: I will bring back particularly to those of you that addressed clinical issues in transfusion. What are the adverse reactions except for infections like CMF, that you would be very concerned that you would do irreversible damage to patients that are in the targeted group? We are all in consensus believe that they should receive leukoreduced group, but that accidentally, here and there, don't receive a leukoreduced group, like today, they receive 95

percent is the cut. Maybe 5 percent of those units are not leukoreduced to the lowest level we can achieve.

Is this bad for those patients? Are we doing damage to them?

DR. DAVENPORT: I would suggest that in terms of alloimmunization in a potential transplant recipient or someone who is going to require long-term platelet support, that there is a real potential for adverse effect, so that if we were to immunize them, so that they have setup for acute organ rejection or that they become refractory to platelet transfusion, then, yes, I would say in that population, there is a real and quantifiable adverse effect.

DR. BIANCO: Of a limited number of units, there is 1 in 20 being not appropriately leukoreduced. I am trying to link these, Rob, to the QC approaches that we are going to discuss probably throughout today. That is how strict do we have to be, it should be a confidence limit of 95 percent, 99 percent, or 100 percent.

DR. DAVENPORT: A very good point, and I don't have data on the top of my head that could address a dose relationship between number of non-leukocytoreduced transfusions and alloimmunization.

DR. ORTON: Yes.

DR. MENITOVE: Hi. Jay Menitove, Community Blood Center of Kansas City.

Dr. Nathens, I was intrigued by your comment about potentially greater workups or attention to patients in the standard group who had febrile reactions. In terms of resources, patient management, patient care issues, and financial issues, is your sense that there is a difference in the overall management of patients who are getting standard versus leukocyte reduced?

I am following up on what I thought your comment was in that the patients receiving standard red cells may, in fact, be getting more intensive utilization of resources.

DR. NATHENS: That is exactly what I was saying. They get more intensive utilization of

resources, and they have clearly a more intense infection workup, because the clinician feels there is something there to find.

We haven't translated this into looking at costs for our patients yet, but will subsequently do that. A lot of federal agencies have an interest now in reducing antimicrobial use, inappropriate antimicrobial use, so if you reduce febrile transfusion reactions, we probably will reduce the use of the empiric antimicrobial therapy for vaguely defined infections.

MR. SIVAN: Yariv Sivan from United Pharma.

Just a couple of comments and then a question. Clarification on the international perspective. Even though 10 out of 29, 34 percent of the countries in Europe do universal leukoreduction, they do represent 13 million of 16 million collections in Europe, which equates to about 81 percent of leukoreduction in Europe, so I just thought that proportion would be interesting also to hear.

Secondly, Dr. Nathens, in your reduction I believe you said that you saw a nonstatistical value of about 12 percent reduction in infection, and if that is correct, if I understood that to be correct, with the 100-plus patients n each arm, you are talking about 12 patients, and I may be incorrect, I may have not heard you.

Then, a couple of questions for Dr. Snyder. Is it possible that the red cell reactions that you saw did not go down when going to universal production? Is it because of platelets that were not taken out in the filtration and went through with the red cells?

Then, a question to the whole panel is if you had a family member that needed to get a red cell, would you or would not give them a universal leukoreduced red cell? Thank you.

DR. SNYDER: Interestingly, throughout all the years, I have always said, well, if my mother was getting a transfusion, no one has ever really been concerned about the father. He can sort of fend for himself, I guess.

I thought that the comment that was made about decreasing microbial use was very cogent. We are always looking for ways to save dollars, and in the 660 patients over five years who did not get worked up for bacterial contamination because they didn't develop a fever, we probably made the entire Northeast safer, MRSA and VRE, so I think we did a reasonable job there, but that is another very important issue.

It is always easier to prevent something than try to treat it, so Celso was asking questions about are patients hurt by this. Well, it depends.

Does drawing two sets of blood cultures from two sites including the port, you know, plus the potential for antibiotic coverage and maybe another stay in the hospital or admitting somebody because they get a temp of 102, some people respond more excessively to the leukocyte antigens than others do, so someone who is getting an outpatient transfusion, gets 102, they probably bought a ticket to the ED or to at least the holding area overnight while they rule out contamination, until

the blood culture reports come back in the morning.

So, I think there are multiple reasons, which are again soft targets, they are not decreased length of stay kinds of things. So, I firmly believe that all these are spinoffs, that if we sat down and thought about it, there would be other reasons for cost savings with leukoreduction.

As far as whether the platelets contained in the red cells, I would probably say not, platelets are not generally well known, they do, but to be that immunogenic, there are certainly platelet-specific antigens, but to cause febrile reactions, I think at the rate I see, when I don't see those reactions when platelets themselves are transfused, I would be hard put to say aha, there is the answer.

I don't know what it is. I was talking with Dr. Klein, and we do see a very low incidence of febrile reactions to platelets, and I am pleased, I am concerned that we see as many as we do to red cells. It can't be that they are not looking, because I can't imagine people are looking

at red cells much more intently, when the platelet is there, they just walk out of the room and don't care what happens.

We see an increased reporting of allergic reactions, so why wouldn't they report febrile reactions? Everything points to the fact that I am seeing actual data is reflecting what is actually happening, but I cannot explain why we see so few platelets and so many with red cells where others have a different type of experience.

It may be the way the center prepares the product. These again, except for the 1,000 outpatient ones we use, these are all pre-source leukoreduced with the Pall System, and we would have to have the Red Cross manufacturing people up here from the Connecticut region, while we still have one, to explain all of this.

DR. NATHENS: Just to respond to your question about the 12 per 100, yes, the relative risk reduction is 0.88 or a 12 percent relative risk reduction. I provided 95 percent confidence intervals there which give you an estimate of the

uncertainty with that 0.88, and the intervals included 1.

So, in fact, the leukoreduction could be more beneficial that 0.88 or actually be more harmful than standard blood. There is a significant degree of uncertainty in that 0.88.

DR. ORTON: Dr. Katz.

DR. KATZ: I wanted to make a similar point. My system is 80 percent leukoreduced, and I would dearly love to go to 100 percent if only to keep things simple in my component lab.

I think Ed Snyder has made the compelling point with his three points. I would point out that in a beautifully done, controlled trial in trauma patients, we heard data from, the relative risk of death was 20 percent higher in the leukoreduced group.

So, I would make a plea that people stop citing statistically insignificant point estimates of this, that, or the other relatively softer endpoint. There was a relative risk of death of 1.2, if I saw your data correctly. It was also

statistically insignificant, so which side of the fence are we going to be on?

Jerry.

DR. HOLMBERG: This is a question for Ed Snyder. Especially coming from the government point of view, we do have to look at the dollars at the end of the day even though our national debt keeps growing.

The comment that you made about your random platelet and your apheresis platelets converting over to the apheresis platelets obviously because the reimbursement was higher there, drives me to another question concerning the reimbursement for leukoreduced blood products.

In your patient population, especially the Medicare population or the Medicaid population that you might have there, how is this covered? Does a physician have to write an order specifically for leukoreduced products? How does the hospital cover the cost in this patient population?

DR. SNYDER: The physician writes an order for a transfusion, and we supply leukoreduced blood

because that is all we have, so he doesn't write a specific order for that. He would need to write a specific order for irradiated, for example, if they wanted that.

We don't deal with reimbursement. All we deal with are just charges and cost, not even cost, just charges. We charge for the transfusion, whatever the fee schedule is for a transfusion, it is not broken down into leukoreduced or non leukoreduced. The reimbursement is through DRG.

The outpatient setting, you do get more. We still lose money, but we lose less with the CMS because they give, for a single donor product, larger reimbursement in the outpatient setting than an inpatient.

I can't explain the economics of all of that, but the physicians do not have to write an order, and, in fact, it would be interesting if someone wrote an order to give non-leukoreduced blood, I probably wouldn't honor it. I probably would have to have a discussion, major discussion, and see what it was that they had wanted. I have

never had that request. The most bizarre request I have received is for washed plasma, which I am sure we have all gotten. I send them an empty bag. I thought that was pretty cute.

[Laughter.]

DR. ORTON: Are there any more questions? Yes.

AUDIENCE PARTICIPANT: I have two questions, first, for the whole group. We heard that there is a 15 percent loss of red cells in the filter. Is anybody aware of any studies on the long term, comparisons of the long-term survival of red cells that are leukocyte reduced versus non-leukocyte reduced, and if the answer is no, that has not been studied, my next question would be for Dr. Snyder.

You made a comment that irradiation shortens--that you are not in support of universal irradiation because it shortens the survival of red cells, if it was shown that leukocyte reduced red cells had shortened in vivo survival, would that change your opinion?

DR. ORTON: Can I just add one thing about the 15 percent? The filters don't necessarily remove 15 percent, I want to make that clear. I look at a lot of QC, and rarely do I see that kind of removal, so I just wanted to clarify that often it is less than 5 percent. That is really more of the norm, not 15 percent.

DR. SNYDER: The only data I have on leukocyte reduction and red cell survival is that we have done a lot of studies, many of which have been published, and several of which are actually going to be probably at AABB this year in one form or another.

For companies that are looking at their filtration, and we are doing radiolabeled survivals along the way, and those survivals have to be above 75 percent radiolabeled survival, which they are.

We haven't done long-term, you know, up to 120 days to see what they are, but the presumption is that if they survive, 75 percent recovery is what you are getting. I have no evidence that there is a decreased survival.

As you can see from our red cell transfusion, when we shift it over, if we were getting decreased survival, I would have expected the red cell rates to go up despite our best efforts to keep the transfusions down, but that is very soft.

I have no data other than the radiolabeled survival which shows that things are fine. If you had a decreased red cell survival due to leukoreduction, we probably would have to rethink issues if, as Rob pointed out, you know, we transfuse by units.

We tried to convert some of the chronic transfusion patients to one-unit transfusions only, because after they got that first unit, their hematocrit rose to a level above the criteria for the second transfusion, and they weren't allowed to get a second transfusion.

That was very difficult to deal with. You could lower the pool rate of platelets from 12 to 4, because you hand out a bag of yellow, and they see a bag and that's fine, but red cells were being

given out one at a time. It was almost impossible to keep people from transfusing a second unit at some point down the road claiming the patient was bleeding or whatever.

So, I don't know what I would do if the leukoreduction showed that that--fortunately, it doesn't have that effect, so I don't have to worry about it, but we would certainly have to think about it if you were correct in your scenario.

DR. ORTON: Steve Wagner.

MR. WAGNER: I would like to make one quick point, and that is, often, a not cited, obscure study that was in an abstract in Transfusion by Rick Davey and Susan Leitman, and it had to do with the effect of leukoreduction on improving the 24-hour recovery of gamma-irradiated red cells.

There is a positive impact of leukoreduction on gamma-irradiated red cells, and the reason for it, the hypothesis put forth is that these white cells undergo apoptosis by gamma irradiation, and they begin to break up, releasing

lysosomal contents, which begin to chew on the red cells.

Recently, in our lab, we have done studies where we compared directly red cell storage properties on leukoreduced versus non-leukoreduced gamma-irradiated red cells, and there are significant improvements on hemolysis and ATP, as well as mean corpuscular volume.

So, there is a number of reasons to think that leukoreduction might help some red cells survive better if they are gamma-irradiated.

DR. ORTON: The panel will be back again this afternoon later.

DR. BUSCH: Two comments. In the VAT study, which was sa large, randomized trial, I am almost sure--I was trying to look it up--but one is that that study actually did show a reduced survival in the non-leukoreduced arm, and it was marginally significant in an adjusted analysis, so there was one study that did show minor, if you will, adverse effect.

The others, I am almost positive that

study did document an increased transfusion red cell requirement in the leukoreduced arm that was quite consistent with the 15 percent estimated loss of red cells.

Just one other comment with Dr. Nathens. We have got a paper coming out next month that one of the findings in trauma patients that is kind of unusual is they develop long-term chimerism with donor cells.

About 50 percent of these patients in studies in Sacramento become chimeric with donor leukocytes, and it persists in about 30 percent, and in some pre/post kind of analyses that we are reporting next month, the rate of chimerism was identical in the post-leukoreduction phase.

We have been collaborating with Dr. Nathens' study, and I know we are seeing chimerism, and I think you indicated during the break that you have got data on the coding now. Maybe you could comment on that.

DR. NATHENS: Interestingly, there is absolutely no benefit in reducing microchimerism

with leukoreduced cells. So, there is a threshold effect, I guess, where we are still transfusing enough white cells that these cells take and persist. So, there is no difference in rates of chimerism between leukoreduced patient and non-leukoreduced, it is still about 30 percent.

DR. BUSCH: A phenomenon, we don't know what it means. It probably is most relevant to this topic with respect to graft versus host, because if you have got enough viable donor cells in these leukoreduced products to engraft, you probably have enough viable T-cells to cause GVHD.

DR. ORTON: I would like to break for lunch. There is a cafeteria in this building. There is also a cafeteria in the building that is across the street.

We will start back promptly at 1 o'clock.

[Luncheon recess taken at 12:05 p.m.]

A F T E R N O O N P R O C E E D I N G S

[1:00 p.m.]

DR. WILLIAMS: Let's get going again. We lost two minutes while the computer rebooted, but I assume we will be able to make that up.

In this afternoon's session, I think we are going to be dealing with an equally interesting area of leukoreduction, which is the practical aspects that blood establishments face in the actual process in defining the QC strategies and the resultant proportion of leukoreduced products that are produced.

As you will see subsequently, this varies somewhat around the country.

Our first speaker on this subject is Tim Malone, who is with Florida Blood Services, and he works with Dr. Leparc, who gave a similar talk at a Blood Products Advisory Committee meeting a couple of years ago, and we found it very useful for a couple of reasons.

One, he has as good perspective on what is practical and what isn't and transmitting that

information, but also, they have done a high level of quality control for a lot of their products for a number of years and can comment on that.

So, Tim Malone is going to speak about Practical Aspects of Pre-Storage Leukocyte Reduction in a Blood Establishment Including Use of Pooled Samples for Enumeration of Residual Leukocytes.

Practical Aspects of Pre-Storage Leukocyte Reduction in a Blood Establishment Including Use of Pooled Samples for Enumeration of Residual Leukocytes

MR. MALONE: Good afternoon and I thank the group for inviting us this morning or this afternoon. I saw a license plate in D.C. this morning that said Taxation Without Representation, so I am glad we are represented here today.

In terms of what leads to keys to successful quality control of blood components, suggests that QC processes should be designed to provide assurance of safety, identity, purity and potency of blood components, which manufacturers do

a great job of providing instrumentation and high grade filters to achieve these goals, but as a blood center, we also are concerned with, and think it very prudent, to minimize the loss of transfusable components, have QC being performed under reasonable logistics, yet only adding a reasonable cost.

In our shop, our pre-stored leukocyte reduced components include platelet apheresis by automated collection and that of both Baxter, AMICUS, and Gambro TRIMA. We are doing RBCs by filtration including AS-1s, AS-5s, and AS-3s, and we are collecting RBCs by automated collections in the leukoreduced product provided by the Baxter ALYX technology.

We also collect Gambro TRIMA red cells, but they are not leukocyte reduced.

In our shop, we average 1,200 collections of platelets by apheresis per month. We transfuse approximately 2,000 transfusion doses per month, and the service area demand is slightly above 3,000 doses per month, the balance being made up from our

whole blood derived platelets.

We have 11 collection sites. We have a total of 39 instruments currently in operations again that being the two types, both the Baxter AMICUS where we have 30 machines and the Gambro TRIMA which we have 9.

Daily, we perform 100 percent QC on our platelet pheresis collected, which includes platelet count and WBC count by impedance. We do bacterial culture for aerobic bacteria and fungi, and we calculate our total platelet contents and yield through volume calculations.

Identifying components for monthly QC, we perform counts on a minimum of 4 units per month for each donation type, that being single, double, and triple dose collections for each site and for each manufacturer's instrumentation.

We rotate those instruments at each site to assure that we have tested or QC'd products from each of the instruments within each of the collection sites.

How do we do all that? Well, hits, runs,

and errors it is not, but this is our monthly QC scorecard where as we go through the month, we capture both single, double, and triple collections by each of the 11 sites and then with each of the machine numbers within each of the sites.

Routinely, we are testing on day 4, but we also recognize end of storage QC by doing 4 AMICUS and 4 TRIMA products that are tested on day 5 or the end of storage.

She also suggests that some of these sites, the larger sites, we have more than 4 instruments per site, so those that do have more than 4, we are rotating QC values from each of those instruments, as well.

Our monthly QC is performed at the end of storage or at time of issue, which requires that we have greater than or equal to 3 x 10

11 in 90 percent

of the components tested. We have a WBC count which must be less than 5 million in 95 percent of the units tested.

We have a pH that must be greater than or equal to 6.2 in 100 percent of the components

tested, and once again for each component code or each component type being single, double and triple collection.

Of course, the units identified for monthly QC are based on our daily QC yield, and they are held again until day 4 of storage and then released if QC is passed. The effect on our component outdate rate has been approximately one-third of these components held for QC or an average of 72 a month expire on our shelves.

I should also state that we operate transfusion services within 5 of the major hospitals in the Tampa Bay area, so this data is also captured including products that are sent to our transfusion sites.

So, this then becomes our monthly QC testing schedule - 4 singles, 4 doubles, 4 triples yielding a total of 24 components for QC per site x our 11 sites. We have 264 components in QC per month held until end of storage or time of issue.

What does that cost? Well, in terms of the QC that I have described, our platelet counts,

our daily counts account for $900 a month. Our residual WBC counts by flow, $7,900 a month. Our pH costs us $25 a month, but the big numbers are the contribute of the outdate of $35,250 per month. Our total monthly QC cost, $44,000, which does not include bacterial detection QC.

Our QC of RBCs pre-storage leukoreduced includes two populations, those RBS leukoreduced by filtration using the Baxter Sepacell R2000, we average 2,500 a month, and we QC 1 percent of the production. We have chosen to QC 30 per month to cover that 2,500 on average.

We do a residual WBC by flow, and we calculate percent recovery using a volume-to-volume calculation. I should also say that this represents about 15 percent of the total red blood cells issued in the Tampa Bay area.

The other population are RBCs collected by automated techniques using the Baxter Alyx. We average 400 donations or 800 units per month where we must ensure that a minimum of 25 donations or 50 units per site have a minimum hemoglobin

concentration of 50 gm/deciliter. Our residual WBC by flow includes 4 units QC per month per collection site.

Questions to consider. Do the current QC requirements offer a significant additional assurance on the quality of components prepared? Given the cost in testing and loss of components, as well as the lack of statistical significance of the current QC requirements, will we be able to use a more rational model?

Beyond QC. Each location where apheresis products are collected requires a separate license. Currently, the licensing of each site requires submission of platelet components to the FDA for each component type collected, that being single, double, and triple doses.

The collection systems that are licensed for the preparation and storage of components with defined expiration time must include this additional quality control.

Additionally, beyond QC, we are performing proficiency testing, and we are subscribers to the

CAP Survey TRC, the Transfusion Related Cell Count survey. The methodology offered or required to conduct this includes either manual counts using Nageotte Hemacytometers or automated flow cytometry.

There exists a dichotomy of these test methods, and the average of the last three surveys indicated 124 labs were performing Nageotte and 39 labs were performing flow.

In looking at that data over the last three surveys,, we see the number of participants using flow versus the number of participants using Nageotte. Each of the surveys seems to have a sample that approaches the cutoff of 5 to 6 million per unit, and we see that the mean residual WBCs in each of the samples of each of the three surveys, at 6.4, 4.1, 6.5 and 4.8, 6.1 and 4.2.

We also see, as a summary, those labs that in using flow, whether or not they determine that unit or that sample to be leukocyte reduced. You can see on the flow side, it is much more consistent in terms of coming out with the actual

value of whether or not that is leukocyte reduced or not, however, in the Nageotte side, it seems there is a real concern for the accuracy of the test results.

In fact, Dr. Jim AuBuchon commented in the last CAP survey, when the actual concentration falls near the distinction of leukoreduced versus not leukoreduced, the manual method appears far less likely to provide a definitive and reproducible answer.

So, the concern should be also not only are we using a statistical model that probably doesn't make a lot of sense, but we are also using a methodology that perhaps does not capture those units that are near the cutoff.

Is universal QC testing in a pooled configuration feasible? Well, we have already set precedents for pooled testing in the way we are doing NAT in 16-member pools. In our shop, we are also doing bacterial contamination detection in a pool for our whole blood-derived platelets.

Retrospective sampling data of our

residual RBCs in our platelet pheresis units over the last 6 months, we have tested a total of 1,555 components, which represents 1 percent of the components produced. In a number of units that failed QC, the n equals 15 or 1 percent failure rate, the failure rate being greater than 5 million WBCs per unit.

This graphic shows the distribution data of those 15 failed units. You can see the ranges from 5.1 million to 60 million over those 15 failed units.

This graphic depicts the fact that a vast majority of those samples that have failed WBC are in the neighborhood of greater than 7 million per unit, and, in fact, 10 of the 15 had greater than 8 million or two-thirds of those samples. One-third or 5 in 15 were in the range of 5 to 7 million.

We are looking at this pool procedure with these highlights. Our individual pool member volume is 1 mL per sample or per unit. Total members in the pool are 10. Our sample volume tested by the Becton Dickinson FACS Caliber Flow

Cytometry System is 100 microliters per sample using the reagent LeukoCount Test Kit.

Initially, we looked at the linearity of serodilutions, and Dr. Leparc thought it initially important to prove that the flow cytometry is, in fact, linear, so this data represents the number of WBC events, which is the operation of the reported value in flow over the dilutional phase of the method. You can see it is a straight line where the WBC events decreased proportionally as compared to the dilutional factor in 5 replicates per sample.

The tricky part about this has been to determine our cutoff calculation methodology and realizing that a certain number of WBC events in Region 2 of the flow test results would equate to the number of residual RBCs in the pool.

We developed this formula to determine the trigger value or cutoff that would create a suspect pool. So, the residual WBC cutoff is equal to the background count, recognizing that even those units that are successfully leukoreduced all contribute

some level or some number of WBC events in the flow plus the WBC cutoff.

Our residual WBC or residual background WBC count is equal to the mean of the WBC events on each of the 10 leukoreduced units.

We have determined that greater sensitivity can be achieved if we take 2 standard deviations from the mean of the WBC events and use that to calculate our background WBC, but the greater pool failure potentially climbs from our current 1 percent up to 5 percent of the pools failing.

This graphical representation describes for us that calculation. The numbers on the left side of the dark line represent those samples that passed QC, and they form a nice bell-shaped curve. Just looking at the mean of those units that pass, and moving forward to beyond the trigger point, would not be as sensitive as if we moved that line back 2 standard deviations from the mean and adjust the cutoff proportionally.

The numbers on this side of the graph

indicate those units that would fail QC. The goal here is to capture the background counts of the residual background WBCs by the number of WBC event to capture 95 percent of the units falling within that bell-shaped curve.

In a limited amount of data, the automated technology demonstrates a relatively lower number of residual WBCs as compared to filtration in the component lab. Therefore, we have not yet done so, but we determined that each of the categories of leukoreduced components will need their own parameters setup to define that trigger point above the number of WBC events that would possibly indicate a suspect member in that pool.

In conclusion, our preliminary data show that universal QC testing of leukodepleted blood components is feasible, however, we recognize that significant additional testing needs to be performed to determine the sensitivity and specificity rates.

We also feel, though, we need to look carefully at the effect of pooling ABO incompatible

samples in the red cell population and performance in comparison with statistical sampling that I think will soon be described.

I thank you for your time.

[Applause.]

DR. WILLIAMS: We have time for just a couple of questions. I will ask the first one.

On that distribution curve that you showed with the results from flow where a proportion were just above the 5 per million cutoff and then the larger bar above 8 per million, it sort of begs the question do you have any data where distributions like that were counted both by flow and by Nageotte?

MR. MALONE: No, we have not from Nageotte. That is all flow data.

DR. DUMONT: Larry Dumont from Gambro.

I was curious, if you are going to pool tenths of aliquots--did I understand that right?

MR. MALONE: Yes.

DR. DUMONT: And then let's just say that the cutoff is going to be 5 million for a

transfusion, so you could potentially have significant dilution of, say, a failed product with the other 9 units, and that is generally the way the failures go for these processes, so that means your cutoff is going to be in half a million range, 0.5 x 10 6, and then if you are going to put some windage on that for guard banding, it seems to me like you might be approaching or exceeding the limit of sensitivity or at least a claim for that assay, which is 1 cell per microliter, so do you have a comment on that, if you followed all that?

MR. MALONE: Well, we are looking carefully at the number of WBC events that equal that threshold of 5 million per unit. I have not looked at the threshold of the capability of the flow cytometer, but that is certainly something we need to consider.

AUDIENCE PARTICIPANT: Why do you do your testing at day 4? Is that because of pH?

MR. MALONE: Yes, and to provide a data that would indicate a worst case scenario yet trying not to outdate more components than we

already are. Yes, for the pH, we look at--we are not doing the WBC counts at day 4. They are done initially with our daily counts after we identify those that are to be reserved for monthly QC, but yes for pH.

AUDIENCE PARTICIPANT: So, actually, it is not the white cell QC that is causing you to outdate your components, it's the pH QC.

MR. MALONE: Correct.

AUDIENCE PARTICIPANT: Have you given any thoughts in a pool as to what HLA antibodies in one of your donors might do to the counts or what impact there might be?

MR. MALONE: No.

DR. BIANCO: Tim, help me with one thing. Even in the CAP survey or in your flow cytometry or Nageotte, what is gold standard? Which one is right? What is the influence that you have in your cytometry when you play with the gating that we all have to do in order to get a count?

You could go a little more this way, a little bit less this way. How do we know what

represents a number of white blood cells?

MR. MALONE: That is a good question. We see from the CAP data that it appears that the flow is much more capable of reproducible results than is Nageotte in determining whether they are leukocyte reduced or not.

We went on the trail where initially, we were doing Nageotte and the volume that we were performing was such that I couldn't get a tech over there to do that, looking at that many slides.

We then went to the imaging procedure, the microfluorometry procedure, yet soon after that, that was taken off the market, so we were left with the choice of either going back to Nageotte, which our volume of QC pretty much ruled out, and that is the reason why we went to flow cytometry, simply for the fact that we can load up 30 samples at a time onto the flow and perform QC on a larger number in a short amount of time.

AUDIENCE PARTICIPANT: By the way, the gold standard is flow.

DR. BIANCO: Why?

AUDIENCE PARTICIPANT: That is how they score the CAP surveys.

DR. WILLIAMS: Thanks, Tim.

In the next session, we actually have representatives from America's Blood Centers and the American Red Cross, who have very kindly summarized the responses for their representative members and regions to a survey that was put together jointly between America's Blood Centers and the Office of Blood Research and Review.

Some of the data collected are similar to the data that you saw earlier presented at one of the Blood Products Advisory Committee meetings, but I think one of the purposes of this workshop was really to update us and you as far as what current practices are and if anything, such as failure rates, have evolved over the past couple of years.

The first report will be by Dr. Dan Waxman representing America's Blood Centers.

Practical Aspects of Pre-Storage Leukoreduction in Blood Establishments

DR. WAXMAN: Thank you very much. As Alan

said, my name is Dr. Dan Waxman. I am the Chief Medical Officer of the Indiana Blood Center, and I am Chair of the Scientific Medical and Technical Committee of America's Blood Centers.

Like Ed Snyder before me, I need to disclose that I do serve on the Medical Advisory Board for the Pall Corporation and recently have agreed to join the Business Advisory Board for Gambro BCT.

Also, I want to thank Alan for shedding his jacket this afternoon. Through a series of events yesterday, I got separated from my hanging bag due to two canceled flights in Indiana, then, I switched airlines and sat on the tarmac for an hour and a half in 85 degree heat with the airline with that plane's engine shut down, so finally, by the time I got here three hours late last night, my garment bag was nowhere to be found.

I can tell you that this shirt and tie combo you can get at the gift shop at the Grand Hyatt Hotel, which is conveniently located on the Red Line at the metro stop as I headed out to

Gaithersburg to the Holiday Inn.

[Laughter.]

So, without further ado, America's Blood Centers is the largest network of community-based blood centers in North America. Seventy-seven blood centers operate more than 600 collection sites in 45 U.S. states and Canada, providing half of the United States blood supply and all of the Canadian volunteer blood supply.

These blood centers serve more than 180 million people and provide blood products and services to more than 4,200 hospitals and healthcare facilities across North America.

The FDA had asked ABC to conduct a survey among its members to determine the status of leukoreduction and unfortunately, with the time being short and the scope of the survey, we were able to receive at least 50 of our centers' responses, which accounts for 67 percent of our collections.

More responses have been received, but we have not yet time to include them in this analysis,

and this survey was conducted in the last two weeks, and I want to thank Celso Bianco and members of the ABC staff for really grinding out for the last number of days and hours to get what we have here today.

The FDA provided us with a set of questions for us to get the answers. The first question was to assess the current proportion of leukocyte reduced components.

As you can see in this graph here, the data was from our initial 50 respondents and it was from the time period of 6 months, so between January and June of 2005, and this is for units distributed.

This is different from manufactured by a certain number of our centers are actually importing cells also. From this chart you can see that 64 percent of the red blood cells from whole blood distributed by respondents are leukoreduced, 94 percent of those RBCs collected by apheresis are leukoreduced, as 100 percent of the apheresis platelets are leukoreduced, and then finally, only

14 percent of platelets made from whole blood collection are leukoreduced.

Now, we did not ask members what percentage of platelets might end up being leukoreduced at the bedside from random donor products, but I think that would be some survey we would have to do actually with our area hospitals.

The second question related to trends in leukoreduction, is it going up, staying the same, or going down. From this graph here, you can see in 2001 and 2002, we did not perform a formal survey. We had actually asked members what they thought the projections would be for those years in leukoreduction, and then we actually, in 2003 and 2004, did not have any type of survey. Then, the numbers are here for what we have just done in 2005.

As you can see, the upper curve is for apheresis platelets, and all of those are 100 percent of the apheresis platelets are leukoreduced, and given the new technology with the various equipment we have, they are coming off

leukoreduced.

For red cells, however, the increase is less evident. Currently, 64 percent of red cells that are being distributed are leukoreduced.

While all ABC members provide apheresis platelets that are leukoreduced, this is not true for red blood cells. A number of centers provide less than 50 percent of the red blood cells as leukoreduced products while others, about half of our sample, distribute mostly leukoreduced blood products.

Among the 50 responders, 19 distributed less than 50 percent of their red blood cells leukoreduced. Now, this probably reflects the fact that among our ABC members, the choice to go toward leukoreduction has been determined by the local blood center and the hospitals that they serve, and we have found that in those areas that many of the transfusion medicine physicians wanted to be selective in how they use leukoreduced products and really gear them towards more of the hematology/oncology patients, while others prefer

due to either choice or by inventory, to go mostly leukoreduced.

I have been at the Indiana Blood Center for the last 8 years, and I have watched as our center has started to transcend through these different groups. Eight years ago, we were right in there between zero and 24 percent. A couple of years ago we were there.

This year, my blood center is leukoreducing around 60 to 65 percent, but one of my largest hospital customers is going to convert, and that will put us into this range of probably up to 80 percent or more.

At that time, we need to determine at 80 percent, is it better just as far as efficiency and inventory to just move to 100 percent leukoreduce.

The next two questions deal with white blood cell counting methods. As discussed in previous talks today, really in terms of the automated type of counters, there is some limitation in that, and the good old Nageotte, what we use in Indiana, in this group, there was only 49

of the 50 respondents, and if we go back to a survey that ABC did in 2001, 44 percent of our members back in 2001 were using automated cell counting methods.

In this survey, we are up to 57 percent.

With respect to QC units, the FDA requested data on QC failures. Now, in this group, we have subsets of the units manufactured, so this is from our groups that are manufactured.

Over 1 million units of red blood cells leukoreduced, nearly 20,000 units were QC'd at a QC rate of 1.84 percent. For apheresis platelets, 13,343 units were of the 2,200 and 47,411 manufactured QC at a rate of 5.39 percent.

You can see for both of these categories, it is less than a 1 percent QC failure. One of the thoughts I have in terms of sometimes the higher QC rate on platelets is whether or not some of this is being triggered by machine flags.

On our apheresis equipment, they will tell us if there is what they think is a white blood cell spillover, and you might actually be QCing

some of those, so I didn't know on some of the other speakers, if sometimes some of the QC rate on platelets has something to do with that.

The FDA also wanted to know if QC and filtration failures were being investigated for cause. Now, when we look at this, and what we wanted to do is just look at only, in this subset, filtration failures, so, you know, 5,683 filtration failures, as you can see, there was no cause identified in 65 percent of them, but we were able to identify in 35 percent a cause, and like previously, I think it was the Canadian data, we could show that 81 percent were due to clogs and clots.

Now, there is some overlap here because people, you know, would stop due to excessive time, well, a certain percentage of those are due to clogs and clots. Also, as previous data showed, about a third of the ones found were due to sickle cell trait, and then we had such things as mechanical priming kinks and other.

The FDA wanted to determine if the use of

automated collection mixers have an impact on filtration failures. On preliminary data analysis, we found no correlation between the use of shakers and filtration failures.

As stated at the onset, this data is preliminary and incomplete and more analysis is needed prior to its use to determine if there is any type of relationship. I know, just as hopefully, there are more cell counters available. At least in the U.S. now, we are seeing a lot more shakers available in various different styles and price.

So, for a center like ours that never considered them due to price, we are now able to find a type of shaker that we could put throughout our system and see how it works. I know other centers were talking about just implementing shakers as a way for a number of issues, but we hope as we go through the data and further analysis, to see if there is any correlation.

Thank you.

[Applause.]

DR. WAXMAN: Any questions, thoughts, places to shop?

MR. ZIA: I am Majid Zia with Hemerus Medical.

Do you see any seasonal variance between QC failures?

DR. WAXMAN: Any what now?

MR. ZIA: Like seasonal.

DR. WAXMAN: Oh, seasonal variance. We didn't ask, I mean we just took this snapshot from 6 months. I remember there were, I don't know if it was the hemolysis that was having seasonal variation or what, but we just asked them for a 6-month segment, and we did not ask them if they saw any seasonable.

Harvey?

DR. KLEIN: Dan, was the 81 percent unknown, was that uninvestigated unknown or was that investigated unknown?

DR. WAXMAN: Sixty-five percent. I think it was they investigated and they could not find a reason.

Thank you.

DR. WILLIAMS: Thanks very much, Dan, and also many thanks to Celso Bianco and also I suspect Jane Starkey who put a lot of work into putting the survey questionnaire form together, and I am sure in providing the analysis, as well. We are very grateful for that.

Similarly, the American Red Cross agreed to conduct the same survey, and presenting Red Cross data is Fred Walker.

DR. WALKER: Thank you for asking us to come.

I would like to summarize. This is all data that we took from the first six months of this calendar year, and we were not able to actually--you will see there are some discrepancies, because we couldn't answer every piece of data, because we didn't collect all this data ahead of time.

But the first is just what we produced in the last year. In the first six months, we produced over 3 million red cells. Of those, 94

percent were leukoreduced. You can see that we also have a similar amount of automated red cells. We only did about 140,000 in the first six months.

This is actually where most of our non-leukoreduced red cells are currently being produced. Our platelet apheresis are 100 percent leukoreduced, and we made about 430,000 random platelets. Of those, 80 percent were leukoreduced.

The distribution numbers look pretty much the same, but let me just make a couple of comments about this. With respect to the random platelets, we have customers who prefer to have non-leukoreduced random platelets. That is one point. The second point is that only 17 of our 35 regions product random platelets.

So, the 8 percent or so of the non-leukoreduced really comes from just maybe 1 or 2 regions and supplying their customers. The bulk of our customers prefer the leukoreduced or random platelet.

The second point that I would just like to mention, the distribution of automated red cells.

We will probably in the next year be making 100 percent leukoreduced for customers that are looking for non-leukoreduced, we will probably switch them to whole blood collections.

This is the trend of our distribution of red cells or leukoreduced red cells since the year 2000. We started in the year 2000. What is when we started a big push to increase the number of leukoreduced red cells in our system. It kind of peaked out at the very most of about 98 percent in 2002, and it stayed fairly constant through this year at about 95 percent or so.

Again, there are some pockets within the Red Cross System where the use of non-leukoreduced red cells is less. There is at least one region that distributes maybe only 70 percent leukoreduced, but we are not seeing any particular trend away from leukoreduced.

Just again to clarify some of the pieces of information in the first two slides, for randoms, regions make one or the other. We don't have mixed inventories for random platelets, and

there is somewhat lower demand at a very few places.

White cell counting, the Red Cross only has one method we used, and that is in the jet chamber. We have been evaluating other methodology and we haven't made a decision to go to those. Truthfully, it's around cost basis and how can we keep it cost neutral and still do all the QC that we need to do.

This is our QC data. Let me kind of explain. We probably did more QC than here. This would be our minimum guess. We don't have good data on the exact number, but we did at least this much. The white cell failure is a fairly solid number, so the percent failure point of 0.2 percent would be on the high side. It probably is less since we probably did more QC.

In our QC, the main reason we see failures is due to red cell recovery, and this is more of I would say an artifact to how we actually do the technique. It is very manual, there is lot of measurements made, all of which are air prone,

leading to I believe errors in determination.

We use a wide variety of filters for the leukoreduction of our products from the major manufacturers. We used Red Cell Inlines from Pall, Whole Blood filters from both Pall and Baxter. We use Sterile Dock filters from Pall and Baxter, and we use the Pall filter for leukoreduction of randoms.

One of the questions we were asked is about hemolysis. We identified in the first six months about 2,900 units that were hemolyzed, and kind of the definition is if someone looks at it and says it is hemolyzed, then, we take their word for it.

It is not something that happens equally throughout the system. There are pockets in our system that have a lot of hemolysis and it goes away after a few weeks. Then, it will show up someplace else.

Some of the risk factors that we have observed for hemolysis are things that others have observed. Of course, age of the unit. In places

where we have had a lot of problems, we have noticed there is definitely a relationship between the collection day and the leukoreduction day.

Temperature of transport has come into it, and there is certainly other factors that have been identified, shear factors because people don't break the cannula have been identified, and almost for every one of these cases, they are multifactorial and fairly hard to sort out.

We asked about adverse events. What I put up here was every adverse event that we had that was associated with a red cell even though most of these also had parts of other components associated with them, and we had a total of 20 adverse events from 3. some-odd million red cells we have distributed.

Failure to filter is another area that everyone talks about quite a bit. Our system, we weren't in a short time able to break it down into the actual reasons, but I thought it was interesting to look at, the different types of filters, because we have observed that failure to

filter is often highly related to how it is used.

You will see that our whole blood and sterile dock failure to filter are relatively on the low side, and the in line red cell has the highest failure rate.

So, in summary, about 95 percent of our red cells are leukoreduced. There doesn't seem to be a trend downward although we do have pockets where people are just sold that they don't need them. All apheresis are leukoreduced. By the way, apheresis comprise 70 percent of our distributions.

Lastly, the issues that we have with leukoreduction of red cells, the highest problem is red cell recovery from the QC, failure to filter followed by white cell failure, followed by hemolysis.

That's it. Thank you.

[Applause.]

DR. WAXMAN: With the advent of your centralized manufacturing facilities, will you have your product QC based at those facilities, or will they be at your NTLs, or have you thought of how

you might do that?

DR. WALKER: We have definitely thought of it. We are still trying to figure out how we are going to do the centralized part. Let me say we have looked at a lot of different models from having just a few QC places, you know, where we do vast numbers. We are going to have 11 centralized sites, doing those there. We really haven't made a decision what is the best idea.

Certainly, we have also discussed moving it our five NTLs to see if that makes sense. There is a lot of logistic and timing issues that come into this, that kind of compound the complexity.

DR. WAXMAN: Then, if you actually centralize it, would you switch from using Nageotte counting to flow cytometry?

DR. WALKER: We would definitely automate it if we had more volume, yes.

DR. SNYDER: So, there are a number of centers or hospitals around the country that leukoreduce red cells, but not platelets, is that fair?

DR. WALKER: There are a number. It is a small number of hospitals that use, that prefer to either leukoreduce the platelets themselves or transfuses them non-leukoreduced, I don't know for sure which.

DR. SNYDER: And the rationale for that is?

DR. WALKER: You would have to call and ask them.

DR. SAYERS: There seems to be at every turn, there is another reason to defer donors. I was looking at your filter failures. There was 15,000 filter failures. Was that for the six-month period?

DR. WALKER: The number is, yes, for the six months.

DR. SAYERS: Are those individuals followed to see if they are repeat failures?

DR. WALKER: No.

Thanks.

DR. WILLIAMS: Thanks, Dr. Walker.

FDA Current Considerations: Pre-Storage

Leukoreduction: Process Validation, Quality Assurance an Monitoring, Processing, Testing and Licensure

DR. WILLIAMS: As is usually the case for workshops, FDA is primarily here to listen and learn, and we are doing that, but I think for the sake of discussion we wanted to share just a few baseline current considerations with respect to the leukoreduction process and validation, quality control, and so forth.

This won't have a lot of detail and I think that is for fairly obvious reasons, but we are splitting the talk into two. I am going to give some of the parameters and then Sharyn Orton will follow by giving a description of some of the potential ways for doing statistical process control including a new technique developed, in fact, at CBER called "Scan Statistics."

Earlier today, I gave a summary of prior draft guidance issued by the agency in January of 2001 and the 1996 Memo prior to that. The product standard introduced in the January 2001 guidance

was 1 x 10 6, and I mentioned the comments that came back with respect to that cutoff particularly since much of the counting for residual white cells in the country is done manually.

Current considerations are that in response to these comments, that we are considering keep the 5 x 10 6 residual white cell per component standard, and proportion to that for single donor platelets 8.3 x 10 5, so when those are pooled, they would meet the 5 x 10 6 standard as well for the pooled product, also 85 percent retention rate for the starting red cell volume or platelet yield as far as retention of therapeutic component.

A lot of thought has gone into elements of process validation--Dr. Orton has been close to a lot of this--elements of process validation that we would plan on defining in future guidance would be installation qualification based on the user manual for the machine, validation protocol development per the existing FDA guideline, which is the Guideline on General Principles of Process Validation. I don't have the link here, but it is

available on the web.

Operator performance qualification, product performance qualification on a statistical basis, and the guidance would propose options that would be acceptable to FDA for a statistical basis, and presumably, those would be associated with a reporting category of annual report or CB30, whereas, a process that you wish to use at a center, that would need complete review would most likely be a prior approval supplement.

Process validation would also include investigation of component qualification failure and requalification as needed, really nothing earthshaking there.

In terms of quality assurance and monitoring, development of standard operating procedures, and then the quality assurance program for component manufacturing, again consideration is that this could be statistically based and again options acceptable to FDA will be proposed as part of guidance and other options will also be considered, but most likely as prior approvals.

The current consideration is that process failures need to be considered carefully and distinguished from non-process failures, and it is really those that come under the definition of process failure that would perform part of the statistical assessment.

There needs to be use of logical process control points. The question earlier actually could be broadly considered as a lumping versus splitting consideration - does your quality control scheme encompass your entire representative sample of your entire production, so that it does represent each protocol that is used, or do you, in fact, do specific QC targeted to a different procedure and look at that individually.

Also, could samples be collected serially or collected randomly. There are arguments for and against each of these combinations, and it often depends just which is most compatible with an efficient process within the manufacturing facility.

There is a consideration that levels of QC

for non-automated or manual leukocyte procedures might be worth having more rigorous given that they are manual procedures, they do inherently have a somewhat higher failure rate and we are considering a differential between manual and automated procedures and perhaps considering as much as 100 percent QC on manual procedures.

That is one of the purposes of today, to just get at the practical implications of some of the things that are being considered.

The quality assurance and a monitoring plan will have a QA plan for equipment and supplies, as well as operator competency, investigation resolution of process failures, and regular quality system audits.

A few other comments related to the leukoreduction procedure and some of them which are specific to the January 2001 draft guidance. FDA is considering a recommendation for use of FDA-cleared mechanical mixing devices during collection to reduce filter clogging and subsequent loss of products.

There are several devices now cleared for this use. FDA, not recommendation as much as encourages the development of more efficient validated methods for residual white cell enumeration.

Admittedly, the pooling method just being looked at initially it perhaps has some promise, perhaps needs more work, but its innovation in terms of looking for more efficient ways to count samples which, for the most part, will be at very low level, but when they fail, would fail and leave a lot of residual white cells that could be picked up by methods such as that.

As mentioned earlier, if binomial distribution is used for statistical process control, it is used as a pass/fail method of samples that are under 5 million versus samples that are over 5 million without actually doing the enumeration.

Movement toward automated technology, I think it has been somewhat slow to occur, but I think that will itself form a more efficient

pathway toward providing QC once more and more technology evolves and the cost becomes compatible with availability.

There was discussion both at an earlier Blood Products Advisory Committee meeting and in comments to the January 2001 guidance that hemoglobin S testing of donors, while feasible at some sites, is unlikely to be feasible at all sites, and, in fact, has certain ethical concerns associated with it.

That said, some sites find that they can do it quite reasonably and bill that into part of their process, so that donors with sickle cell trait, in fact, do not go on to--the collections from those donors don't go on to leukoreduction.

The current consideration is that FDA, the Blood Products Advisory Committee recommendation to the FDA is quite reasonable, and that while sickle cell trait testing of donors may be useful for defining the process, that it probably would not be specifically recommended.

There is the recognition that donors who

fail the leukoreduction process at one level or another tend to do this in more than one occasion when they return for donation, and there is consideration of a recommendation for diversion of donors for other types of collections. One, units do not filter successfully on two occasions, so that subsequent attempts to donate by that individual would not be lost.

Acceptance of the comment was made that a dual inventory representing 100 percent QC of products destined for CMV-susceptible patients was probably not practical given the separation between the component laboratory and the transfusion service in most instances.

So, that recommendation in the draft 2001 guidance most likely will not remain in future guidance although, as I said, we are considering that in some cases, that high level or perhaps even 100 percent quality control is indicated either for manual procedures and/or targeted populations if a feasible method can be found.

Future guidance will also include helpful

hints and description of what is involved with the registration and licensure process, or what types of supplements are appropriate for prior approval, supplement versus CBE-30 and annual report if an establishment chooses to follow an FDA acceptable procedure, chances are that level of application would be in the realm of a CBE-30 or an annual report and save some time.

Comparability protocols. A licenseholder submits a protocol reviewed as a PAS and then provides a basis for similar procedures to be put into place at other sites under the same license with a lower level--not a lower level--but a CBE-30 review versus a prior approval supplement also is an efficient way to bring other sites on line under the same protocol, and some practical guidelines on how to submit license supplements.

So, I think we need to look at today's workshop as really just a review of some of the key aspects that have been permeating the discussion with respect to quality control and some of the practical considerations.

Today's workshop discussions will be carefully considered and we urge a good discussion on each of these areas.

Guidance with respect to leukoreduction, we will reissue as draft guidance because it will be changed considerably from the January 2001, although it is often not wise to put timelines on issuance of guidance for a lot of different reasons, but I would say the current target would be sometime the latter part of this year.

At this point, I am going to turn the floor over to Dr. Orton, who is going to say a little bit more about some of the statistical process control options.

DR. ORTON: I am going to talk about quality control testing or leukocyte reduced blood components.

You have heard us allude to the statistically sound sampling plans, and, in fact, in the regulations 211.160(b) for Laboratory Controls states that, "Laboratory controls shall include the establishment of scientifically sound

and appropriate specifications, standards, sampling plans, and test procedures designed to assure that components, drug product containers, closures, in-process materials, labeling, and drug products conform to appropriate standards of identity, strength, quality, and purity."

The current recommendation, which is in the 1996 Memorandum, is that QC "be performed using a sampling plan that includes 1 percent of monthly production, 4 per month for establishments producing less than 400 units."

Alan has gone over the acceptance criteria. That criteria, we don't anticipate to change.

The labeling of leukocyte reduced components is, of course, based on the criteria that was noted above.

The question has been asked, Should FDA continue with the same monthly testing paradigm to monitor for nonconformance? We believe it is appropriate to consider other scientifically and statistically sound QC plans.

One example of this is the use of scan statistics, and that is what I am going to talk about today.

I would like to state that Dr. Epstein has worked with Dr. Tony Lachenbruch in our Office of Biostatistics and Epidemiology very extensively on these plans, and Alan and I became involved with it. For those of you who have heard my presentation on what is under consideration for the platelet pheresis guidance, we also have consideration scan statistics for that QC, as well.

For nonconformance, nonconformance rates are generally expected to be low, and it is estimated that it is less than 10^{-2} for manual procedures and less than 10^{-3} for automated procedures. I think some of the data that has been shown today supports that, in fact, it is true. Failures, in fact, may be clustered.

Now, the power to detect nonconformance as we know is going to be lower for very small sample sizes, and so we want to pick a sample size where we can get at this nonconformance adequately.

Some biological variables, usually donor related, cannot be controlled by current technology, and we have discussed hemoglobin S-related failures.

So, one statistical method, as I said, under consideration is the use of scan statistics, and scan statistics assess events that cluster in time and space, or are non-random. We can use a rolling window of test results for this nonconformance assessment.

We calculate the number of test failures required to trigger an investigation of an unacceptable level of nonconformance. To do this, we use the following considerations: an estimated nonconformance rate. Now, for automated methods, as I have said, it is about 0.1 percent.

We want a greater than or equal to 80 percent power to detect a failure rate of 5 percent, and that 5 percent failure rate then is, in fact, a delta of 50 compared to what we considered the acceptable nonconformance rate or the no nonconformance rate, and an acceptable false

positive rate.

By that, I mean that we will, in fact, in the course of doing our QC, determine that it appears from our QC that we have nonconformance when, in fact, that is not correct, that we do have conformance.

We need a total percentage of collections to be tested, and in this case, for scan statistics, we use 10 percent of total collections.

QC monitoring is done on a rolling basis. All failures should be evaluated and corrected for attributable cases, so non-process control failures are not counted.

So, in the example of platelet pheresis, if the donor has a reaction while they are on the machine, and that was the collection you had targeted for QC, you wouldn't include that. The machine alarms for other reasons.

If you have someone who has hemoglobin S, these things that have attributable causes are not counted in the QC as failures.

I am going to give you an example on how

this works. Let's say that 24,000 platelet pheresis are collected per year at your blood center. So, based on our 10 percent, you are going to test approximately 2,400. We are using random selection from total collections, and for this example, calculations use a window of 120 tests. This is done actually by a statistical program, and I will give you some examples on sample sizes at the end.

It turns out that you can have 3 failures in this 120 test window that would trigger an investigation of an unacceptable level of nonconformance. Now, keeping in mind if you know in advance you have a failure and you know in advance you can detect what a problem may have been, these get removed out of what contributes to that trigger.

With this particular sample size, the false positive rate would be 4 percent.

So, for this example, let's say you perform 10 QC tests on any given day. As long as you have less than 3 failures within this 120-test

window, the level of nonconformance for the process is considered to be acceptable.7

After 120 tests are complete, the window "rolls" forward and the next 120 tests now include the testing of the samples from days 2 through 12, and a new set of 10 samples, those that are going to be tested on the 13th day.

So, here is an example. Here is your first 120 tests. Over the course of 12 days, as I said for this example, 10 tests per day are done. On day 4, you have a failure, on day 9, you have a failure. At the end of 120 tests, you have 2 failures. That does not hit the 3 trigger, so this particular window is considered to have an acceptable level of nonconformance, and now you move up a day.

So, for your second 120 tests, the first 10 are not counted, days 2 through 12 are, and now you have a 13th day. On that 13th day, if you have a failure, you now have reached your target of 3, and that failure should trigger a full investigation.

In the event this QC failure, this trigger is reached, a complete failure investigation should be initiated.

Now, one of the things that Alan has alluded to is, for instance, failures can be caused because the whole blood isn't being mixed properly during collection because you don't have an automatic mixer.

We have talked about some donor-related failures. We all know that there are some device-related failures, but there are a few other things that I think are very important to stress when you are doing these investigations, and that is, whatever your methodology is for counting your cells when you are doing QC, it is not uncommon in the course of our reviews to find that that is a major part that people do not investigate when they are seeing an increase in QC failures.

They look only at the device, and they even have the manufacturer come in. They can't figure out what is wrong, the device is running properly. This is really an entire system and all

the pieces need to be investigated.

Because we are allowing the removal of things that have what we call "attributable cause," you really should be down at this point, needing to talk to the manufacturer that something is perhaps wrong with the device, because we have eliminated donor-attributable causes and things like that.

Corrective action and follow-up should be performed when you have reached this trigger, and if resolved, the QC should be re-initiated, and the count of tests restarts as a zero or starts again at day 1, so you don't have to keep recounting, moving that window and counting your previous failures.

if it is not resolved, it may be that revalidation needs to be performed.

Now, this is an example of the sample sizes. The N here represents 10 percent of collections, so for a QC of 400 or 600 tests, it turns out that the window that you would look at is 60 tests, you would have a trigger at 2 failures. This gives you a false positive rate of 2 and 3

percent, and greater than 80 percent power to detect this nonconformance that we consider not acceptable.

When you get into the larger sample sizes, 1,200 through 4,800, those test windows are 120 tests long. The 3 failures is considered the trigger, and you can see the false positive rates remain below 5 percent and the power remains above 90 percent.

If you would like references, I have stuck them on here. There is a book on scan statistics that is done by Springer Publishing. Dr. Lachenbruch, Foulkes, Williams, and Epstein have a published article that is about the potential use of scan statistics in the quality control of blood products that I think you would find very useful.

Thank you.

[Applause.]

DR. WILLIAMS: I think I would like to just add that any recommendations that FDA does put out which incorporate statistical process control, I think we would probably include numerous examples

as appendices, so hopefully, we can get as close as possible to a plug and play system for most centers that would be converting to this, and then for sites that have more sophistication, would be developing own systems, you would have the freedom to do that, as well.

DR. ORTON: Yes, for the platelet pheresis guidance, we did include a lot of detail on scan statistics as an example. We didn't give a lot of other examples, but it very clearly states that if you submit a statistical plan to us that is sound, we will evaluate it.

It turns out that for the platelet apheresis, unless you already have a comparability protocol, those are all PAS submissions, as well, so they would be included and we would certainly entertain any statistical plan submitted.

DR. WILLIAMS: I will deal with the informational piece. Please let attendees know that copies of the Nathens and Waxman presentations are on the front desk. I imagine they will also be posted on the web within a couple days of the

workshop, so you can get access to PowerPoints for all the slides.

We are scheduled for a break, but I think it would probably best use of our time if we just had the questions for the afternoon speakers so far, so if we could ask the speakers to come up to the front and then we will have the break and come back with the panel consideration of the two questions that we have put forward to them.

Questions for Afternoon Speakers

DR. KATZ: I am Louis Katz, Mississippi Valley Regional Blood Center.

Sharyn, the question that I get most often when I talk to people in blood centers and laboratories is what do you guys do to investigate a quality control failure. Is it the intent of the FDA to specify some level of minimum content for a failure investigation, or is everybody going to be left up to their best devices?

DR. ORTON: One of the things, I can only speak for what is under consideration in the platelet pheresis guidance, and Alan and I have

tried to set the leukoreduction considerations up very similarly is to give you give some guidance.

Now, we don't say you need to look at this many real specifically, but we do give the various topics that are areas within an entire system that we think need to be looked at, particularly based on the QC failures that we see when we are doing review and we ask what people do for investigations and find large, large pieces missing.

So, I think we have a fairly exhaustive list of things that should be considered for those failure investigations.

Does that answer your question?

DR. BIANCO: Sharyn, I think you are going to be on the spot with all the questions now. It is very interesting, your approach, but I want to follow the previous question and then I have another one.

I want to follow because it makes it very reasonable when you remove all the failures for cause that you have identified, so you are looking really at the background of what is happening in

your system.

But that is when also what I see with this survey and other things, that is when very often you don't identify a cause, so what do you do. You do a re-validation. Yes, you call the manufacturers, you do a re-validation, you recount things, there essentially, you are going to restart without knowing what the cause was, because there are many factors that we really don't control.

DR. ORTON: I agree. Alan just wrote exactly what I was thinking is how hard people actually look for what the cause is, and, in fact, like I said, I do a tremendous amount of review, so when we get some of these reviews in, and you see a trend or you see a problem, and I call and say, well, did you look at this, never thought of it.

So, I am not sure that when I see 65 percent that couldn't find the cause, I am not sure how good a job they did looking for starters, and the second piece is, if you notice like with the scan statistics, we expect a 0.1 percent failure rate. We are allowing a 5 percent failure rate. I

mean that is a 50-fold increase. I mean that really is fairly large.

So, I don't know that if people using the guidance or the guidelines that we are trying to give them do a more thorough job in their investigations. I think perhaps you will attribute a lot more causes, and with those removed, then, you end up with quite a few failures.

DR. BIANCO: Maybe what we are asking you is since you have seen so many applications and so many explanations of failures, if you could either in the guidance or collecting some way where you post on the website that collection of issues that came up, that would be an experience that would be helpful to all of us.

DR. ORTON: That is why, like I said, in the guidance, what is under consideration for both, there is a fairly exhaustive list of things. I never thought about looking at whether my cell counter QC was drifting or not, things like that.

DR. BIANCO: The next question is I am not a regulator, I have read the regs, but not as good

about it, but when you say 100 percent QC, for me that is a release test. We don't have a licensed test for release of units on the basis of cell counting, so how do you marry those issues?

DR. ORTON: I think like you do your platelet yield on every pheresis collection. I mean those cell counters are not cleared for release testing of platelet pheresis. You use a cell counter to essentially do that at collection to get a yield. In fact, there is a regulation that says that you need to do your yields. We consider this in some ways a yield.

So, perhaps 100 percent QC, may be a better way would be, what I think I call it in the platelet guidance is really a daily specification, what is the yield of residual white counts in the component if you were to do all of them. I am only the messenger.

MS. WILKINSON: Susan Wilkinson. Sharyn and Alan, just a follow-up to Celso's question. On Alan's No. 4 slide, your bullet point, 100 percent for non-automated manual leukocyte reduction

procedures, which obviously is the vast, vast majority of what we are doing, I mean you were talking in the millions of units, I think, that is, if I am understanding your definition of manual correctly.

DR. ORTON: You are. I have done this, I understand the difficulty, and we have a lot of discussion and this is what is under consideration. As you know, as Alan said, it is draft. We spent a lot of time thinking both logistically, scientifically, clinically, but again, as Jay and Alan have pointed out, which is why we are here today, so we don't take any requests for you to even consider 100 percent specification testing, we don't take that as something very lightly.

Steve.

DR. KLEINMAN: What I am trying to get my arms around is thinking what the increase in QC staff would have to be to go, let's say, to 10 percent or 100 percent depending on the product. The extra cost that that would entail, would there be some good that would come out of that with

respect to patient health, and if not, I understand the desire to have a very tightly manufactured regulated process where you would be able to identify failures, but you also have to look at the good that comes out of it.

It is very difficult for me to see how the increased work and cost would result in appropriate patient safety.

DR. ORTON: One of the things I would have to say, at least for some of the things we have talked about, whether it be scan statistics of using the binomial, is that they are, in fact, statistically sound.

So, I understand there is an increase and what contribution is that going to make. I guess I have a concern that if we have arbitrarily chosen numbers for QC, what is that telling us. It may, in fact, not be telling us what we need to know.

DR. KLEINMAN: I meant from a clinical point of view with respect to patients. Lots of times you can show that things are different statistically, but when you get into the practice

of medicine, you figure that that statistical difference wasn't very meaningful from a patient perspective.

I am just thinking about the people who get the products, whether the increased cost would be justified.

DR. WILLIAMS: I think two responses, Steve.

First of all, I think it raises the question that I introduced earlier and we are going to hear a little more about in the next session, that is, how do you work with this dichotomous concept that you need rigid, high-quality QC for a product that is going to a patient who really needs the leukoreduced unit or it could become a safety issue versus the high-volume, high throughput that a lot of blood centers are currently doing where rigorous QC may, in fact, not be feasible.

I guess the second concern is there is a tendency to think of all of these processes in the current paradigm, that if you do 100 percent QC with Nageotte chambers, you would have to probably

quadruple your staff.

That is certainly a logistics problem. On the other hand, there are technological advances possible that could stimulate better technical solutions when there is a problem to be solved, and I think that's one direction that potentially the field could take if rigorous QC was, in fact, put forward as a recommendation.

DR. BIANCO: I think, Alan, that the question that Steve is asking you is the amount of effort that is going to be invested and the clinical benefit that comes from it.

Sometimes I have the feeling that we are treating a very biological system of which we have very little control even over the counting methods. I know flow cytometry is very nice, but if you played with that gating, you can get double, you can get half, you can get whatever you want.

There are standards, but they are relative standards, and all the clinical studies that have been done, even the CMV studies, were all done with bedside filters that we know didn't work as well as

the filters as we do them pre-storage today.

Sometimes I feel, and I am just giving you a feeling, that I hope you will take into consideration, that we want to treat leukoreduction in the same way that we treat the serological tests, for which is something that we have a clear marker, we have to set up clear cutoffs. If you miss somebody that is positive for hepatitis B, you don't want to miss that person.

Very differently with leukoreduction where if came down to 1 million or 5 million, we don't know really the difference between 5 million and 1 million, and as we even heard, we affirmed today with tons of data that are there, that still we have complications like GVHD and chimerism that we don't know what it means, that they are not being prevented by what we are doing.

So, I am hoping that we look at it more as a biological system. We are trying to do the best. Those are all population studies, we are trying to do the best for these patients that we think really need it.

In many communities, it was possible to go to 100 percent leukoreduction, it makes the system simpler to deal with. Even if, as Ed Snyder says, it is cheaper, it may be cheaper in Connecticut, but it is not anywhere else.

Even if more expensive, we are being able to do something, but I am thinking about the others that are still at a level that is different if we have to bring them to migrate with us and to the levels of burden that the additional QC and all that will bring particularly if they move, if this is not going to be inhibitory to the migration of people from the low levels of leukoreduction you mentioned in your talk to more leukoreduction.

DR. ORTON: Merlyn.

DR. SAYERS: Sharyn, you can say that you are just the messenger. Well, I am just a questioner. Against that background, if your proposal sees the light of day, then, I think at our shop we would be tempted, I mean reluctantly, but we would be tempted to start that complete failure investigation at failure one, the reason

being that you might well lose valuable time if you must sit back waiting for two and three to fall within the 120 days.

So, we are really going to need some pretty good guidance to understand what the nature of that complete failure investigation is going to be, and as diligent as you might be on your part, I suspect that that guidance is going to be incomplete, bearing in mind that, you know, Alan, you conceded that many of the failures for reasons that are poorly understood or have yet to be identified.

DR. ORTON: I think part of the point was at failure one you investigate, that that really is the point. Just from my own blood center experience, we had a situation with a particular component person, so right upfront, before we had to worry about failed QC overall, if you investigate thoroughly upfront, you can fix problems as they come along instead of, you are right, waiting until you have three failures.

I can't in a document, like I said, say

you need to do this this many times, but I really do believe that a lot of thought has gone into these with consideration that will give you good guidelines on the kind of things you need to look at in your system, and that is the best that I can tell you at this point.

DR. WILLIAMS: Dr. Kleinman.

DR. KLEINMAN: Steve Kleinman, AABB.

I wanted to ask you about the automated procedure. You talked about random sampling. I am assuming that the current requirements for different product types and collection sites would then go away, is that correct or not correct?

Secondly, do you really mean random or do you mean representative?

DR. ORTON: The proposal with the scan statistics and the proposal for the statistically sound sampling plan is for collections, it is not by machine type, by product type, by site at all. That has been taken out of the mix.

As far as random, what we are trying to get at, and my biggest experience is with platelet

pheresis, is when I say random, you are not selectively picking anything. For example, you don't wait until you have your at-collection count to determine if that is the one you are going to QC.

So, we are trying to get--you know, some places are doing the first four from the machine that they need in a given month, so when I say "random," I am not talking about using a random number generator to do it, but to really have it non-selective.

DR. KLEINMAN: Yes, so I suggest maybe the word "random" could be thought about and maybe a more precise term could be chosen.

DR. ORTON: Jerry, your hand has been up for a while.

DR. HOLMBERG: When you mentioned about looking at your entire process, it raised a question concerning what the international community looks at as far as quality control. I know that all of us have gone through the exercise last holiday season, December, with the hemolysis.

Do you plan to put anything in there about hemolysis? The European guideline is 4 units per month to determine hemolysis.

DR. WILLIAMS: I would say there have certainly been discussions in that area. I am not sure, you know, our current considerations are focused enough to address that here, but we are aware of that situation and sort of the absence of objective methods to define hemolysis, so we are considering that carefully, but not to the point of addressing it as a current consideration that might end up as a recommendation.

AUDIENCE PARTICIPANT: One of my problems is that we should be doing random, and the thing that we won't do is random when we do red cell leukoreduction. It is taken out of the normal production because you have to weigh sample and go through that whole process.

Is there any consideration to just going for a minimum red cell content of the final product? That would probably improve our process control in my opinion.

DR. WILLIAMS: I think that is a good point and, you know, as was raised earlier, defining the therapeutic content of a unit, or else labeling the unit as far as what the content is specifically, has value.

I think there are a lot of things to consider before going in that direction, and I think what you are suggesting would probably be dependent on some ratio to what the therapeutic content was and ended up in the final product.

Again, I think there is interest in that area, there has been discussion, but there needs to be a lot more.

DR. ORTON: I don't know where to go. Lou.

DR. KATZ: I can't resist. I don't want to ask a question of the panel, I want to ask a question of Ed Snyder. Is that okay?

There is two questions here obviously. There is the desirability of more leukoreduction, and I am probably pretty convinced and it is obvious that is, so there is a good thing that we

can do and that Ed has identified three indications that we all agree upon that provide clinical benefit. All the rest of it is extraordinarily controversial, remains controversial after many, many good people have attempted to answer the question, so I suspect we will not answer it.

Ed then shows us some really beautiful data over a long period of time that shows pretty clearly he is accomplishing the major goal, and I think all of us who transfuse patients recognize that alloimmunization is much less frequent than it was prior to the TRAP study blah-blah-blah.

So, Ed, let's say that we give you that 100 percent leukoreduction is a good thing, so Question 1 is answered, and we can all go home after you answer this question. Are the quality control parameters that Sharyn is talking about useful to you after your experience with doing things the way we do them now?

DR. SNYDER: Yes, I think it's useful. We don't do the actual processing. That is done by our blood center, so easy for me to be cavalier and

say, you know, do whatever it takes, yes, we will pay for it, right. Well, we always pay for it eventually anyway.

DR. BIANCO: So, you pass the buck, right, Ed?

DR. SNYDER: My feeling is that as Joe Bobee [ph] often says, you know, do the right thing and whatever it takes, if the feeling is--often what happens is the FDA puts out some very, very high bar waiting to see if we twitch and if we jump, they turn the voltage up.

You guys aren't supposed to listen. They waiting to see if we twitch. If we really feel that testing is too excessive in some area, I think we should be quite vocal and quite uniform and pointed in our combined response to say that we feel a more appropriate result would be blah-blah, and then they will go back and then a miracle will happen.

So, I really think that the level to which quality is a very ethereal type of a concept, so whatever makes Celso happy makes me happy.

DR. ORTON: Ed, would you want your father to get a leukoreduced unit that hadn't been counted?

DR. SNYDER: No.

DR. ORTON: The gentleman behind Lou Katz.

DR. STROMBERG: Bob Stromberg. Quality control, of course, is only as good as the testing, so Dr. Waxman, Dr. Walker, you both indicated that you are using the jet chambers, I think Red Cross exclusively, and, Dr. Waxman, I think you said you used to a smaller extent a flow chamber and then the jet chambers.

Do you have quality control of the people doing the quality control sampling?

[Pause.]

DR. STROMBERG: Did you understand my question?

DR. WAXMAN: I guess I can speak for our center. We have a very defined training program set out with procedures and policies, and we do annual competency assessments on our staff, and then we do a variety of proficiency testing that we

follow to see how our methods are doing.

So, I would say in terms of my center, and I don't know, Celso, whether we have asked the question, but I know that at least the centers I have been involved in, we have training programs and annual competencies to see how the staff do perform.

DR. ORTON: I can say from the review standpoint we get very, very detailed procedures on sampling and testing for QC, and if they are not very detailed and thorough, we usually remind them they need to be detailed and thorough. So, they do go to as much trouble to sample properly and run those cell counts as they do anything else.

DR. WALKER: I would say that in the Red Cross, everything is true. In addition, we have our quality organization on almost a daily basis comes around and audits the QC Department.

AUDIENCE PARTICIPANT: When they audit you, do they actually count the samples, and does somebody else count the samples?

DR. WALKER: Probably not, but they do

audit against following the procedures.

DR. MENITOVE: Jay Menitove. I am just curious, is there very good, good, or any alignment--I assume there is alignment--between what the manufacturers of the devices are held to and what you are attempting to hold us to, because if the devices only get to a 95 percent confidence interval 95 percent of the time, then, how can we do better than that?

DR. ORTON: Dr. Epstein will answer that question.

DR. EPSTEIN: Dr. Vostal [ph] can probably answer it better than I can, but one of the paradoxes here is that the failure rate in the hands of the manufacturers is extraordinarily low. It is orders of magnitude lower than the experience reported by the user community, which is why we think that most of the problem has to do with the larger process, not a failure of the filter itself.

That is why we are focused on process validation and quality control monitoring of the process, because there is, as you are suggesting, a

very large disconnect in those two measures. It is true both at the level of frequency of failure and it is true looking at the distribution of the expected residual white counts.

In the hands of the manufacturers, the expected residual levels are often like 5 x 10

4.

You are not worrying about 5 x 10

6, and yet we know that that is not the experience in the field.

So, it has to do with process conditions and the quality assurance of the entire process. That is why there has been this sort of, if you will, shifting of burdens, because the user community has said to us forever and a day why are we worrying about this if we know the filters are validated and work.

But the answer is that the use of them is not robust, it depends very critically on the conditions of use, and that is why you need a quality control and quality assurance program at the user level.

So, I think that is the answer. As far as exact numbers, I would have to defer to others.

DR. ORTON: If you are referring to a procedure where you have to have 100 percent compliance, is that what you are talking about?

DR. MENITOVE: I am just trying to see if the arithmetic lines up. I agree with what Jay is saying completely, but the problem is there are small numbers that are used to have devices I assume approved, and so the data they are supplying are going to be I am sure very well done, but when you broaden it out to the larger perspective, and I guess that is why we are having this discussion because we want to see how it works in the field, but there is a precision, I assume, in the instrumentation that gets you only so far, and I just hope we are not pushing that precision farther than the field can take it to, because then we are going to be in a loop that we will never get out of.

The other thing, Sharyn, is I think your answer to Merlyn's question is very sage, and that is, investigate after the first failure, because we don't collect 24,000 a year, we are about half of

that, and if you do the arithmetic on that, then, one failure puts you in the nonconformance loop--well, one and a half, which really means one if we are going to be on top of it.

I think the standards you are setting are extremely high, going to be very difficult for us to do, and I am concerned about the precision of the devices in terms of the scale-up, can we really do it, because you have seen the data and you know where the data are among us when we submit it, and then where you expect us to be.

I think we should be as good as we possibly can, i don't disagree with this one bit. I just hope we can get there.

MS. SYLVESTER: Ruth Sylvester with the American Blood Centers. I understand the FDA's desire to put in controls to help us improve our processes and the quality, safety, purity of the products coming out.

I didn't understand, though, on the current considerations on Alan's last talk, where he says diversion of donors whose units do not

filter successfully on two donations. Why would that be under the purview of the FDA to recommend that we defer these donors? Wouldn't that just be an economic hardship and then it would be just for the centers to make that decision? I don't understand why that would be in a guidance document.

DR. WILLIAMS: To answer at least partially, it is unlikely there would be any recommendation to defer the donors. It would be just to not use collections from those donors in the future for leukoreduction processes.

MS. SYLVESTER: But that is still an economic decision, it's not a scientific decision on the safety, purity, potency. It is just that it is not filtering properly, so you would be wasting perhaps a unit or a filter. I don' understand why that would be in an FDA guidance document.

DR. WILLIAMS: I think in each of these biological processes, you have a consideration for what the specificity is of a process. I mean if you are looking at IVDs, you need to meet a certain

level of specificity so as to not needlessly collect product that doesn't end up being used.

Where the authority comes from, I think probably that ultimately would need to be looked at more thoroughly by the legal side of the agency, but I think there is precedent for not only considering the safety aspects of policy, but also the preservation of a resource.

Larry.

DR. DUMONT: This is a point of clarification. I think I understood you to say, but I am not sure, that you were going to consider, say, in your scan approach, that you would consider everything in a blood center, all the products coming out as one lump, and not differentiate between different processes or different machines?

DR. ORTON: If you have all the different apheresis machines to collect platelets with, all those platelet collections go into the QC together. If you have multiple red cell devices, those apheresis red cells all--right now the QC for red cells is all in a pool. It is per collection site,

but it is 50 no matter what you draw it on, so what we have done is we are eliminating the per-machine type, per-product type, per site type of stratification. It's your collections.

DR. DUMONT: So, that would also apply in apheresis settings to things like doubles, triples, and singles. You are just considering the unit that you issue.

DR. ORTON: The collection.

DR. DUMONT: So, in fact, if that stands, then, there may be a reduction in the QC burden.

DR. ORTON: In fact, at several of the presentations I have given previously, when people have first said 10 percent, and then went back, and actually Florida Blood Services was one, that went back and looked at the fact that right now for platelets, they were QCing I think it was 18 or 20-something percent.

So, in fact, for them, it is going to be less and it made more sense for collection. So, certainly, it is an example of a way to do it that might work for a facility, and if not, if they have

another statistically sound plan that would work better for them, we would consider that, as well.

DR. DUMONT: So, then, for example, an investigation of a failure on what do I do next, may be then directed more specific at the process that failed, and not all the platelets or all the red cells or something, it might be very specific to a piece of equipment.

DR. ORTON: Exactly. I mean if you took it off of this Device No. A, and it was at this facility, and these are the people that counted it, you look very specifically at that piece.

DR. WILLIAMS: I think up at the other microphone, there is a question for the other folks at the table.

MS. JETT: Betsy Jett at NIH.

In my experience in the lab with small numbers of failures, it is very difficult to find the root cause unless it is something really obvious. I have had lots of experiences where we just ripped the place apart, retrain everyone because we are trying to figure out what happened.

If you can assign cause 50 percent of the time, I think that is pretty darn good, but what I have found is that after we have like ripped the place apart trying to figure out what happened, changing all the reagents, and doing all sorts of stuff, we find out that oh, there has been a lot of places that had the same problem and we didn't know about it. It was a device manufacturer issue, not ours.

So, one thing I would like to see is some impetus for the device manufacturers to share their complaint files with us, so that we have a sense of is it us or is it everybody.

DR. ORTON: I think at the end, when I was talking about kind of a follow-up, with what we have proposed, looking at the different part of the entire system, by the time you get down with scan statistics to that trigger, as I said, you are at a point where perhaps it is call the manufacturer because you really have looked at everything else.

As far as them sharing their files with you, I don't believe I have any say in that matter,

but clearly, that is something that you should consider.

When I looked at everything that I could think of, again, I have many, many years of experience actually developing root cause platforms, so I agree. A lot of times--not a lot of times--you may not always find it, but once you put together really good protocols on what to follow through, it is really quite extraordinary how many things you do, in fact, find from my experience.

MS. JETT: One of the difficulties is a lot of your evidence is gone by the time--well, we send our testing out, so when we get our answers back, the filter has been thrown away, the sample is not there. The tech forgets, you know, if there was anything unusual.

So, I am just saying that the evidence often isn't there in this scenario, and if we are at the point where we need to do a root cause analysis based on one or two or three events, and we don't find a cause, are you going to provide

some recommendations for that in terms of what if we don't know what happened and we are starting over with our QC, is that a problem with the FDA that we said we tried, but carry on?

DR. ORTON: Jay is going to respond to that.

DR. EPSTEIN: I think that there is a perspective that is being missed here, which is the assumption of the zero failure rate. The idea is that we understand that most filtration failure is unexplained, but it leads to a certain expected rate of failure, and the statistical test that is being put forward is looking for a significant deviation from an expected rate.

So, if what you are left with is the baseline rate of unexpected failure, it should just not exceed the norm. So, in fact, the burden is not to figure out more than, say, 35 percent.

Also, this concept came about because at the BPAC, when we reviewed this, and I think there were data from the Red Cross, there was about a 1 percent failure rate, but if you backed it out for

simply failure to filter due to clots, the intrinsic failure rate, if you controlled for clot formation, dropped about a log.

So, what we are really saying is we are looking for systematic deviations from an expected baseline rate. So, it is less important to focus on the fact that we can't explain all failures than to get a consensus view of what is the right expected rate of failure given the current state of the art, and then that is the baseline against which one does a test for deviation.

And it is a loose test, mind you. Again, the example that was put up, which is only an example, was a test to look for a 50-fold higher rate of failure than that which was expected.

Now, those numbers were back of the envelope numbers, you know, the 0.1 percent versus 5 percent, but we think that they somewhat conformed to reality, but the debate should really focus on whether that is the right set of baseline expectations.

DR. BIANCO: Thank you for putting it into

that perspective. The only thing that I would like to say yes, and we are looking for that deviation, but then the burden comes with the investigation, how extensive it is going to be, how many resources, what is the burden of the investigation.

That is where we have to limit also as part of the knowledge that we know that we are not going to identify the cause in many situations, and that we are going to restart the process. That is why I asked a few minutes ago, that is, what should we do when we come to a point--and I am looking forward to your list--when we come to a point where we say we tried, and we didn't find anything, that is, we just restart and what is the FDA inspector going to say when they come here and look at my books.

DR. ORTON: I believe if you have a thorough description of your plan and you follow through with it, and it includes involvement with the manufacturer, and you follow through with that, and that is well documented, the inspectors aren't going to hit you for something.

What we found is generally, the inspectors are concerned when the plans are clearly not thorough and, you know, the QC didn't work, so we retrained the person is not a thorough investigation, and I have to tell you we see that a lot.

So, I think you are going to be surprised. I mean I have done this personally, and does it take some time? Yes, once you get it under your belt, you really--you know, who said, Ed, doing the right thing.

I mean doing a thorough investigation really it works, and I think if people look at this list and develop a thorough plan, I think you are going to find it is not that difficult. Call me, I will help you.

DR. WILLIAMS: What I think we will do is take a couple more questions, particularly folks who haven't spoken yet, and then we will take our coffee/cookie break. When we come back, we have asked some senior people in the field to sit here as a panel and give an approximately 5-minute

digestion of a couple of key questions related to what we are talking about, as well as any other observations that they want to make.

I think it will be kind of a focus continuation of the discussion that we are having here.

MR. SIVAN: Yariv Sivan, United Pharma.

I think that there is an aspect here that sometimes we are not perceiving in terms of sometimes we can overmanage processes, and they can bring to risk the safety and the compliance and the standardization, because we will spend so much time trying to regulate ourselves that we won't be available to do the business at hand.

Sometimes there are systems in place, for example, I don't know if it is a good example, but the hemovigilance of material, vigilance system that is available in Europe, that collates information from blood centers under a regulatory body, to have that body look at a good sampling of data rather than have the overall burden on each and every blood center to a great degree, and let

them do their job appropriately.

The second comment is that sometimes you have to accept a certain level of nonconformity, that we are, you know, so long as you are proving you are doing the right thing and the right job, and you are filling all the requirements, and so on, that you will have an imperfect world. That is not at 95 percent confidence or the 95 percent of the cases most of the time.

DR. ORTON: Steve.

DR. KLEINMAN: Just a follow-up to the comment about sometimes the problem is at the manufacturing end, and not at the end of the operator. I think we have all had that experience, and what I would suggest is maybe if FDA can't do anything about this, maybe an organization like ABC should have people call their complaints into the Central Office, so that there could be a way to check and find out, well, somebody else actually has had that problem with that same piece of equipment, because in my operational experience, the manufacturer doesn't suspect itself until there

are many, many calls, and most of the times people in the field suspect the manufacturer before the manufacturer does.

So, I think it is up to the blood centers to try to set up a mechanism by which to gather that data quickly and be able to go to the manufacturer with a convincing case.

DR. ORTON: Steve, when I said that I didn't think there was anything we could really do about the manufacturers sharing their information, and Gill Conley is here today from the Office of Compliance, not infrequently when there has been a fairly nationwide begin to a problem, the call has come in to us, and we have followed through, so certainly at any point in time, if you think you have a problem, you can certainly either call my office or Gill in the Office of Compliance, and we will investigate it.

Just as far as if they have information, sharing it, there is nothing we can really do about that.

MR. CONLEY: If I could just chime in,

also, remember there is a voluntary reporting system through Med Watch, so if you have a low frequency, but something that you think should be reported, those reports do eventually reach us, and we compile them, in addition to if you have a most notably then and you make a couple of phone calls, we do follow up immediately anything that put a patient or donor safety at risk, we would follow up immediately.

For those lower incidence issues, Med Watch reporting is available to you.

DR. WILLIAMS: Thanks, Gill.

Last question, Deborah Dumont.

MS. DUMONT: Two questions actually. The first question is regarding scan stats, and if you reach your trigger for QC failures of 3 or more, then, between the time of investigation and finding a root cause of the failures, would you have to do 100 percent QC?

If so, if you have to do 100 percent QC, could you include that as your number for the next scan? That is the first question.

The second question. With the advent of wonderful leukoreduction guidance, which includes guiding us towards good process control and quality monitoring, what is this going to do to bedside leukoreduction?

DR. ORTON: As far as the scan statistics go, we didn't get into the level of detail if you reach your trigger, do you do 100 percent QC. I mean clearly within your own facility and depending on your medical director's input and things like that, there is only so far I thought we could legally guide you certainly.

If you decide you want to do 100 percent QC at that point, because you were very concerned that certainly if it's in your SOP, it would be up to you, but clearly when QC starts again for your scan, it should start fresh.

So, I think that if you are saying, well, I did 100 percent QC, what part of that can I count, you wouldn't count any of that. You would need to get to resolution to start again.

DR. WILLIAMS: Let me see if I understand

the second question. It was if the agency issues recommendations that really define a very tight process, will it exert negative pressure on leukoreduction as a whole, so it happens less frequently.

I think, you know, that is something that FDA has been quite clear about, that as Dr. Epstein mentioned in his opening, the fact that there has been support for white cell removal in blood components for transfusion for a long time, and it is expected that will be continued.

So, I think that would be a major factor of balancing any standards or process recommendations that are put in place to try to make sure that it doesn't diminish the overall proportion of leukoreduction particularly since in many cases, it appears to be that rather than economic pressures or other pressures, that it is simply easier to maintain 100 percent inventory.

If that is, in fact, a true trend, I think FDA would probably take the position that it's a good one and we will want to put factors in place

that would reverse that.

AUDIENCE PARTICIPANT: Just one more quick question, and maybe Dr. Epstein is related to this, are clots considered to be a failure in this process, in things in which the filter is actually stopped, because those are 100 percent checked right now, aren't they?

DR. ORTON: That is not considered a QC failure. So, again, I have pulled this red cell for QC, it doesn't filter, that gets taken out of the mix of the failures that go towards a trigger. Actually, in the documents under consideration right now, we do give examples of the things that would be removed as we consider non-process, they are not related to the filter.

AUDIENCE PARTICIPANT: But clots might be considered a failure of another manufacturing process, which should be duly investigated.

DR. ORTON: Yes, again, it goes back to investigating everything that doesn't work, but as far as what goes into your trigger, all your attributable causes are removed from that.

DR. WILLIAMS: Let's break and return at 3:35 or thereabouts and we will have the panel.

[Break.]

Panel Discussion

DR. WILLIAMS: It is getting a little harder to get everybody back in the room as the day goes on, but we are going to go ahead and get started.

I think this should, in fact, be a very interesting portion of the program. What we have done is assembled a panel of six individuals who were already registered for the workshop, but whom we recognized as not only being senior and well experienced in the field, but having some unique perspective to some of the issues that we have been discussing.

What we have done is supplied to them in advance two questions related to the subject of the workshop, and asked them each to give about a 3- to 5-minute response to the questions, and because I think Question No. 1 on universal leukoreduction has been worked pretty well so far, I would also

invite them to comment on any other perspectives that they took away from the discussions today.

The panel members are: Dr. Harvey Klein from Department of Transfusion Medicine, NIH; Dr. Dana Devine, who heads the R&D program, Indian Blood Services; Dr. Celso Bianco with ABC, America's Blood Centers; Dr. Michael Busch, Blood Centers of the Pacific and BCI; Dr. Larry Dumont, and Dr. Gary Moroff with American Red Cross.

The questions being posed are:

1. All things considered, is there evidence to demonstrate that leukoreduction of relevant blood components for all recipients would advance overall public health?

We will do this, I think consider the questions together.

2. Please comment on the best manufacturing process control strategy or strategies to, one, meet the needs of targeted patient subpopulations whose safety may depend on a leukoreduced component, as well as, two, provide reasonable process control efficiency for larger,

non-targeted recipient populations.

I am not providing any further structure to this. I think if you want to just go down the line or speak up when you have something further to say, go ahead, but I would like to turn it over to the panel just to provide their answers to these questions and any other perspectives that they would like to introduce.

Following this panel, we will then move right into the discussion of prion removal by filtration. Dr. Luisa Gregori is here from the University of Maryland, and Dr. Jerry Ortolano from Pall Corporation, and that will be the home stretch for the workshop.

So, whoever want to start off, feel free.

DR. BIANCO: I will start with a protest. I preferred it when you called us luminaries instead of senior.

I think it would be easier if we start with the one question and we all talk a little bit about it, and then go to the second one that is much more specific, much more technical.

What I want to do is just in one minute iterate one position that we have had at ABC and that you saw reflected in the practices that are there in the slides that Dan Waxman presented.

It has been not an issue of public health, it has been an issue of medical practice, and the policies have derived from the interaction between the blood centers, the physicians at the blood centers, and the communities that they served.

Every attempt among our centers to just impose leukoreduction in a community had a backlash. It was a gradual thing that happened more and more at different centers as practices changed, as hospitals realized that there were some more benefits that they could rearrange their systems like, for instance, Sharyn did, and Ed presented it very well. They did the right calculations and they felt that they could afford it, and those systems moved gradually there.

I still don't see it as a problem of public health. I see it as an issue of medical practice, and I see that the data that I heard

today shows in terms of populations and all that, there is still an issue of medical practice.

It was very easy to decide that there was a great benefit to reduce febrile reactions, a great benefit to patients with targeted diseases, and for the other patients, it goes both ways.

I would like to put this as a position to start the discussion.

DR. KLEIN: I couldn't disagree with you more. I don't think it's an issue of medical practice at all, and I will speak from the standpoint of a transfusion service director of a small hospital. This is not the NIH position, because NIH has no position on this issue.

But as a clinical hematologist, I can tell you that one really can't in real time identify people, and on a regular basis, who require leukoreduced components. We think we can, but then in every study that has ever been done, if you look at the individuals who are excluded from the study, there are individuals who have already been transfused, by and large, if you are looking at

leukoreduction for alloimmunization or leukoreduction for CMV, there are individuals who have been transfused before coming to the tertiary care center that is conducting the trial.

Why is that? The reason is that the diagnosis has not been made in real time. We, as physicians, simply can't diagnose the patient fast enough, by and large, who needs leukoreduced blood, and so many people who could benefit from that are not getting leukoreduced blood unless their system is providing all leukoreduced blood.

I think the issue of medical practice has been put up there as a straw man, simply because of the cost to transfusion services of adding leukoreduction, and the issue that was political at one point of whether they would have free will in terms of getting a component that they want or component that was being forced upon them by a regional blood center.

Now, in the 30-something years that I have been practicing hematology, I have never heard anyone ask for leukoreplete blood. If someone else

had, I would be delighted to hear about that. By and large, the only way that you will be able to treat many of the patients who will benefit form leukoreduced blood is to have a leukoreduced system.

If I could just transition a little bit into the second question, I think the data on who benefits in the three categories that we heard earlier from Dr. Snyder, are without any question valid. There are clearly a lot of other benefits, I think. Nobody will deny, I think, that other cell-associated viruses will be reduced. Some of those are important, some are only important to a small subset of patients, but those, too, will be reduced although we will never have randomized controlled trials to demonstrate that.

So, I believe that we shouldn't set aside a certain group of patients and say we need very carefully controlled leukoreduced components for this subset, but for all of these other individuals, many of whom will end up in this subset in two weeks, two years, or ten years, we

can be a little bit more sloppy with them and we don't really care if we know how many white cells.

Now, I am not going to tell you I know the exact number of white cells, and I am not going to tell you that I know the statistical approach that we ought to take, but I believe we should take a statistically valid, practical approach to quality control and have one standard for leukoreduced components.

DR. MOROFF: I am not a physician, but increasingly, I have been convinced or I am becoming convinced that there should be universal leukoreduction. This is my own viewpoint.

Over the last two, three years, there have been an increasing number of publications, and Dr. Davenport summarized a lot of them today, and my interpretation is that there are real trends to benefits for leukoreduction, and I agree with what Harvey Klein just said, that you can't pick out the patient, from the way I understand it, who would benefit.

So, I really agree with what Ed Snyder

said this morning. I would love to replay what he said this morning in terms of the reasons why he has utilized leukoreduction for a number of years at Yale.

So, that is my response to the first question.

DR. DEVINE: Maybe I will jump in. I am the token Canadian on the panel, and Alan carefully placed me here between Harvey and Celso, because Canadians are the peacekeepers of the world. I haven't brought my light blue helmet.

Perhaps as the Canadian who is here, I could offer just a little bit of perspective and history on this. To address the first question, from the perspective of the Canadian regulatory agency, our FDA equivalent, which is called Health Canada, they did view leukoreduction as a public health issue.

I think that there was no single driver that took the country toward universal leukoreduction. The blood operator in Canada made the elected decision to leukoreduce all of the

random donor or the whole blood derived platelets and we were fully leukoreduced for platelet inventory by February of 1998.

Although the blood operator had been discussing leukoreduction for red cells, we were trumped by the regulatory agency who came along and said thou shalt implement universal leukoreduction for all bicellular products.

We have been, in Canada, fully universally leukoreduced since July of 1999. So, we are now six years into this process. I would say that it has evolved from a public health issue, which it was six years ago, to one of medical practice, and also to a related issue around the standard of care.

We heard earlier from one of the manufacturers about the situation in Europe where the vast majority of products that are provided for European patients are, in fact, leukoreduced products, and obviously, all of the Canadian products are, as well.

It is interesting to me to observe that

this is an unusual set of circumstances where the standard of practice is actually not being led by the U.S., and this is very unusual to me. As a Canadian, we are always looking over the border saying what are the Americans doing and following suit. So, it is fascinating to see that the tables are turned a bit on this particular issue.

DR. DUMONT: First of all, under the full disclosure, if you don't know, I work for Gambro, so in case there is any concern about bias, there it is. They bought my plane ticket here.

I think we had a great review of the data this morning and I was especially impressed by the Yale data, and, of course, the data there confirms other data, recent data that has been reported. There was a "before and after" study out of Edmonton that showed similar effects.

I think there is a very strong argument to say that this is a useful thing just for that one indication, not to mention the others. I actually just did a review of this a couple of weeks ago, and I think some of these effects that we are

looking at are small and they may be very specific to certain indications, but to be smart enough to parse that for every patient in a large blood supply, personally, to me, seems like a huge task, and that there is a good indication for universal leukoreduction.

Now, a lot of times I do statistics, and statisticians, usually they look at a problem backwards and inside-out when they ask their questions and develop their hypothesis, and along the line of Dr. Klein, if we were sitting here saying we should add white cells to our red cell transfusions, or we should give platelet transfusions that have all these added cytokines to them, you know, what would be our answer and what would be our burden of proof and efficacy for those things?

So, I kind of leave that to you to think about, but I think there is a real indication to go for it.

DR. BUSCH: I actually voted against universal leukoreduction, I guess it was about five

or six years ago at the ACBSA meeting. Having worked in some focus studies to try to evaluate whether it was beneficial, and, you know, well-powered studies and not seeing an effect, but these were, you know, some of these more esoteric effects.

Scientifically, I am disturbed that there is not more definitive data on benefit of leukoreduction, but looking at the data today en masse, and just seeing these studies emerge over the last few years, I think there is enough evidence for benefit certainly in terms of febrile reactions and the benefit to the patients who suffer those reactions, the consequent alloimmunization.

I do think the point that the rest of the world, the developed world, really has moved forward with universal leukoreduction, the overall mix of data to me does support that the U.S. should move to universal.

DR. BIANCO: I don't want you to take my statement and particularly Harvey that I am against

it. I think that it is a positive process. The reason why I left it to medical practice is because pragmatically, I don't see how, except by fiat or FDA, and at the same time of HHS providing the adequate reimbursement, I don't see how it is going to happen. It is happening, but it is not going to happen overnight.

Maybe it is becoming more and more of a standard of practice. There will be no legal pressures here, but as I mentioned before, I think that leukoreduction is very different from all the infectious diseases that we dealt with. We don't know, there is no marker on the patient to say if the patient got leukoreduced or non-leukoreduced product. There is no specific disease associated with it.

Alloimmunization happens even with leukoreduced product. I think it will happen, but I don't think that we are at a stage where the scientific information is such that it should be mandated, as for me the question implies.

DR. MOROFF: I want to add something to

what you are saying. I think these studies, the way I understand them, are very difficult to conduct. There are all sorts of factors. I think there is all the confounding factors.

I have always felt that if some of these factors could be dissected with large enough studies, the studies would show a much greater benefit. It is really hard to say why there isn't more effects. My feeling is it is because of the confounding factors that go into these studies.

DR. BIANCO: One more thing to the point that Larry raised, adding back leukocytes. I think that we don't ask that question, Larry, only the statisticians ask them, is because that is what we have been doing for the past 50 years of transfusion is transfusion with leukocytes.

So, we have an experience, we see some bad effects that have happened particularly before we did at least partial leukoreduction for specific patients, and those patients in the vast majority survive without the specific event. So, there is no break there. With our experience, a lot of

what we do with blood is good despite the fact that is has leukocytes.

Yes, there has been a natural progression. We started with the whole blood and then we started transfusing only specific components. That makes sense as technology allows us to do it, but again, I think we are going to see with the second question, that the burdens are, as we try to make this a very specific and very well characterized component, that creates a sequence of additional events that we have to deal with.

That is why I want us to tread carefully in that direction.

DR. KLEIN: I would like to make one other point if I can, and that is the issue of errors. We know that errors never occur in hospitals. We read again and again that for a patient who is ordered a specific medication, because you know that is what medication that patient needs, that patient receives the wrong medication or the wrong dose.

So, even when we have identified people

who should get, whether it is leukoreduced or some other component, there is a great risk, in my experience, and my guess is that probably most of the treating physicians in the room, that they may, in fact, get something they aren't supposed to get. We have seen that with autologous blood, and you see it with leukoreduced blood.

One of the additional benefits of universal leukoreduction, pre-storage leukoreduction, is it will do away with bedside filtration, and I think that is a very, very, very good thing for a lot of reasons, one of which is that when you find the bedside filter that you have issued with your unit of blood on top of the refrigerator in the nurses' station where the unit was transfused, it probably hasn't benefited that patient at all, or when you go to the outpatient oncology clinic and find that the well-trained expert oncology nurse is pushing blood through the leukoreduction filter with a syringe, so that the patient can go home earlier, it probably hasn't benefited the patient either.

So, I think a lot of the issues of errors, mistakes, would simply be eliminated by having a relatively quality controlled component that you issue, and you take that out of the hands of the areas where errors can be made.

DR. BIANCO: The ABO mismatched bloods will still be transfused, Harvey.

DR. KLEIN: We are not going to cure everything here.

DR. MOROFF: In terms of the second question, if we can move on to that, I think there can only be one standard for leukoreduction, otherwise, you would need two inventories of blood, and I don't think two inventories of blood would work either at blood centers or at hospitals. It just complicates things and makes the situation--I like to use the word impossible.

So I think there should be one standard for leukoreduction for all products that are leukoreduced.

DR. DUMONT: Along the line of number of inventories, the question that was first posed to

us was the dichotomy, and I think actually the major dichotomy I see is the bedside filtration that Dr. Klein just brought up, that there is still a fair number of large institutions that do that.

So, if you come with a rational, sound, rigorous guideline or rule even for pre-storage leukoreduction, and it doesn't apply to bedside, then, what kind of dichotomy do we have in the clinical setting. So, that is a concern.

DR. DEVINE: I guess I will just share with you a little bit of our six years of QC experience for better or worse. We use a flow cytometry method in Canada and have right from the beginning, so we don't have enough technologists in the country to do Nageotte counting on all of our QC for blood products, and you probably don't either.

The issues that I think are important to consider about this is really what you are trying to achieve with your process control. The issue for us in Canada, someone had showed some of the data that we shared at a BPAC meeting a few years

ago, and we find that the process control around the red cell leukoreduction is actually very good.

We do close to as well as the filter manufacturers do, so this sort of 99.9 percent of the products really having the white cells removed is our experience over the last six years. Where we run into difficulty more often is with the leukoreduction or platelet products.

Canada has basically the exact opposite numbers of the U.S., and that about 75 percent of the transfusions we give in the country are coming from whole blood-derived platelets, so obviously, we are doing a lot of leukoreduction, in line filtration using the Pall System on our whole blood-derived material.

We have been finding that that product is where we are having a filter failure rate or at least an increased residual white cell rate in the product above what we would expect to have, and it's a place where we have been starting to really look very carefully at the why.

One of the things that we have done, and I

am not sure how this fits into what Sharyn was describing for the new guidelines about where you sample and how often you sample, but each one of our blood centers does a sort of standard type of QC along the lines of what you do down here. It's 1 percent or 10 per month.

What we have found is as we have tracked this QC data over the last 6 years, we have sites for which if you sort of were to think about if you are still within meeting the standard, but how far are you, what is your actual white cell count, and you sort of were to take that distance and chop it up into tenths, we find that a number of our centers are sitting down around the 10 percentile.

So, they are doing very well, they have always done very well for 6 years now, and we continue to have no problems. However, we have two or three of our manufacturing sites that started out for 2 or 3 years right down at the baseline, everything was great, and then you started to see a little bit of fluctuating noise.

So, I would make a very strong argument

that one of the things you really need to keep an eye on is you need to keep an eye on trend analysis, so you have to look at the real numbers, don't just look at what percentage of your products are passing, you know, you standard, which is way up here, because this is where we started to see our problems and our drift.

We think it relates mostly to the training of the operators, which was something someone else had raised earlier today, but that has been a real take-home lesson for us, and if we had paid attention to that sooner, we would not have the problems in one of our centers that we have had over the last year.

So, I would sort of throw that at least small voice of experience into the mix. As far as how much of what you test, we are, in Canada, in the process of changing over a manufacturing method from the PRP method for whole blood-derived platelet production to the European buffy coat method.

As we have been undertaking that task, we

have had to sit down and rethink the whole QC piece, what are we trying to measure, what are we trying to accomplish, and once we had done a fairly careful critical control point analysis on buffy coat manufacturing, we started to address again what do we want to be doing about our QC.

So, we have proposed to our regulator that we will be implementing primarily the European standards around residual white cells, so we are headed toward the 1 x 106 per unit of pooled platelet product.

We are also in the process right now of discussing 100 percent QC testing to that standard, and I don't know whether we are going to go there or not, but that is an active discussion that we are having.

DR. BIANCO: How do you analyze your trends?

DR. DEVINE: Well, at the moment, we are trying to understand what the best statistical method is for doing that, but the eyeball analysis alone is very informative.

DR. DUMONT: Just a comment on trends. I know that England has implemented this approach now for several years, and, of course, it is a little easier for them from the standpoint of having a centralized transfusion service, but they have a group that monitors that and trends it, does the training, and as they have implemented this and learned over the years, they have been able to identify counting methods that have been out of whack, and they have been able to fix those or eliminate them.

They have identified training issues, of course, and they have been able to fix those. They have identified certain devices that weren't meeting their expectations or that were not stable, and they fixed those by throwing them out of country, so, you know, it works, and when I started worrying about white cells a while ago, since I have aged now, is that what you called me--senior, right--nobody asked me or told me that I was looking well today, but when I started worrying about this, most of the field had no clue what they

were doing.

They weren't counting white cells, and if they were, they were doing it in a way that wasn't telling them anything. So, over the years here, a decade or so, we have made a lot of progress, because people are using pretty good analytical techniques. They are actually looking at their processes, they are actually responding to them, so there has been a lot of progress.

So, that is a comment on that. You might expect I had a lot of comments on processes, but one of the questions was should we go to 100 percent QC, and in some situations, I think that is manageable, but in total, right now, my personal view is we don't have the right analytical method. We don't have a low complexity test that is low cost that we can go out and test every single one of our units.

I mean it would be great if we did, because then all this process QC stuff would kind of take a second seat. We would still have to look at trends, but we wouldn't have to worry about

sample sizes, and et cetera, et cetera.

But I don't think we are there yet. I am not sure that a guideline or a regulation is going to force invention on that either, because a lot of pretty good people have looked at that over the years, and the technology is what we have, and I think it is because that's the only thing that can be paid for, but I could be wrong on that.

So, I think the main thing with looking at our processes is to make sure that they are stable over time, and there is 100 ways to cut the pie. Sharyn has shown one way this afternoon, and there is a lot of ways to do that, but the key thing is to look and to see the stability and verify that you are where you want to be. So, whatever works for that, works for me.

DR. MOROFF: I want to just echo what you are saying about methodology, Larry. I think we need some new methods, some simple methods to count white cells before we do a lot more QC.

I agree with Celso, we really don't know what the gold standard is for white cell counting.

I heard CAP uses flow, but I am not sure that is the gold standard or should be the gold standard. This is always a very tough question, what is the gold standard for platelets or for white cells.

So, it would be nice to have some type of joint effort to look into all these issues, and hopefully, some new ideas can arise or could be used to develop some simple techniques that could be used for white cell QC.

DR. DUMONT: We and the Dutch have done studies on white cell QC using PCR type methodologies and they work very well, and they can be very sensitive and quantitative, but when you start to run the numbers, they are very costly especially with the small proportion of products that are QC'd currently.

But, to me, seeing the data and the FDA approach of saying manual leukoreduced or filtered products, because the distribution is higher than apheresis or automated pheresis that we are going to require 100 percent QC just because they are closer to that, what in truth is a pretty arbitrary

limited database cutoff of 5 or 1 x 10 6, and when you see the breakthrough cases, some of the data today showed it is really two different kinds.

There is basically the upper edge of the normal distribution of what gets through, and then there are literally failed filtrations, and it is really those failed filtrations that are, to me, the worry with respect to patients really potentially suffering consequences, be it febrile or alloimmunization, et cetera.

It would seem like if we are going to go universal, it would be attractive to have a simple, cheap release test that was not requiring that very low level standard, but would pick up this gross failure, 10 8, or something like that. It would seem like if that were something, a target that manufacturers could build a very simple test that could achieve that.

I still think you need the process control. You need to look at actual values on the low end, but similarly, on the high end, when people get, be it Nageotte, which actually Simon

Glynn told me that stands for swimming pool, it's a French word for swimming pool, these low levels clearly are important to monitor trend for process, but similarly, I believe Nageotte and certainly flow give you the high values, too, and it doesn't seem like the algorithms to investigate are sorting based on whether your, quote, "breakthroughs" are just above the limit versus gross failures. It would seem like the algorithms should be designed to work through the types of failures that are observed.

DR. BIANCO: Actually, I think that this discussion is very good. We all have thought a lot about the maximum contents of a unit in terms of the ideal process, and when there was the desire to go to the European standards, the European standards are tough, that is, 10^{6}, but they are not as strict, at least at the current time in the performance of their QC as we are. That is, they still don't have to close a process because they failed QC as we do.

But maybe if we can migrate somewhat, and

it is just an idea that is starting in my mind, to a process that is a little bit easier in terms of what we do, we will do more. I think that there is a balance there on how much can be physically done in terms of QC, trending, and all that, and how much centers do, in the same way that the more demand that we make, regulatory demand and applications, and not CB-30s, but prior approval supplement that we require, the less we see centers trying to apply if their hospitals are not demanding more.

So, I really ask that Alan, Sharyn, as you think about the processes, Jay, that is, the simpler, the better, the more people are going to adopt it faster. We are going to migrate to things that are better for patients.

DR. DUMONT: I have another comment that I think is unique to machine-prepared components, and I hope that FDA, when they--I don't know if it is going to be in the leukoreduction guideline or the apheresis guideline, or both, or how it works, but there is one question regarding a stability of a

process, and is the equipment and the process running the way it is intended to run.

That is kind of a population question. There is a second question. When you have a bag of platelets in your hand, and you are going to transfuse them to someone, and how many white cells are in that bag.

The reason I set it up that way is because many of the apheresis products will make, as you know double and triple platelet products, and it is very conceivable that you might get a double product that might have 8 million white cells in it.

Well, that may or may not be okay for the process stability of that piece of equipment that depends on that process, but when that product is divided into two therapeutic doses, there is less than 5 million white cells in each of those bags.

To me, and based on the consistency of standards and guidelines in Europe and in the U.S., that those are individual products and they seem to meet the specification, if you will, of leukocyte

reduction, so there should be a consideration, proper consideration on how to handle those.

DR. MOROFF: I just wanted to make a comment about the fact that I was impressed from the data that was presented earlier this afternoon, how low the failure rates are with the current devices and current filters.

I think we have some good filters and good devices, and I don't think we can forget that.

DR. DEVINE: I guess one of the other things that I sort of took away from the discussion that we have had so far is this whole issue around trying to develop a standardized way to investigate the QC failures. I think that would be of great value to the community.

I would certainly love to see what gets developed because my laboratory tends to be on the receiving end of large numbers of boxes from various Canadian blood services manufacturing centers saying there is something wrong with this, can you fix it.

So, to have some more standardized

approach to these kinds of failures, whether they are actually leukoreduction failures or they are issues around, you know, dare I say white particulate matter or other kinds of things that require investigation, to have more standardized ways to look at these, that we share these learnings amongst ourselves would be of great value.

I would certainly be happy to participate in developing such a thing.

AUDIENCE PARTICIPANT: I have one comment and one question about the comment associated with the first question. Today, the U.S. is approximately 80 percent leukoreduced, and it is a free market, so it seems that the market is voting towards universal leukoreduction, and it might be just simpler or easier, if we are going to go to QC to the levels we are talking about, to standardize production in the U.S. by enforcing universal leukoreduction.

You would have one stock, you would have one product, you have simple, single method of

production, and it takes away a lot of the curios you see out there.

Then, my question is related to the QC and the definition. I am not sure if I understood it correctly. I think the question is for Sharyn.

Are we also talking about 10 percent QC of the 13 million or so red cells instead of 1 percent, because all I have heard here is apheresis and automated red cells? Are you also recommending, in your QC process, that instead of 1 percent QC, it would be 10 percent QC of all the red cells that are leukoreduced in the U.S.

DR. ORTON: Yes. Right now the guidance document for QC of just red cells, apheresis red cells, is 50 per collection site per month, which in many cases means that collection facility QCs is essentially to 100 percent QC.

So, the 10 percent for scan statistics would apply to that grouping. For the apheresis platelets, the 10 percent would apply to that grouping. Again, many places will tell you they are doing 18, 20, 25 percent.

For leukoreduction of whole blood red cells, are you asking if it is going to be 10 percent now versus some--yes, the answer. Well, if we did 100 percent QC, no, it would be all of them. If we put them into a scan statistic program, yes, it would be the same kind of principle.

Again, that is just one option. We are just giving you one statistical option. There are others available, as Larry brought out, there is many that could be better fitted to your facility.

But I do want to make it clear that when I earlier said we didn't have the kind of stratification, we are not lumping all components together for scan statistics. It would be apheresis platelets, whole blood derived, et cetera, and we can use it for the platelet count and pH. We can use it for the leukoreduction. We can use it for any QC parameters that we test for.

AUDIENCE PARTICIPANT: So, for the QC, it seems to me that it would make more sense to go in the direction of (a) centralized collection of data that would allow trending like Dana is proposing,

or like it is done in the UK, that is more useful than getting actual percentage of pass data, if you understand my point.

DR. ORTON: Yes. In fact, in consideration, the platelet pheresis guidance does give information on tracking and trending. That was already in there. So, yes, we do consider all of those things.

DR. BIANCO: Sharyn, as a follow-up to these questions, so I can sleep tonight, is the guidance going to come out as a draft?

DR. ORTON: Yes.

DR. BIANCO: This morning, Alan, as he introduced the meeting, mentioned that in the past, there was no mandate for leukoreduction because the legal people at FDA felt that these should part of a rule, and not a guidance.

Could you tell us if you are working on a rule?

DR. EPSTEIN: If we were, we couldn't tell you.

[Laughter.]

DR. EPSTEIN: But it is not in the works now.

DR. MOROFF: Sharyn, could you clarify about the two guidances that you are talking about, you are talking about platelet pheresis guidance and a leukoreduction guidance, are they both going to come out at the same time, or will one come out before the other?

DR. ORTON: Gary, where have you been? No. The platelet pheresis guidance, it's for automated collection of platelets pheresis, has been in the works for quite some time.

Now, in its defense, right now I have most recently been working with Kate Cooke in our Office of Chief Counsel, and we really wanted to look very thoroughly at what we were recommending, whether we were overstepping our bounds legally or not.

We wanted to make sure that anything that we thought we were recommending that, in fact, is in the regulations, that we are making it clear that you must do, so that has gone through some iterations.

The last section I worked on with Kate has to do with what you would need to submit, so we are really in the final process.

The other is the leukoreduction guidance, which is what Alan has been working on. What we are trying to do is standardize the different component guidances to cover the same kinds of things, the validation, the quality control, sections on what we see missing and reviewed generally to make sure you have them in your SOPs, et cetera, so that there is consistency across these and that they are useful for you. So, those are two different guidances.

AUDIENCE PARTICIPANT: So, they will both come out at the same time?

DR. ORTON: I hope mine comes out before Alan's.

DR. DUMONT: Just one more comment that I wanted to make on the numbers game. I know it is real easy to get lost in numbers, so I would encourage the agency and then also the people that implement these things that we need to keep the

numbers in balance with Steve Wagner's earlier point about what are those numbers really helping us do.

If that is what we need to do to really demonstrate stability of processes, then, that is probably the right thing. If we are just driving numbers because it makes us feel better, then, that is probably the wrong thing, because we are not helping patients out.

DR. BIANCO: As a complement to what Larry just said, it would be very nice if you could pilot those proposals. I am sure that many of the ABC Centers, I am sure the Red Cross would give you access to data that you could use to test and to see what actually, in real life, those approaches are going to generate.

I think there was a question here.

AUDIENCE PARTICIPANT: It may be a bit naive, I probably should have asked it at the beginning. A lot of this is new to me today. I am looking at the data that were presented, and I don't see a huge advantage, but certainly the rest

of the world has already done all of this.

So, going back to Dr. [inaudible] comment, again, my naivete, but why are we behind, is it purely economic? We are not going to have huge benefits or certainly we have jumped on the bandwagon a long time ago. [Off mike.] Is it economic, is it we are waiting to give an opinion?

DR. BIANCO: It is a very important point definitely. I think somebody said it today already. I think it was Harvey, that if it wasn't for the cost, we would all be doing it. Yes, I think that is a big, big factor.

DR. KLEIN: If I could just comment on that. Again, I am talking from a relatively small hospital, however, we have five physicians who have been in transfusion medicine probably a total of about 130 years, maybe more than that, all of whom are internists, hematologists by training, and have taken care of patients and many still do, and about eight years ago voted unanimously to go to universal leukoreduction. I don't know that I can say any more than that.

AUDIENCE PARTICIPANT: If I could on another aside, would come to some closure at that point, one of the things I have heard over and over again today is a desire for the FDA to provide some guidance essentially on how to an effective root cause analysis.

It is something that I have seen from the field over and over again, and I can also tell you that when we have been investigating product problems in the past, and we are interviewing people who use the product to see how serious the problem is, I have seen a broad spectrum from everything, while if it is not this one item, what could it possibly be, to people who have done a very elaborate brainstorming of a fishbone diagram and a very deliberate root cause analysis to eliminate all potential causes.

I think it is difficult for the FDA. We can certainly, in guidance, say if you do these things, we have seen that work, but it is never the bottom, and it is always thin ice for us to go out, and someone says I did everything FDA told me to,

and I didn't get the right answer.

The bottom line is there is a lot of training about root cause analysis, and because of the unique aspects of anybody's systematic approach or their own unique circumstances, we can't provide good, detailed, complete guidance on that. It is something that I would hope that organizations who represent the industry can maybe collaboratively help more than FDA can, or perhaps we should, because too often when we say it, it bounds it and people want to do what FDA has told them to do and no more.

So, I would advise--I will get a chance to comment on these documents as they come around, but I would advise caution in how much we try to lead such things as a root cause analysis.

DR. DEVINE: I was just going to say, since I brought that up, I didn't mean to imply that I thought that the FDA should actually be providing that guidance. I think that has to come from the community, and we have to take collective responsibility as manufacturers of components to

actually learn how to do the analysis properly and share what we have learned.

Now, if you guys want to participate in that and provide us with some off-the-record, you know, go think about this type of information, that would be very useful, but I didn't mean to imply I thought it should come in a regulatory context.

DR. BIANCO: And to complement what Dana said so well, you have a repository of information that we have no access to. You have a lot of reports from manufacturers and from centers, and all that. There must be in the same way that you post on the website, reports on donor callbacks or these types of things.

If we could have at least some of that information in an aggregate form, that could help us, direct us in that way, but I agree with Dana, but just as the bottom line, it is always the FDA's fault.

[Laughter.]

DR. WILLIAMS: That can't be the final word. Anyone else? Merlyn.

DR. SAYERS: Well, I am not going to dispute anything that Celso would say. As far as root cause analysis is concerned, I don't think we were asking the question how do we do root cause analysis as much as we were asking how do we investigate failures satisfactorily to the FDA's liking given the fact that we do not have a comprehensive understanding of all those matters that can contribute to the failure. In regard to what Celso said, I suspect the FDA has got a better handle on understanding what the contributing factors are than we do.

DR. KLEINMAN: One issue that came up in the discussion before is if you do fail your statistical process plan, and you have to do some sort of investigation, can you continue to manufacture leukoreduced products when you are seemingly out of control.

That brings up a whole host of issues, you know, as to labeling. It may take a while to do your investigation, so I hope that there is some sort of clear indication as to how one would handle

that once one gets into that out-of-compliance or out-of-conformance situation.

DR. WILLIAMS: That is very true, but, in fact, don't most large facilities have alternate pathways if, say, for instance, they get a bad lot of filters, they could switch to an alternate filter lot? Perhaps that is a misunderstanding, but are you tied to simply one production pathway?

DR. BIANCO: Alan, you wanted something different as the ending. I think at least for the community, I want to thank FDA for having organized this discussion. This was very good, I learned a lot. I hope you learned a lot, too.

I wish that when you have these guidance out, maybe we can find another forum like this instead of just submitting--well, we will submit the written pieces of paper, but where we could hash it out, and I think that that would be a very productive process for all of us. But thank you.

DR. WILLIAMS: Thank you, as well, specifically Celso, for helping with the survey, and to all of our speakers and panelists, I think

it has been a very informative session.

I think we did learn a lot and we will certainly carefully consider the transcript and everything that has gone on.

We have the final session still to come. Dr. Luisa Gregori is here. Dr. Gregori works with Dr. Rohwer at the University of Maryland on some very exciting prion technology, and she is going to talk to us about New Technologies in Filtration, Prion Reduction from Blood by Filtration.

Dr. Gregori.

Before I forget, please, you have evaluation forms in your folder. We would appreciate it if you could complete those. It helps us to make better workshops in the future, so please complete your evaluation.

New Technologies in Filtration

Prion Reduction from Blood by Filtration

DR. GREGORI: Thank you. Actually, Dr. Rohwer sends his apologies, he could not be here today. He really wanted to, but he had an emergency, family emergency, so I am here to

present the data from our laboratory.

The presentation is divided in update on TSE blood infectivity. We will talk about leukoreduction, leukoreduction and PRDT technology in the context of control of TSE pathogens. Then, if I have time, I would like to spend a couple of minutes just on talking about diagnostic still in the context of TSE removal.

There is a large body of evidence at this point that there is TSE infectivity in blood. This evidence comes from experimental animals, from natural TSE infections in diverse strains of the TSE agents, and all this information are consistent with, and predictive of, transfusion-transmission of TSE in humans.

Unfortunately, we have seen already two cases. This is a summary slide of the UK TMER study. We obtained the data from Dr. Robert Will in the UK as a personal communication, but he allowed us to show this data.

What you see here, those are all the recipients of blood from donors who later on

developed variant CJD. There are less than 50, I think there are 49.

Those are the years since transfusion. The blue dot corresponds to individuals who died, and the red dot are the individuals who are still alive.

This is the first case of variant CJD transmission that is reported in the literature. This was an incubation period of 7 1/2 years. This is the second case, the heterozygote individual who died without showing signs of variant CJD, but later on PrP-res was found in the spleen of this individual.

So, what this picture says, one way of looking at this data is let's say that the incubation time of variant CJD in blood is five years. All these individuals here, they cannot be counted in this calculation because they died before five years, so they could be incubating the disease, but they died too early.

If we just count the individuals who died and after they had enough exposure to the

infectivity, longer than five years, then, there are already 2 cases out of 7. That is a very high percentage of transmission.

If we include also the living individuals, that is still a very high transmission, 10 percent, this is what we see. This is not what we see with animals.

If we take 2 years as the incubation time, that is still a very high transmission rate that we find in variant CJD individuals. So, this could mean that either the titer of the variant CJD blood is higher than we have anticipated based on the animal models that we use, the mouse or rodent models, or the virulence of the variant CJD strain is higher again than the mouse or hamster model.

The other new piece of information that came out last year is this study by Hilton and Coworkers in which they looked at the presence of PrP-res, the marker for TSE infectivity in appendix and tonsil. They started with a very high number of samples. Assuming 100 percent ascertainment, this study concluded that there are about 3,800

cases of incubating variant CJD in United Kingdom at this time.

This is very different from the number that we actually have now. There is about 150 cases that we know of, so there is something there. Perhaps these individuals are incubating, but they are symptomatic, so that means there is some silent potential transmission that we have to take into account that perhaps is going on.

Also, estimating that 10 percent of individuals here donating blood, then, we have currently 380 variant CJD infected blood donors in the UK.

So, this is not to make the picture too grim, I think in these days it needs to be looked at for what the numbers say, and we have to try to understand what they actually mean.

In our laboratory, we work with the hamster model of blood-borne TSE infection. We have done a lot of studies for several years. I will show you some of the studies, some of the results, but before I get to that, the results, I

just want to spend a couple minutes describing the various ways in which we titer hamster tissue.

If the tissue is brain, for example, brain has a lot of infectivity, so then we use the endpoint dilution titration method, which is a conventional method in which the inoculum is diluted in a 10-fold serodilution that you see here. At each dilution, a cohort of animals are inoculated. This is a little syringe, IC inoculation.

Then, after a year, we just look. The gray animals are the ones that died, the yellow, the ones that are still alive, and then we use the Spearman-Karber method to calculate the titer. That is a very conventional method to do. We can do this with brain because brain has a very high titer of infectivity.

Another way to look at it is also using the incubation time in the dose response curve. There is an inverse correlation between the titer of infectivity inoculated into the animal and the time that the animal takes to develop the disease.

The higher the titer, the shorter is the incubation time.

This gives a correlation that is very linear to some degree, and this can be predictive of the titer of the infectivity. However, when we go down to very low titer, high dilutions, then you see that the infectivity, the incubation time doesn't really correlate with infectivity. This is very flat line. This is the dose response that disappears limiting dilutions.

This here, each dot corresponds to an animal that was inoculated with blood, and you can see that starting from 150 days to 450 days, these all are animals spread out almost randomly.

So, if we need to titer blood, we cannot use the dose response curve, we cannot do the serodilution, we have to use a different method, and the method that we use, we call it dilution titration, a method that was developed in our laboratory and basically, with this method, we take a 5 mL aliquot of the test material that we want to titer. This 5 mL are inoculated, 50 microliters

each, into 100 animals.

At the end of the study, about a year and a half later, we just count the number of animals that are positive. Their number correspond approximately to the number of infectious doses, and then in this case we divide by the volume, and it gives a rough estimate of what the titer is.

We can get a very precise estimate if this titer is then corrected for the Poisson distribution that takes into account the probability that one animal gets more than one dose of infectivity.

So, this type of titration is dictated basically by the Poisson distribution because the titer is so low that you can anticipate that 50 microliters either has one unit of infectivity or no unit of infectivity.

So, all the infectivity studies done with hamster blood or blood components are done this way. We have done many of these studies over the years, so this is a composite of some of those. The red corresponds to the pool and you see that

they are all clustered around about 10 infectious dose per mL although when we looked at individual animals, we found the most variation. Those are the blue symbols.

So, usually, we found around 10 infectious dose per mL. We also looked at titer in blood of animals incubating the disease at different times during the incubation. We found that there is infectivity early on before the clinical signs of the disease.

We find it here after 80 days, 100 days, and 120 days where the clinical manifestation of disease occurs. This is some sort of type of linear correlation. One might think that this is very low level of infectivity.

Up here it is about less than 2 infectious doses per mL, but if you consider that a unit of blood has 450 mL, then, you can calculate this, already something like 800 infectious dose per unit, so it becomes a significant amount of infectivity.

In terms of control of TSE pathogens, what

we have used at this point is sourcing and deferral, but this is more a moving target as we learn more about TSE infections, how they spread and how to control them, it is a good option that we have, but cannot be the only option that we apply.

Screening, of course, it would be very useful if we had one, but the screening at this point is technically problematic for blood. Inactivation, it is incompatible with blood products, so we are left at the end with one option, which is removal.

Removal is relatively low risk and is technically possible, so we focused on removal, and we think that this the best option that we have at the moment.

Removal in a certain way, leukoreduction is a form of removal of TSE infectivity. You are expert here on leukoreduction. We only looked at leukoreduction in terms of TSE removal. Leukoreduction was implemented in Great Britain several years ago.

The idea was, the rationale was the infectivity is concentrating in buffy coat, PrPsc, which is the marker for infectivity, and infectivity itself was demonstrated in lymphoid tissue and some TSE infections, and also there was some involvement of B lymphocytes.

So, they were the scientific basis for leukoreduction. Since then, other countries are also implementing universal leukoreduction. Three years ago we did the study with Health Canada. We worked with Tony DuLeve [ph]. They had just implemented the universal leukoreduction in the country, and he came to us because he just wanted to know whether leukoreduction actually removed TSE infectivity in blood or not.

This is the study we did for Health Canada, together with Health Canada. We prepared a human size unit of hamster blood, that is about 140 hamsters, the blood from 140 hamsters was collected in one bag. We filter it.

This is the Pall filter that is used currently. I believe it is currently used in the

Canadian blood centers, and we collected and leukoreduced the whole blood. Then, we titer the blood pre- and post-leukoreduction.

We had to confirm and verify that the leukofilter performed according to specifications and that hamster blood behaved similar to human blood, and so on. I am not going to go through that because that work has been published already last year, so I just go to the bottom line.

This one is the distribution of animals. This is the day post-inoculation. That is the number of animals, and this is just to emphasize what I said earlier, there is no dose response here. These animals came down from 150 days to 550 days, pretty randomly.

We calculated the titer pre-leukofiltration, post-leukofiltration, and what we find is that the leukofilter removed 42 percent, that is, 58 percent of infectivity went through the filter.

In different words, if infectivity in the unit before leukofiltration was 4,500 ID, after

leukofiltration it was 2,600 infectious doses.

The way we concluded the study, leukofiltration is necessary for TSE removal because it targets a specific blood cell type that will have to be removed anyway in terms of TSE infectivity, but obviously, it is not sufficient to reduce the risk of transmission by TSE by blood transfusion.

So, we propose to look at alternative methods together with leukofiltration. One method that we feel very strong about, as I said, is the removal. The advantage of removal is that it removes also infectivity that cannot be detected by diagnostics. Even if we have a diagnostic test, there is still going to be a limit of detection for that test, so a removal of infectivity.

If we have a device that removes infectivity, will it remove also for dose unit that escaped diagnostic. Also, for removal, we don't need to differentiate, discriminate between the abnormal form or the normal form of PrP, so that is another advantage.

So, we think this is more comprehensive and perhaps less costly, but I am not sure about that.

In terms of removal, we start a collaboration with a company called PRDT, and actually, to be perfectly clear, Bob Rohwer is one of the founders, Dave Hammond, the American Red Cross, Ruben Carbonel at the University of North Carolina. This company is a joint venture of American Red Cross and Prometic [ph] Corporation, and more recently Maco Pharma enter in partnership with PRDT for the manufacture and marketing of the final product.

For full disclosure, the study that I am showing you that we did with PRDT was fully funded by PRDT.

One of the things that interested us about this company was that they were using combinatorial peptide libraries to find ligands for specific targets, so that technology was very appealing to us, because we thought that we might be able to use that to capture PrP-res or PrPsc, the target

protein for infectivity.

The peptide library can be actually this many combination. We only looked at the subpopulation on this combination, and they went through different screening.

I am not going to go through many details on this screening, but you can see that at each step, there was a significant reduction. Now we are down here to one ligand, and this ligand has been tested now for removal of endogenous infectivity.

The primary and secondary screening was done in vitro, looking at PrP proteins with Western blot. The tertiary or final screen is done with infectivity. I showed you just to give an idea of what the secondary screening was.

The 200 ligands that were found from the primary screening were immobilized on resins and then we tested those resins. We challenged the resins with brain homogenate spiked into red cells. Then, we looked at what was captured on the resin, and the darker the signal, the better the resin was

because we must have captured a lot of PrP.

You can see here we tested with humans, with hamster, scrapie, and with different forms of mouse, mouse-derived TSE. From this study, a handful of ligands turned out to be very interesting, so we moved those ligands to what we call the tertiary screening, and that involved infectivity, brain spiked infectivity.

At this level, what we wanted to know is to verify that those ligands performed well in vitro, removing PrP-res. They actually also removed the infectivity. The study that we did, we started with one unit of human leukoreduced red cells that were spiked with hamster scrapie brain homogenate.

Each ligand was challenged in a series of 5. We collected the effluent from each step and we used the incubation time as the measurement. We don't usually use incubation time, we think it's not very accurate, but we thought that for this study, since we were looking at very dramatic reductions on the order of 2, 3, 4 logs of

reduction, so we thought that it is a suitable method, and the number of animals that they needed for incubation time compared to endpoint dilution titration is much less.

We also used an empiric endpoint using the animal weight loss that we have used for the first time, and it worked very well. For the incubation time, we had to make a dose response curve, and I showed you the results with that.

This is very briefly schematic to clarify what you are going to see later. This is the challenge, red cells in a homogenate passthrough, ligand chromatographic column format. There were actually five of them in line, but I showed you only the results of the last one.

What we did, we looked at incubation time of the challenge solution and incubation time of the effluent, and we compared the two and looked at the level of removal.

This is the dose response curve that I was talking about earlier. We need to have a curve, so that we can compare and determine the level of

removal from the incubation time only. The challenge was 10^{-3} dilution compared to brain. It was a 0.1 percent scrapie brain homogenate.

This was serially diluted 10 times. We inoculated a cohort of animals. We determined the average incubation time, which is this blue symbol, and this gave this curve that you see here.

The next step was to titer the effluent. Actually, the challenge was 89 days post-inoculation. Then, we started looking at the effluent. This was our negative control. So, the negative control showed that the animals inoculated with this effluent from the resin 4 did not have a decrease in the incubation time indicating that the infectivity that was in the challenge was still present in the flowthrough, in the effluent.

But then we found other resins that performed differently. This one had 99 days post-inoculation, 123, 140. To make a long story short, the one that we focused the most were on these 3 resins here, 8 or 3 or 1. They showed the most reduction in infectivity titer because they

had the longest incubation time.

So, if we now look at this incubation time here and report on the axis here, it looks like the incubation time corresponds to the brain homogenate at 10 -7, so we started from 10-3, now we get 10-7, we got 4 logs of removal with those 3 ligands.

So, the conclusions for this infectivity study is that ligands showed around 4 logs of removal of TSE infectivity in red cells. The negative control did not remove infectivity, so it was not a mechanical or some other artifact going on during the chromatography.

The infectivity in the challenge was 200,000-fold higher than in 1 unit of infected blood. This was necessary because we had to use brain, so that is what we had to do, so obviously, it was overloaded.

Also, what we found maybe wasn't--I forgot to mention here--all the animals died. That means not all infectivity was removed. So, there is some infectivity that is still going through, and when we calculated, it was 1 part per 10,000 unit of

infectivity. So, the filter removed 4 logs, but the leftover infectivity that went through the filters is on the order of 1 part per 10,000.

What is the implication for this for an endogenous infectivity, we really don't know, because it depends on how blood infectivity is distributed. If the blood infectivity is distributed in the same way as in brain, this 1 out of 10,000 units, it doesn't correspond to a lot. Actually, it will leave 0.5 infectivity per unit in the blood after the device.

On the other hand, if blood is enriched in that type of infectivity that did not get trapped by the chromatography columns, then, it is more problematic because we will have to then look if this actually is an effective device.

So, the only way to distinguish these two very different scenarios is to just do the experiment. We had to look at endogenous infectivity in blood as the proof of principle and also to validate the relevance of our studies.

The endogenous infectivity study is still

ongoing. I just showed you what we have at this point, but first I want to just take you, step by step, how we get to do what we actually did.

The first thing that we had to do was to choose the test material. We went through, there are ideal test material and then there are realistic test material. The ideal test material that we really wanted to use obviously doesn't exist. It would be 1 unit of variant CJD infected blood from human patient.

Such material doesn't exist and even if it existed, we don't really know how to measure infectivity in that blood, so it would have been a problem anyway. The second best choice will have been variant CJD infected with blood from a phylogenetic human mouse.

The problem with that is that the human mouse, the humanized mouse doesn't seem to be working very well with variant CJD, so that also didn't work very well. Sheep blood, of course, we could have studied with sheep blood. The advantage is that we can produce 1 unit of sheep blood with

no problem.

The disadvantage would be where are we going to titer it. Well, we can titer it in sheep, but in the same host, that is possible to do, but it will take more than five years before we know the results, so that is a little too long.

We could have done it in the mouse, transgenic sheep mouse. There are several laboratories including our laboratory that has the transgenic sheep mouse, but it has not been characterized enough to know if there is enough sensitivity to do these type of studies.

So, at the end, we ended up with a rodent blood model. This is what we are very familiar. We can inoculate the blood into the same host. It takes a year and a half. This is a long time to wait, but it is still better than five years, so we settled with the hamster.

The second choice we had to make was the challenge. We had three options: whole blood, red cells, or plasma. What are we going to challenge these ligands with?

We ended up, we decided to use whole blood. That is because it represented the worst case and contains all the infectivity that are present in blood. Also, we had already experience with leukoreduction. We already knew that there would be enough infectivity in the leukoreduced blood to be able to run this experiment.

We also looked at the interference of protein in plasma. So, at the end, the model was hamster-infected whole blood. The titer was done with the limiting dilution method, and I just want to clarify and point out here an important point, that the demonstrable removal function on the volume of the sample assay.

We usually test 5 mL. This is in 100 animals. This gives a limited detection of 0.2 infectious dose per mL. We could have started with 1 mL. There would be much less animals, but it would give us much higher limits of detection.

So, we prefer to use, we are convinced that this is the best option, the model that we have, 100 animals is a good balance between a study

that would be too huge if we use more animals, and a study that would be too small and won't give us a very clear answer.

So, the endogenous infectivity was basically done this way. We started with a PRDT leader ligand. It was a scaledown. We are going to do another experiment with the full 4 units of blood, but this was a pre-prototype, so we just want to have an idea whether these resins actually remove endogenous infectivity.

Leukoreduce whole blood in the challenge. It was the challenge, and we did limited dilution titration on the challenge and on the effluent from the PRDT devices. The study is ongoing. It is 87 percent completed, and it is going to be completed in January 2006.

What we have now. I notice it is not a month old, but not much happened in the past month. This is the not leukoreduced blood. This is the titer that we found, that we have extrapolated to 100 percent completion.

This is the leukoreduced whole blood and

this is the titer. The final flowthrough, we found no animal that came down with the disease as of 330 days, and so we have reduced the level of infectivity to the limit of detection on the assay, which corresponds to about 1 log on infectivity reduction.

I will come back to the leukoreduction results in a moment, at the end of the presentation, and this is what the filter looks like. It has been prepared by Maco Pharma.

So, the summary at this point is that PRDT has a lead resin defined. This ligand appears to have a high affinity for prion protein, and it works with brain of rodent and human with different forms of TSE strains.

It works in vitro with red cells, whole blood, and plasma. In the removal of infectivity, we show 99.99 percent removal using brain that infectivity corresponds to 4 logs, and we also showed about 90 percent of removal, which is 1 log of the infectivity. These ligands appear to have no impact on blood.

I said I thought that this audience might be interested in what we find here. I mentioned the Health Canada study with the leukoreduction earlier on. Those studies concluded that 42 percent of infectivity was removed by the leukofilter.

In the study we did now with PRDT, we find that the same leukofilter, I mean the same type of leukofilter removed 71 percent of infectivity, not 42. So, we are trying to understand why there is this difference.

Of course, it could be the filters behave different because there were two different filters, but that would be too obvious. I think there might be something else going on, and we tried to address this point by looking at some other data that we have in our laboratory, and I am going to just briefly mention to you.

When we spin blood to produce the three fractions, and we titer plasma, buffy coat, and red cells, we find that most infectivity is in buffy coat, 45 percent of the total infectivity in whole

blood is in buffy coat.

That matched very well with the infectivity that was removed by the leukofilter. The leukofilter removes white cells. It was about 42 percent. This is most in white cells is 45 percent, so we thought that we got everything clear.

So, there would be two pools of infectivity in blood. One is in plasma, one is in white cells. The leukofilter removes infectivity in white cells. We just have to go after the infectivity in plasma. We thought that the two pools of infectivity could be separated in separate compartments.

Later on we did another experiment in which we took buffy coat from blood, separated by centrifugation and washed the buffy coat extensively. We just wash away with PBS, nothing special. Then, we titered the washed buffy coat.

What we found is that after washing, most of the infectivity was gone. As much as 80 percent of infectivity was gone from buffy coat. So, our

conclusion at that point was that was, well, maybe infectivity is not very tightly bound to white cells, and it is easy to wash it off.

So, if that is the case, then, that could explain the difference in leukoreduction. After all, the white cells are trapped in the leukofilter, there may be some factors that we haven't identified yet, that removes more or less infectivity from the white cells that are trapped in the filter.

So, that is one possibility that we are at this point considering. Also, I just want to point out that we did the leukofiltration reduction only twice, so it is not that we have a large number of data in our laboratory that we can interpret, did it two times, and we got rather different results.

Finally, just to mention how we see removal. We see removal as a form of concentration. Once infectivity is removed, we have a device, we have a filter or something where the infectivity is basically concentrated, and this concentration may be useful for diagnostic

development.

So, there is a lot of interest in diagnostic on the TSE field at the time. We think that this interest should also be placed on looking at ways of removing infectivity and use that removal step as a concentration step.

We all know we need to concentrate PrP-rs from blood if we want to have a diagnostic. So, we are proposing that plan, too.

Thank you.

[Applause.]

DR. WILLIAMS: I think we have a little time for questions.

PARTICIPANT: I am just curious. In your washing experiments, do you retain the platelets with the white cells, or do you wash the platelets away with the plasma?

DR. GREGORI: In the buffy coat wash? The platelets are mostly in the buffy coat, and they stay in the buffy coat, so it's in the buffy coat, yes, they are retained.

PARTICIPANT: Thank you.

DR. GREGORI: But I mean in terms of infectivity, we have done a study a couple of years ago, looking at platelets purified from hamster blood, and we showed that there is no infectivity in platelets, so that was one of the reasons why I wasn't really looking at the platelets.

I know in the study also that I didn't mention is during the wash, white cells were not lysed. We were very careful not to do that, so we think that the loss of infectivity is not because we lysed white cells and infectivity was inside the white cells and that is how it got lost. We think the infectivity, the majority of it is localized on the surface of the white cells.

PARTICIPANT: Would you expect that the kinetics of removal would be the same in high-titer material than low-titer material? You did most of your experiments with high-titer material, so that you could actually detect it at the end, but with low-titer material, would the time for removal be much longer, because the collision between the prion protein and what is absorbing it would be

less frequent and limited by diffusion?

If so, and if that is the case, are the conditions that you are using the filtration and the time it takes for filtration going to be an accurate estimate for what might occur actually in blood before someone becomes symptomatic?

DR. GREGORI: The studies, in both cases, the spiked study and endogenous study were done as chromatography, so I think the diffusion might be initially for we are doing it benchwise, but in terms of, as chromatography, and the flow rate we used, we think we gave enough time, contact time, for the prion protein to bind the ligand that was immobilized on the resin. We don't really know.

The other question, I am not sure I remember what the second question was, but the brain infectivity might be very, very different from blood infectivity. Actually, it is different. So, the extrapolation from the brain results, one has to be very, very careful of how to interpret the brain results experiment.

I think that is very important data

because it shows that the ligand in that conformation has the capacity of binding that much infectivity, 4 logs of infectivity, it is huge, but in terms of how that translates in terms of removal of infectivity in blood, it could be a very different story.

At this point, it looks the infectivity in blood is being removed by the ligand, but we still have six months, and six months is a long time, and we can get even one animal coming down and the whole experiment is not useful, I mean it is not working then.

So, we hope that we are going to see the same situation six months from now. We have to wait. I am not sure I am answering your second question because I don't remember your second question anymore.

PARTICIPANT: Well, basically, what I am thinking of is that I guess the objective is to remove prion protein in individuals who show no symptoms, and so you would expect that the titers would be moderate or low.

I guess the concern I have is you have a process that is being driven by affinity, and that is dependent on the number of collisions of the prion protein with its ligand to take it out. If the ligand is very rare, that is not going to occur very quickly, for example, at a rate of a leukoreduction filter, so you may not get down to a level that would not necessarily transmit BSE or infectivity.

DR. GREGORI: I guess then we will have to look at different flow rate for this type of filtration. Also, the final device, the way Maco Pharma--it is not going to be like a chromatographic column, it is going to be a large surface area. I guess it would be not the best solution.

I don't really know how they are going to solve that issue. I understand what you are saying and I think with flow rate, maybe it is just a matter of sending it slower, but we haven't done that study yet.

DR. EPSTEIN: Is there any feasibility to

make a concentrate of the hamster blood to use as a higher titer inoculum for a clearance experiment? Can you use the affinity ligand to generate a concentrate, or can you use centrifugal method to generate a concentrate?

You might need hundreds of hamsters, but if you were able to spike hamster blood, not with pooling units from infected hamsters, but with concentrates from a larger number of hamsters, you might be able to raise the titer inoculum at least 1, maybe 2 logs, because then you would have a more convincing experiment.

DR. GREGORI: That would be nice, but I don't know a way to concentrate blood without incurring so many other problems. One way of concentrating infectivity in blood is by preparing buffy coat. Buffy coat has a tenth of a volume of blood and has half of the infectivity of whole blood, but the buffy coat, because the device that we are using will be placed after the leukofilter, so obviously, we can't make a buffy coat. So, I don't know if there is a way to concentrate blood.

What I was mentioning at the end is that we could use the concentrated infectivity in the PrP-res presumably in the device and use it as a diagnostic concentration step. Whether that could be used in some other purposes actually, I never thought about it. Maybe it is possible.

DR. BIANCO: Can you elute your material from the resin?

DR. GREGORI: Yes, the method that we use at this point is a denaturation method, so that will work as a diagnostic, but it will not work for anything else. We didn't really evaluate that, but one could imagine a milder way of eluting out and trying to spike into blood. I never thought about it.

DR. WILLIAMS: Thank you very much, Luisa, very nice work.

[Applause.]

DR. WILLIAMS: Our final presentation will be by Dr. Jerry Ortolano with Pall Corporation, and he is going to describe the Pall Leukotrap Affinity Filtration System.

Pall Leukotrap Affinity Filtration System

DR. ORTOLANO: The best part of going last is I get to say anything I want.

I do want to make a comment about the cost of universal leukocyte reduction because it really prompted our interest in prion removal. One of the things that we became very well aware of is the fact that ULR has been estimated to cost the American healthcare system some $600 million for full implementation.

It also turns out that about 75 percent of the blood product is already paid for right now in one way or another with adoption of universal or nearly universal leukocyte reduction, leaving about $150 million to be spent.

If you divide that $150 million by 6,000 hospitals, we are talking about $25,000 per hospital, which in the grand scheme of things seems to be a pretty small price to pay.

That notwithstanding, we are constantly looking towards ways of improving leukocyte reduction and adding attributes to it to kind of

improve or increase the value of leukoreduction, and our pursuits with respect to prion removal are really spirited by that thought.

So, I would like to share now with you some results of our prion removal capability, which was targeted for a leukocyte reduction filter, but as you will see when we talk about this technology right now, we have broken down the project into two phases.

The first phase was really for a more immediate use in Europe where the pressing need for prion removal was higher, and for that, we have really characterized this filter for us with an already leukoreduced blood product.

The second phase of the research, I am not going to speak about today, is really encompassing leukocyte reduction with prion removal. Suffice to say that we have made some sufficient progress on that, as well, and we expect that to be coming up pretty shortly.

Dr. Gregori has really provided a wonderful background for my presentation, which

means I can spend a lot less time and you can get out a little bit earlier than originally planned.

The Pall Leukotrap Affinity Prion Reduction Filter really targets all prions, both cell and non-cell associated. Even in a leukoreduced blood product, there are still some cells remaining, and with this technology, since it was based on integrating prion removal with leukoreduction in its early concept, still has leukoreduction capability, so we also effect leukoreduction on top of the removal of non-leukocyte associated prions.

Surface modification technology does not impact red cell stability. We did some survival studies, 42-day storage studies, and demonstrated that to be true, and the filtration is a commonly used process, as you all well know, so integrating the two is ideal for use in the American blood centers.

Dr. Gregori has provided you with some information about the kinds of testing that can be done, and I would just like to summarize for you

the advantages of each. The Western blot is the most economical approach for screening. Basically, what that involves is taking hamsters that have developed disease, taking the brains of those hamsters and preparing a 10 percent homogenate, and then spiking an aliquot of the 10 percent homogenate in the blood product of choice.

In this case, we talk largely about previously leukoreduced human blood. You can then filter that blood product and then try to measure a pre-filtration aliquot by Western blot compared to a post-filtration aliquot to get some idea of the magnitude of prion removal.

I am happy to admit that spiking prion brain homogenate into blood was probably not going to be the same as blood that has become infected by a more natural route, but nonetheless, there is some value in doing the Western blot with respect to screening.

The exogenous bioassay really is an extension of kind of the Western blot format. What it involves is taking very homogenate, putting it

into blood, filtering the blood, and then taking an aliquot of pre-filtration product, as well as the post-filtration product, and then serially diluting those, and then injecting those intracerebrally into hamsters, so that we can get some idea of the proportion of animals that die over a fixed period of time to see at what dilution animals do die in both the pre-filtered sample compared to the post-filtered sample.

This will give us some idea of the log reduction overall effected or accomplished by filtration.

The endogenous infectivity study, which Dr. Gregori explained so well, is also really a very valuable tool in that now the nature of the prion is much more closely aligned with what we might expect to see in an asymptomatic or symptomatic blood donor. Obviously, blood donors who are symptomatic wouldn't be donors, but you are getting very close to the very low levels of prion that you would expect to see in those blood products.

If it isn't obvious to you already, please do appreciate that the concentration of pathogenic prion present in a brain homogenate is about 10^9 infectious unit per milliliter, and you can contrast that with what Dr. Gregori has shown in a publication about 10 infectious units in blood when endogenous infectivity has elaborated.

Here is an example of the Western blot studies that we have performed. Again, this is scrapie-infected hamster brain homogenate spiked into human blood, filtered, and then determined log removal using the Western blot.

Keep in mind now that, as Steve has pointed out, this is a very high concentration that we spite into the blood. There are some limitations with respect to understanding these data or interpreting these data, not the least of which is the fact that we may very well be saturating the leukocyte reduction capability by virtue of having such a high concentration of prion in that blood.

That is a requirement, however, because

the limit of sensitivity of the Western blot is actually not very low at all, it is not very sensitive at all, and without sophisticated or I should say the more technologically advanced approach used recently, effective 2002, the limit of resolution capability was about 2 logs, and now we can get it to be a little bit better than 3 logs>

What you see in the panel on the left is leukocyte filtration. There is a pre-filtration sample Western blot and a post-filtration sample Western blot, and no surprises here, 42 percent reduction in infectivity, as Dr. Gregori has published, barely shows any change in the Western blot, both pre- and post-filtration with standard leukocyte reduction technology.

If we look at the prototype design for a prion removal filtration technology, again, this was done when we didn't have as sophisticated a Western blot assay as we have now, we see that removal is at least 2 log, if not greater. That is attested to by virtue of the fact that as you look

at the Western blot on the right, in the middle panel, the post-filtration sample doesn't show any image at all on the gel.

In terms of the final filtration design, here we have just completed a series of studies totaling 48 samples, which looked at Western blot data and calculate removal to be about 2.9 log overall.

In our bioassay in hamsters, again, just to refresh your memory, we are spiking now the brain homogenate into blood and then taking a pre-filtered aliquot, serially diluting it along with a post-filtration aliquot, and serially diluting that, and injecting all of those serial dilutions into naive hampsters, and then looking for disease.

You can see here that with respect to the concentrate with the dilution required to start to impact on the prevention of manifestation of disease, we are at about 10^{-9}. If you compare that with post-filtration, it is about 10^{-5}, so the total infectious prions removed here in this example is

about 3.7 log. Again, it is not infectivity, it's an exogenous model.

Here, in our infectivity studies, again, with this particular prototypical filter, we have scrapie-infected hamster brain homogenate injected into hamsters, 100 hamsters were used in this case. Then, the blood was pooled after manifestation of disease was apparent. Blood was pooled, and then an aliquot of the pre-filtration sample was injected into a series of animals, as well as post-filtration aliquots. These are also intracerebral injections into animals.

The Western blots, 3 of the animals that we see in a pre-filtration sample is shown below in the panel on the right. In total, we had 6 out of 43 hamsters that were infected, and that contrasted with zero out of 38 in the post-filtration sample, giving a p-value of 0.0384, which does establish a level of statistical significance.

So, in summary, with respect to the various types of assays, our prototype and the Western blot gave us greater than 2 log removal, or

equal to or greater than 2 log removal. The final design gave us 2.9 log reduction.

With respect to the exogenous bioassay, we had a 3.7 log reduction with an N of 1. The final design, the study is ongoing and is planned to end in December of this year.

With respect to our endogenous infectivity study, the prototype again showed significant reduction in infectivity, but the final design data will not be available until the spring of 06.

Some additional studies you may find of interest involve taking hamster brain homogenate, injecting them intracerebrally into normal hamsters, and then taking the blood from those hamsters, pool them, separate out the components, and filter the packed red cells, and then subject that to filtration.

As you might expect, these animals have a very low level of pathogenic prion in the circulation. We were at least able to detect a little bit of it in the panel on the left, so before filtration, you do see some signs there of

pathogenic prion on the Western blot, and PrP-res post-filtration is clearly not evident on the Western blot, but if you take that filter and actually backflush it with a small volume, you can recover and concentrate pathogenic prion, so much like Dr. Gregori's model, our system appears to be working pretty much the same way.

It is possible to use this filtration technology as a way to concentrate sample, so that we can utilize existing assays for pathogenic prion, and actually increase the overall sensitivity of the system.

In terms of safety studies, we actually CE-marked this in Europe in May of this year, and we had to subject our filter to a wide variety of tests. All those tests are standard tests for filtration products and all have passed.

In summary, the prototypical filters show a 3.7 log in the bioassay, and the Western blot data showed equal to or greater than 2 log. The final filter showed a Western blot of 2.9 log, so we expect that the bioassay data should be actually

higher than 3.7 log.

The quality of the blood cells is unaffected by prion reduction filtration. The safety study showed no cause for concern, and the 24-hour single and double isotope red cell survival data is unaffected by filtration.

On top of that, the residual white blood cells were further reduced by filtration to levels less than 1 x 10
5, such that 98 percent of the time we are able to ascribe that this occurs at 95 percent confidence, which is significantly lower than the current standard for leukoreduced blood.

I would be happy to entertain any questions you might have at this time, and I think I gave that in record time.

[Applause.]

PARTICIPANT: I am just curious. Could you tell us something about what the mechanism of removal of the prions from the blood is?

DR. ORTOLANO: How much of that broaches outside the area of being proprietary, I don't know, but I will tell you this much. It is well

known in the literature that lots of things can tie to prions. We tested early on a wide variety of agents that are known to be able to latch on to prions.

Some of that information was useful and some of it led us in a certain direction, but the direction ultimately wound up in that we have now, not a ligand, not an affinity ligand, not a protein, but rather a physical/chemical separations technology.

So, if you look at the physical properties of prion, that is basically where we honed our work, so I will let you think about what that means.

PARTICIPANT: So, is the mechanism therefore somewhat nonspecific?

DR. ORTOLANO: Yes. It removes pathogenic prion and it removes PrPsc. We have checked it for a wide variety of proteins and found that with the exception of factor IXA, it doesn't appear to remove very many other proteins. As a matter of fact, IXA might be a good surrogate QC for removal

of pathogenic prion.

PARTICIPANT: In the short time you have on the market in Europe, what is the feedback you have gotten?

DR. ORTOLANO: Well, Europe does things a little bit differently than we do. We have the FDA to keep us honest, and they go through a different process. They go through a process of internal validation. So, you can expect to put a product in Europe and not expect a year to go by before you actually sell anything. They undergo study, and the Prion Working Group, which is a group of four nations, the scientists from four countries in Europe are actually convening now and reviewing our data, much like the FDA would, and they are prepared to initiate their own trials probably the end of this year.

PARTICIPANT: What is your blood loss per unit?

DR. ORTOLANO: It is about 40 mL, and if you add that on top of the blood loss you get with a standard leukoreduction product, it is excessive

in the sense that it is just not something you are accustomed to.

If we were to integrate it together with leukofiltration as we plan to, particularly if it needs to be released in the United States, you know, if it's an issue in the United States and it becomes important enough, we can pump up the developmental process I am sure, you know, making it a higher priority, and get that technology integrated into one single filter, so your loss would be no more than what you would see now with a standard leukofiltration.

PARTICIPANT: You showed a Western blot in which you found protease-resistant, protease-resistant prion protein in serum of hamsters, if I understood correctly.

DR. ORTOLANO: Yes.

PARTICIPANT: Nobody else has been able to do that.

DR. ORTOLANO: I know, and I don't know what to make of that either. We have been questioned by everyone, Neil Cashman [ph] and

others, and I mean the data are what they are. Could it be a contaminant? I don't know honestly, but it's what we got.

You know, a lot of these studies, we have an N of 1, pooling hundreds of hamsters together to give you sufficient sample size is difficult, so that is why we look at the Western blot as an alternative.

PARTICIPANT: Conventional Western blot or is it some sort of--

DR. ORTOLANO: This was the--I am blanking on the paper--it's the enhanced phosphotungstic acid precipitation

PARTICIPANT: Jerry, were these stained with Coomassie or Silver stain?

DR. ORTOLANO: The readings were done by densitometric readings on Western blot.

DR. BIANCO: What I heard from Dr. Gregori and you is that we are in the range of 4 logs. Dr. Jay Epstein just left the room. I wanted him to hear the question.

DR. ORTOLANO: He will read about it.

DR. BIANCO: Is that enough? Can we get rid of the deferral of donors, geographic deferrals that gives us only 1 log?

DR. ORTOLANO: You know donor deferral is not going to work, you know that. But we asked the experts and we did convene a panel of experts in this field, and they seem to think 3 to 4 log will be good. Whether or not it is going to work, I don't think they have a crystal ball, I am not sure. But that is what we are targeting.

DR. GREGORI: The 3 to 4 logs of infectivity that you said the filter removes, that is not endogenous infectivity.

DR. ORTOLANO: I understand.

DR. GREGORI: That is brain infectivity.

DR. ORTOLANO: Yes, and we did an endogenous infectivity study, and we showed that 6 animals out of 43 came down with disease, and none out of 38 came down with disease. The question is how much was that. We can't answer that with that assay.

But does it attenuate infectivity? Yes,

it appears to.

DR. GREGORI: Yes. So, in your calculations, maybe I missed it, what did you say the calculated log of removal from endogenous infected hamster red cells?

DR. ORTOLANO: I didn't say.

DR. GREGORI: Oh, you didn't say.

DR. ORTOLANO: No, because we haven't calculated it.

DR. GREGORI: You could calculate it based on the data you have.

DR. ORTOLANO: Yes. That was a prototypical experiment, an experiment of the prototype filter. The next series of experiments is being done in 600 animals, 200 per group per filter.

PARTICIPANT: What level of log reduction do you think ultimately you will need to prevent infection, human infection?

DR. ORTOLANO: What level of log reduction do we need to prevent? As I mentioned before, I am not the expert on this, but when we polled the

experts, they seemed to think that 3 to 4 log is acceptable. I hear Dr. Gregori saying how do you know. I don't know.

DR. GREGORI: The experts, I think they will agree that whatever you can do, the best you can do, they will work around that, because basically, the infectivity in brain, you can huge amount of infectivity removal, 4 logs, even more if you work hard to prepare a filter that will specifically do that, but who wants to remove infectivity from brain?

From blood, you cannot demonstrate 4 logs of removal, it is just impossible, because what you can demonstrate is if you inoculate 5 mL of blood, and none of the animals come down with the scrapie, then, you can say that there are zero or less than 1 infectious dose in 5 mL, that is all you can say. You cannot say there is no infection in that unit because you didn't measure the whole unit.

If you want to titer the whole unit, you need thousands and thousands of animals.

DR. ORTOLANO: It's impractical.

DR. GREGORI: So, the maximum you can actually show is I think it's a log and a half, 2 logs at the most. That is if there is no infectivity in the flowthrough of your device.

So, the agencies in Europe, and I am sure here, too, they are perfectly aware of this limitation. That is on what we can do, and they accept it.

DR. ORTOLANO: Thank you very much.

DR. WILLIAMS: Thank you, Dr. Ortolano.

[Applause.]

DR. WILLIAMS: I think before we get off on a discussion of geographic deferrals, we will call it a day. Thanks. You have been a great audience, great speakers, and I want to thank again Rhonda Dawson, Susan Zula [ph], Marty Edwards, and our unnamed audiovisual support person who did a great job.

It has been a good workshop. Thank you.

[Whereupon, at 5:37 p.m., the workshop concluded.]

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