on that at all? No? Other comments? Dr. Salman?

DR. SALMAN; This is for clarification and excuse my naiveness. How many donations do you need then of one single pooled unit?

DR.SCOTT: Each manufacturer really has their own unique batch size but I think the lower limit in the CFR is 1000 or something like that but typically, I am not sure I understand your question, how many do you need.

DR. SALMAN: When you have one pooled unit how many donors will contribute to that pooled unit?

DR. SCOTT: Right, so the manufacturing pool is comprised is plasma of a lot of donors and if you want to a 500-liter pool and you have recovered plasma and you have say 200, 250 cc's per recovered plasma you can just do the math and figure out how many donors that you would need to make up that pool size.

DR. SALMAN: But that will be a very important question for the model and maybe Dr. Anderson can answer that as we heard like the model will take that into consideration. So, the number of the donors contributes to the unit will be unimportant aspect to the model especially when you look on one of the three slides. You said they are very important and you skipped them and --

(Laughter.)

DR. ANDERSON: Do you want me to actually show

those slides?

DR. SALMAN: One of them you said, "Well, there will be 60,000 donors pool and 10 percent of those may be contaminated.

DR. ANDERSON: Yes, if you had a disease prevalence of 1 in 500,000 and you had 60,000 donations per pool it would take about, in 10 pools one of those potentially would have an infected unit, contaminated unit.

DR. SALMAN: If I understand the model and you present it well I think what will contribute to the contamination is the number of the infectious donors that participate in the pool.

DR. ANDERSON: That is correct.

DR.SALMAN: So, the number of the donors for that pool will be an important aspect. Is that correct or not?

DR. ANDERSON: This is an important aspect of the model and we are specifically modeling it. So, let me just sort of emphasize that and we have one option that we put up here is we have a range of 20,000 to 60,000. So, we are going between that range based on the information that we have.

The other thing we can do is we have recovered plasma that we are interested in and source plasma. We could generate different distributions for each of those. The problem here is that we have very limited data by which

to make these estimates and what we are doing is we are saying that we know it is between 20,000 and 60,000 and our anecdotal information is that most pools lie at for the most part at either end of these ranges. So, we are proposing a bimodal distribution. So, most of them will be either 60,000 or 20,000 in our model.

So, it is important and it is figured into the model.

DR. SALMAN: But is that reflecting reality like in any given pool unit? I am just asking the question for people who --

DR. ANDERSON: Manufacturers from our understanding different manufacturers have different pool sizes from which they make these products.

DR. BROWN: I think about 6 or 7 years ago when this whole thing with the FDA and precautions and so forth got going at that time manufacturers were using 100,000, 150,000 units for a plasma pool, sometimes. It depended on the product they were particularly interested in. After this story broke it was proposed that that was a too high limit and since then I believe in fact I think all manufacturers, there was a guidance. Is that not right?

DR. ASHER: No.

DR. BROWN: There was no guidance?

DR. ASHER: There is none, not a guidance.

DR. BROWN: There was advice. There was something in the air, don't go over 50 or 60. Is that right?

DR. ASHER: Sixty. That is again a completely voluntary --

DR. BROWN: Exactly but manufacturers pay attention to things that are in the air when they come from the FDA and so you can bet your bottom dollar that manufacturers paid attention to that. I know they did and virtually the maximum now that is used is 60 and I think this is probably a very realistic range, 20 to 60 at the moment.

DR. ALLEN: In actual fact from using source plasma donors however, you might have multiple donations from a single source plasma donor. So, if you had one infected donor there may be multiple donations from that one donor that go into a single pool.

DR.BROWN: That is actually an interesting questions. Would they in fact go into the same pool if they were donating say, I don't know once every 2 weeks? Sometimes they donate once every 2 weeks. So, they could wind up in the same pool.

DR. ASHER: And that is something we can model as well.

DR. SCOTT: I think part of minimizing the donor pool is actually trying to use some of the same donors in

pooling from certain selected centers into one pool all the time. You see what I mean? So, it certainly could happen. It would be consistent with current practice.

DR. BROWN: I think the rationale was to limit the damage. If you had a pool that was contaminated with smaller pools it would be distributed to fewer people. I think that was the clear --

DR. EPSTEIN: That is correct for the infrequent product user. The problem that you get into is that the chronic product user will simply be exposed to more product lots made from a larger number of smaller pools and so those phenomena offset each other which is part of our motivation for trying to look at an annual patient risk but for the rare or infrequent product user yes, a smaller pool would have a lower probability of having an infectious donor.

DR. BOLTON: I just have a question. Is there a uniform relationship between the number of donated units and the units of Factor 8 that are manufactured from that? There is a critical relationship there in terms of translating donations to product vials.

DR. ANDERSON: Right, and we have that actually from the literature and manufacturer information. So, we have a range actually for that and I actually don't recall what that range is offhand, but we actually have put a

range in for that estimate and it is using a yield calculation essentially.

DR. BOLTON: Is it pretty uniform across the industry or is there --

DR. ANDERSON: There is a little bit of variability from what we can see from the information that we have. So, we are incorporating that into the model.

DR. PRIOLA: Dr. Leitman?

DR. LEITMAN: This is getting off on a tangent but isn't there a voluntary hold practice in the industry so that a donor's plasma is held 6 months until their next visit to confirm they are not in a window period?

PARTICIPANT: Sixty days.

DR. LEITMAN: Sixty days. I knew there was a six in there. So, potentially a donor's unit could get into the same lot 60 days apart?

PARTICIPANT: Yes.

DR. PRIOLA: Okay, any other discussion from the Committee?

Mr. Bias?

MR. BIAS: It is exactly because of that hold that you can get several infectious donations into a pool and because there is no rule, hard and fast rule we don't exactly know how many factors are handling that on site. We learned from the eighties that it was possible for them to

in making batches leave a little bit of a batch from a previous batch at the bottom of the pool that would increase the infectivity if that previous pool was infected and previous batch was infected and therefore instead of 60,000 you had a pool that suddenly had donations from 120,000 people in there and because there is no hard and fast rule we have sort of a gentlemen's agreement with the manufacturers that they are going to lower the pool size. I would be very concerned that that still occasionally happens because it is a manufacturing process and a manufacturing process is one that produces a product that produces something they are going to sell and become income and they are certainly not going to pour it out.

DR. SCOTT: I can say that that practice is highly discouraged and I, personally, am not aware of any use of tailings anymore and it is an inspectional issue. Anybody that is found to be processing things this way will definitely get a problematic inspection.

MR. BIAS: I am sure they are all playing appropriately in the sand.

DR. ASHER; I want to just make certain it is on the record that this again is a voluntary system and the 60,000 is voluntary. It does not mean that there are no manufacturers that have gone above that.

DR. PRIOLA: Okay, so, are there any other

comments from the Committee?

If not do we agree that it seems to me the range that the FDA proposes is okay?

All right, let us go on to question 9.

DR. SCOTT: Can a cumulative effect from repeated exposures to low doses of the vCJD agent be incorporated into the risk model and we propose to allow for the theoretical possibility of cumulative effects by having the model provide a cumulative risk for a 1-year period for these different types of patients.

DR. PRIOLA: Dr. Brown?

DR. BROWN: Yes, that is a good idea. In fact, the good news or shall I say the bad news first? The bad news is that in our model it can happen. It has been shown. Now, we were wondering about that for a long time. The answer is now on the table.

The good news is that despite that fact hemophiliacs are not dying and that is another way to look at it but absolutely it is almost more than a theoretical possibility now. It is something that really has to be included in any model.

DR. PRIOLA: Any other comments from the Committee?

So, we are in agreement that the 1-year cumulative is a good idea. I think it is.

All right, let us go on to the final question, question 10 which is the voting question.

DR. SCOTT: I don't think we will have a final solution today but we do want to understand the Committee's feelings about everything that you have heard and this is a question that we are asking you to consider now.

Given the present scientific uncertainties that you have heard about today in the underlying assumptions of the Factor 8 risk assessment do you believe that the risk assessment model could provide a useful basis for risk communication to patients, their families and health care providers?

DR. PRIOLA: Dr.Salman?

DR. SALMAN: I think the short answer is yes but I will put a condition like the sensitivity analysis should be done as part of the risk assessment model and that should become part of the communication with the public.

DR. PRIOLA: Dr. Brown?

DR. BROWN: It really depends on how well it is articulated to the patients' families and health care providers. We can't expect them to understand clearly what has been happening this morning with all the caveats and this, that and the other thing but the fact is that all the evidence to date indicates that sporadic CJD No. 1 is not associated with infectivity in the blood, the evidence to

date.

Second, to date we have no cases of variant CJD in this country.

Third, the infectivity present in cone(?) fraction in the precipitate already has a low amount of infectivity. In fact, four is that processing currently in place for Factor 8 is more than adequate to take out any infectivity that might theoretically have been present.

I think the risk assessment will probably validate this overall scheme of safety.

DR. LEITMAN; I just want to second that. When you start to talk to patients and their families about risk assessment they assume you are talking about very real risk. This is theoretical risk or hypothetical risk because there has not been a case even in the highest-risk population which would have been UK hemophiliacs before the screening procedures were put in place.

DR. ALLEN: Let me concur with the previous comments and say that I think the FDA needs to move forward with this model development and to look very carefully at the results that come from it.

The subsequent steps as one tends to want to go public with the information, however, are to look carefully at how you approach this with the media because you can't keep it just to patients at risk, providers and the small

community. It is going to and has got to be involved with the general media and I have got real concerns about that because they like to hype everything regardless of what the actual risks are.

The experience we had with HIV infection more than 20 years ago now clearly tells us that we can't just sit on this and wait. I think the risk is likely to be extremely low. Fortunately we have had enough experience. We have got much better surveillance systems than ever in the past but the answer is not going to come down to zero risk. We know that and we are going to have to look very carefully at how this is communicated so that it is useful and reassuring and educational rather than frightening.

DR. BRACEY: Again, I concur with the previous statements and I think the statement that was made earlier was very important and that is that there are groups already that have been discussing the risk with the members of that community and I think that rather than start anew it would be good to try to partner with those individuals to continue the counseling that has already begun.

DR. PRIOLA: Mr. Bias?

MR. BIAS: Although I want the risk assessment developed I have real concerns about how it gets communicated to patients and health care providers.

I am concerned about the possible stigmatism to

patients as related to not their primary caretakers at per se hemophilia treatment centers or someone who is familiar with their background in hemophilia but their outliers; one of the weaker parts of comprehensive care is dental care. If their instruments are at risk they may choose not to treat patients with bleeding disorders. I think that other agencies within the government have to be alerted and have to, if we are going to publish this information there has to be some provision so that patients can continue to be treated and guaranteed that treatment on some level.

States are moving toward preferred product lists where they are limiting patients to one type of clotting factor and we are fearful that in some states they are going to select a plasma-based product because it is cheaper and if that is the case that is going to leave that family without any alternatives for care.

So, I am very concerned about how this risk assessment gets applied to the public and any slow news day, we are in a 24-hour-7 news cycle now. If the earth isn't cracking open this will be the major story of the day and it will run. If it is a holiday weekend it will run for 3 or 4 days and what you will have is a group of patients who are without care and without access to care and without alternatives because the Federal Government hasn't protected their right to have care or have access to other

product choices, if their state says that this is the product that we have for you.

So, I am very concerned about how this is applied and I would strongly recommend that before this information is published in any way that there is major consultation with hemophilia organizations both in the world and nationally so you can get their perspective and guidance as you go forward and in addition to that that we work with HHS to ensure that there are going to be alternatives for patients to continue to receive care including saying to medical providers, "You don't treat these patients; you don't have access to Medicaid."

DR. PRIOLA: Dr. Hogan?

DR.HOGAN; Relative to that I am sure we are going to be hearing about the results of this model in this Committee. I think it would be possible and we would ask the FDA for this Committee to discuss those results relative to how they would be dispensed.

Obviously this is an open forum and the media is here but we can certainly stress that there are the uncertainties that are involved and hopefully have some sort of oversight as to what Mr. Bias is talking about.

DR. PRIOLA: Dr. Johnson?

DR. JOHNSON: I am concerned about the same thing that Val was talking about and that is the nature of the

audience this is being released. There is a huge percentage of people who are well educated, well informed in this country who don't differentiate mad cow disease from anything else and think it is here and the level of confusion they have already undergone is enormous. Trying to explain something like this on top of it reasonably is going to be very hard.

I would like to hear Florence's comments on that. She deals with it every day.

MRS. KRANITZ: Thank you. i totally agree with Val. I could not agree more and as simple as you may make the explanation or as hard as you may try to show how this risk model made the assessment and realities of it, you still are going to have some panic, probably a lot of panic on the part of not only the patient but of the health care provider.

So, before you take on the project of informing publicly any part of the population you need to know that you might even have to do risk assessment on top of risk assessment as to what possible damages you are going to create by releasing this information.

DR. PRIOLA: So, the basic point that the risk model could be a basis for communication to family and health care providers, that is okay, and it is the way in which that information is communicated that is the biggest

concern. Okay.

Any other comments?

DR. BRACEY: This may be a somewhat naive question. I think it may be a matter, well it is a matter of perhaps economics and a matter of supply, but you know the entire issue revolves around the current methods for preparing the product. If we were talking about recombinant products aside of course from the risk of the albumin that it is resuspended in I think we would be having a different discussion and so one of the things that I wondered about and I would like to hear from Mr. Bias or Val about this is if you indeed did have this risk model that you could address that looked at plasma, recovered Factor 8 wouldn't that potentially be useful in making an argument to bolster the use of the recombinant product?

MR. BIAS: It probably would be but my concern is the reality that we are facing on the ground and currently we are in a battle state to state to maintain the access to the care that we have and I am just concerned that without the force of the Federal Government behind it the publication of this information would not have the same impact on every patient in every state and there will be people who will fall through the cracks. There will be discrimination and so on and so forth. So, my guidance is that if we are going to release this information that other

parts of the Federal Government that are responsible for health care and health care provision also be prepared to put laws into effect, put statutes into effect so that we can guarantee treatment for people with bleeding disorders. This is a disease that we have worked at high cost of lives for many years to make very livable for people. People are living very full healthy active lives now that we have gone to recombinant clotting factor and the plasma products are very clean. It is such a difference from when I was a child to today.

A child today plays on their school basketball team. I was not allowed in the gym and because of the switch of power from the Federal Government to the state government that understanding isn't there. That history isn't there and we see our access to care being rolled back in states all over the country.

So, I just want to make sure that if we are going to release this kind of information it can be used as an argument but we have got to have the Federal Government's power behind that argument.

DR. BROWN: The text says as I read it carefully, "Provide a useful basis," and let us vote.

DR. PRIOLA: Dr. Weiss, you had a comment you wanted to make?

DR. ASHER; Yes, I just wanted to clarify the

element here that in fact there are no recombinant von Willebrand's containing factors and so there is a definite need for plasma-derived materials and secondly there are current studies going on there about potentially the advantage of using plasma-derived for immune tolerance. It is unclear whether or not this is really preferable to recombinant but there is some evidence that is being investigated now.

DR. PRIOLA: Let us go ahead and vote on the issue because we are voting on is it the basis for a reasonable risk communication.

So, Bill?

DR. FREAS: For the record there are 17 voting members at the table. Dr. Bracey is a non-voting consultant at this meeting.

I will go around and call the roll.

Dr. Bolton?

DR. BOLTON: Yes.

DR. FREAS: Dr. Johnson?

DR. JOHNSON: Yes.

DR. FREAS: Dr. Telling?

DR. TELLING: Yes.

DR. FREAS: Dr. Creekmore?

DR. CREEKMORE: Yes.

DR. FREAS: Dr. Lillard?

DR. LILLARD: Yes.

DR. FREAS: Dr. Sejvar?

DR. SEJVAR: Yes.

DR. FREAS: Dr. Hogan?

DR. HOGAN: Yes.

DR. FREAS: Mr. Bias?

MR. BIAS: Yes.

DR. FREAS: Dr. Allen?

DR. ALLEN: Yes, with reservations noted during the discussion.

DR. FREAS: Dr. Priola?

DR. PRIOLA: Yes.

DR. FREAS: Mrs. Kranitz?

MRS. KRANITZ: Yes.

DR. FREAS: Dr. Geschwind?

DR. GESCHWIND: Yes.

DR. FREAS: Dr. Leitman?

DR. LEITMAN: Yes, with the reservations noted during the discussion.

DR. FREAS: Dr. Gaylor?

DR. GAYLOR: Yes.

DR. FREAS: Dr. Ghetti?

DR. GHETTI: Yes.

DR. FREAS: Dr. Salman?

DR. SALMAN: Yes.

DR. FREAS: Dr. Brown?

DR. BROWN: Yes.

DR. FREAS: The vote is unanimous.

Agenda Item: Topic 2: Labeling Claims for Filters Intended to Remove TSE Infectivity from Blood Components

DR. PRIOLA: Okay, let us move on to topic 2. The Committee will have noticed there is no break scheduled for the afternoon. So, I have asked the speakers to keep on time and if possible we will try to take a 10-minute break or something after the first couple of speakers if everybody is on time. We are about 20 minutes behind time now which isn't too bad.

So, our first speaker of the afternoon session will be Dr. Vostal.

Agenda Item: Prospects for Reduction or Removal of TSE Agent Infectivity from Blood Components by Filtration and Criteria for Allowing Claims: Introduction - Jaroslav Vostal, MD, PhD, OBRR, CBER

DR. VOSTAL: Thank you for the opportunity to share with you some of our thinking in terms of validation of labeling claims for TSE reduction studies with blood processing filters.

Now, my talk actually starts off with several introductory slides and since these topics have been very

well covered earlier today I am just going to go through them very briefly.

As you can see this is a brief schematic of the prion protein. It points out that there is different conformation of the protein. One of these is the pathologic form of the prion. It has protease resistance and less soluble.

The main point of this would be that TSE infectivity can be present even in the absence of PRPSC and so therefore PRPSC is only considered as a surrogate for TSE infectivity.

Now, this is a schematic of the organs of an animal and in terms of pathophysiology just very briefly if there is oral inoculation the infectivity goes through the LRS system and through the peripheral nervous system and migrates into the central nervous system.

As was discussed by Dr. Asher earlier today these models have demonstrated that there is infectivity in rodents during the asymptomatic phase of the disease and so it is pretty much agreed that at least in the rodent model there is infectivity in blood.

Those earlier experiments raised concerns that there is transmission by blood transfusion particularly for BSE. This was confirmed by the key experiments by Houston and Hunter where they used the sheep model and they had an

asymptomatic BSE-infected sheep. From this animal they were able to collect a full unit of blood and transfuse that to a healthy sheep which then went on to develop or some of these animals went on to develop BSE.

This model is interesting in that respect that it uses a full-size animal that is capable of donating a full unit of blood that can then be processed by the devices that we are going to be talking about later on today.

Moving on from the animal experiments there is now currently epidemiological evidence for variant CJD transmission by blood transfusion in humans. This was reported by Dr. Llewelyn and Dr. Peden. As we already heard earlier today there is a national CJD surveillance system in the UK that identified 48 individuals. Actually these are numbers taken from these publications. So, those numbers are slightly different, current numbers are slightly different. Basically there were 17 individuals identified that are still alive who received products from 15 donors who later became diagnosed with variant CJD. Two of these living recipients were subsequently diagnosed with variant CJD. One died from symptoms of vCJD. One died of unrelated causes and was later identified as having PrPres in his spleen and both of these patients received non-leukoreduced red cells.

So, if there is infectivity in blood, TSE

infectivity in blood we are very interested in looking at devices that are capable of reducing this and what we would like to do is to establish a system to validate the claims for these devices.

Some of the issues to consider for validation of the devices are the distribution of TSE infectivity in blood in humans or in the particular animal model, whether this infectivity is cell associated, whether it is intracellular or extracellular, whether the infectivity could be free floating in plasma and then we have to consider the physical attributes of infectivity in plasma, whether these are aggregates, fibrils or microvesicle.

We need to consider the interaction of the individual units of infectivity with the devices and finally to look into the distribution during and after processing to make sure that the devices actually eliminate infectivity and do not only distribute it such as would happen if you had a microvesicle formation from the infected cells.

So, the steps to validate the TSE claims of these devices would include in vitro spiking studies and this is as was already discussed before, these involve spiking of brain material usually homogenate into these products and detection of infectivity either by surrogate markers for PrPres, PrPsc or by bioassay again in an appropriate

animal.

Complementary to this is a model that uses endogenous TSE infectivity and here because the levels of infectivity are so much lower usually the detection is done by the bioassay.

Now, when we discuss animal models there is always the question of whether these are predictive to the human situation and some of the things to consider in terms of deciding whether these animal models are predictive are the comparability of the animal blood to the human blood, for example, to look at the type, number, size of these cells and the physical properties of the blood cells. Also, we need to look at the interaction of the animal and human blood cells with the different materials that they are going to be exposed to and also there are questions about the transmissibility or infectivity of a TSE agent that can be influenced by the strain of the agent, the dose of the agent, distribution of infectivity in blood particular to the agent and also and sometimes we are concerned about the distribution of normal prion, whether that can influence a distribution of infectivity in blood cells.

Now, these are just a small table comparing the hematologic values for various species. The species that we have listed here are human, mouse, hamster and sheep.

You can see the human value here on the bottom

line, the red cell volume and the platelet count. For the most part the platelet counts in these animals are equivalent and so are the white cell counts. The key differences between the human and the animal models comes in looking at the size of these cells. The human cells have about close to tens of liters of volume per red cell. You can see that in the mouse it is about half and particularly in the sheep these are very small cells and we always wonder whether these cells, whether the size influences the way those cells are processed by the devices and whether they can influence the way the infectivity can be distributed after it has been processed by the devices.

It appears that the small size of these cells is compensated by the increased number of these cells. So, the sheep and the mouse have a significant number more red cells than you find in human blood.

This slide talks about the normal protein expression on blood cells of different species. This is the difference. We are wondering whether the expression of prion protein on these cells would make a difference because there is a physical association between the pathologic conformation and the normal prion proteins in terms of propagating the infectivity.

So we looked at distribution in human cells by flow cytometry and for humans there is expression on

platelets, erythrocytes, and particularly on lymphocytes and monocytes.

If you look at some of these animal models that have been used to show TSE infectivity in blood it is striking how different they are in terms of prion protein expression, particularly for the hamster. We were not able to detect any prion protein expression. The mouse has comparability at least in red cells and sheep again platelets and red cells are devoid of prion protein. So, we are not exactly sure how this can influence the distribution of infectivity but it is something to consider when deciding the appropriateness of the animal model for this purpose.

So, besides looking at validating the removal of TSE infectivity these devices will also have to be evaluated for their impact on the transfusion product quality. The evaluation of the red cells, platelets and plasma will have to be done after they are processed through these devices and the FDA follows a standard evaluation approach to each transfusion product based on previous experience with the devices that process transfusion products.

For example, we have a lot of experience with leukoreduction filters and the next couple of slides will just share with you the evaluation process that we go

through to evaluate leukoreduction filters.

So, leukoreduction is the process of reducing the total number of leukocytes in the transfusion component. The methods that are employed are filtration or apheresis.

These leukoreduced products have been associated with the reduction of febrile non-hemolytic transfusion reactions, alloimmunization and reduction of CMV virus. Even though there is an association or beneficial aspect of these products none of the manufacturers actually claim these beyond meeting the criteria for leukoreduction and in the US the criteria for leukoreduction is less than 5 times 10 to the 6th leukocytes per transfusion product and in Europe this is reduced to less than 1 times 10 to the 6th leukocytes per transfusion product.

So, to validate leukocyte reduction filters for efficacy we look at the quantitation of leukocytes in a particular blood product before and after filtration. So, there is leukocyte count and the whole blood, red cells, platelets and plasma before and after.

We, also, look for definition of the timing of leukoreduction from the time of collection of the product. This is because leukocytes actually disintegrate within several days and in order to be able to remove the whole cells it is best to filter early on in the storage period.

We, also, explore the effects of temperature on

filtration efficacy whether it is done at room temperature or cold temperature and we also look at validation of the efficacy for a particular anticoagulant which can influence the way those cells perform as they are being processed through the filter.

In terms of validating these products for safety we look at biocompatibility and integrity of the materials. We look at their effect on cellular products. In particular we have criteria for in vitro recovery which is 85 percent. This means that we expect to see 85 percent of the products, of the red cells that are passed through that product to be recovered after filtration. We also have criteria for hemolysis at the end of storage and it should be less than 1 percent and we do in vivo recovery of radiolabeled cells in normal human volunteers and this is done for platelets and red cells.

For plasma we look at levels and function of plasma proteins and also complement activation.

So, this slide here summarizes our proposal for validating a claim for reducing TSE infectivity in human blood products. We would like to see a demonstration of a reduction of endogenous TSE infectivity by bioassay in two animal models and we suggest that this would be a rodent model as well as a sheep model.

We like to see a full-scale blood unit and

leukoreduction filter used. We would like to see the TSE infectivity to come from a BSE or variant CJD strain of TSE diseases.

The reduction of PrPsc in blood products will be considered supportive but not sufficient for a claim.

Study should be performed at two separate sites to minimize the issues of cross contamination and differences in laboratory practice and finally study size should be sufficient to support statistically valid conclusion from those experiments.

So, that is my introductory talk. Here are the questions that we are going to be asking you to comment on today. The question is are the FDA proposed minimal criteria for validation of TSE infectivity reduction by filtration adequate and appropriate? And we would also like you to comment on the following points. The rationale for use of specific animal models to study the properties of blood-borne TSE infectivity, are the experiments in rodents sufficient or should experiments also be done in sheep?

Is it necessary that each experiment should be done in two separate laboratory sites to ensure the reproducibility and accuracy of the clearance and are general descriptions of informative scaled-down processes for reducing TSE infectivity in blood?

There are several more comments that we would

like your input on, for example, levels of clearance acceptable for claims of reduced TSE infectivity in blood components as used in clinical settings, the estimated logs of clearance of TSE infectivity required to conclude that blood filters have effectively removed infectivity from blood components and the methodology appropriate to use in evaluating TSER agent clearance, bioassays for infectivity, Western blots or other assay for prion proteins.

So, those are comments to the initial question. The follow-up question is does the FDA's proposed labeling for a filter meet the appropriate criteria for a claim of reduction of TSE infectivity in blood or blood components and here we have several options. We have an option A which is this filter or device has been shown to reduce TSE infectivity in blood from an infected animal model. Now, A would then be coupled with labeling C which is a disclaimer that states that due to lack of feasibility studies have not been performed to validate this claim in the human population and the other option for labeling would be using part B which is this filter has been shown to reduce transmission of TSE infectivity by transfusion in an animal model and this again would be coupled with the disclaimer C.

So, if you can help us out by commenting on some of these issues that we presented we would be very

appreciative.

Thank you.

DR. PRIOLA: Thank you, Dr. Vostal.

The next presentation will be by Dr. Marc Turner who will discuss evaluation of prion reduction filters.

Agenda Item: Evaluation of Prion Reeduction Filters - Mark Turner, MB, ChB, PhD, FCRP(Lond) University of Edinburgh

DR. TURNER: Thank you very much, Madame Chairman. I am going to speak to you about briefly the UK evaluation process for prion reduction filters. You would probably be aware we are really somewhat on the front line on this issue and that we probably have put quite a lot of thought into these issues over the past 6 to 12 months.

The first comment is that you are probably aware that there are four UK blood services, English National Blood Service. SMBTS, and the Irish Blood Service, the Non-irish Blood Service along with the Seven Irish Blood Service and all accountable to different jurisdictions and so we have elected to approach this problem from the collaborative power of a working group which really is aimed at reducing the points of contact for the companies themselves.

So, they have one joint service rather than five different services also reducing the amount of duplication

of effort between the services.

So, the purpose of the group is to get a primary point of contact with any blood services or the manufacturers to foster a dialogue which I think has been very successful with those manufacturers providing expertise and advice from our end of the business on the clinical development requirements we require from these systems to liaise with regarding in-house operation evaluations, to liaise with other parts of the UK, Joint Professional Advisory Committees for Blood Services with regard to implementation of these devices and to ensure that the appropriate UK Department of Health decision making properties are also kept apprised and up to date and in the UK that formally means the UK Spongiform Encephalopathy Advisory Committee or SEAC and also the Committee on the Microbiological Safety of Blood, Tissue and Organs.

Now, this is a, I apologize for the complexity of this diagram but this is a diagram showing you the pathway that we have devised just to simplify it down for your comprehension. It really breaks down into three parts. The first part on the top left here is a process of establishing the technical specifications and that is being done through the JPAC process and there are three lines for that. One is the technical specification around

efficacy and the second is the technical specifications around quality and third is an operation and technical specification and that work is being doing and has been brought together and approved by the JPAC approval process.

A very important aspect of this is a risk assessment which has been carried out on our behalf by colleagues in the Department of Health,Economics and Operational Research, Statistics and Operational Research and I will speak to that issue in a few minutes.

Really that is this point now in the process. On the right hand side are what I would describe as preclinical evaluations around independent evaluation of prion removal and also around the independent evaluation of component quality. Those will then lead into the bottom left hand corner which will be clinical safety evaluation studies which I will describe to you.

The key issue from the point of view of the risk assessment was the issue of how great a reduction in infectivity is going to be needed to be clinically useful for us and we have made a number of assumptions around this issue. First of all I think it is worth pointing out that the current generation of prion reduction filters are applicable to red cell products only and not to plasma and platelets at this stage. We assume a red cell concentration in optimum added solution with prior leukodepletion and 10

to 30 mls of residual plasma and I will just make an aside comment here that the two transmission events, e.g., prime ones occurred almost certainly using an earlier generation of red cell products and not only were they not depleted but they probably almost certainly weren't in optimum additive solutions or they would have been in an anticoagulant with probably about 100 to 150 mls of residual plasma.We are making the assumption that total residual infectivity of greater than 2 ID50s transfusing into one infectious dose will transmit for certain and we are also making the working assumption that prion removal is going to occur mainly from the plasma.

Now, in terms of background in risk assessment we have used a starting proposition of 10 IDs per ml of infectivity with the assumption that a 450 to 500 ml unit which gives you between 4-1/2 thousand and 5 thousand infectious doses per unit. We have made a pessimistic assumption of no differential between IV and IC and we have followed Bob Riller's work in assuming for the purposes of this assessment that about 60 percent of infectivity goes forward in the plasma and about 40 percent associated with the leukocytes. So, in general terms there are about 3000 infectious doses associated with a plasma component and about 2000 infectious doses associated with the lymphocytes.

During the component processing as you have heard the units are subject to leukodepletion and although it is not shown but it is true it is about 1 times 10 to the 6th residual leukocytes per unit. In point of fact in practice we are finding that we normally get down to around 2 times 10 to the 5th residual leukocytes, so about a 4 log reduction and that is where you get this residual leukocyte infectivity figure of 0.2 IDs and in addition to that the residual plasma that is probably about 225 mls in a unit before component processing and depending on processing technique that is reduced to somewhere between 10 and 30 mls. So, traditional top-top component processing will give you in the order of magnitude of 25 to 30 mls plasma and a more modern bottom to top processing will leave you in the order of magnitude of 6 to 10 mls of plasma and in some scenarios that differential is actually of some importance. I have to say that those various assumptions that we have bought into with risk assessment are going back to the UK SEAC probably in this month for evaluation. So, they will be addressing some of the very similar questions that you are addressing at this table, ladies and gentlemen and I am vaguely hopeful that they will come up with similar kind of answers but we shall see.

In this illustration you can see that where you are only getting say for example 1 or 2 logs of reduction

of infectivity and in the plasma you have still got enough infectious doses to infect the patient for sure given the relatively large volume of plasma or large volume of the product and it is really only if you start getting to around 3 logs of reduction across the prion reduction filter itself that is in addition to any other effects of plasma reduction or leukocyte reduction you start to make a significant impact on the risk of infectivity. As you don't get beyond that in this model you find that reducing the plasma infectivity even further doesn't assist you that much because you have still got the residue of infectivity associated with the residual leukocytes.

So, the conclusions from that assessment are that 1 to 2 logs are likely to give value as clearly highly dependent on their route of infectivity and highly sensitive to that in the plasma. I haven't shown you this but the estimates are that if we were to achieve 3 logs that might amount to 75 percent reduction in the incidence of secondary transmissions in the UK and I think important further reduction in residual plasma could augment reduction in infectivity over the prion reduction filter and therefore the incidence of secondary transmission and so there is an issue as to whether if and when we implement these technologies we should really be looking also to from top to bottom in processing with maximal plasma reduction

and in addition any further effect on residual leukocyte count could be of additional significant benefit. So, if your second filter serves to drive down the residual leukocytes that might be of overall benefit as well.

So, that is obvious. The analysis has driven the prion reduction specifications and we are looking for a 3 log reduction to be shown and we then are sponsoring these immunoassays and bioassays. We would also like to see data from endogenous infectivity studies up to a limit or the model and we are asking that the model be capable of demonstrating at least 1 log reduction by immunoblot if possible but certainly by bioassay and asking the manufacturers to look at the issues of process, their levels including we have processed some blood at plus 4 degree C and some at ambient temperature. We need to know the filters work in both those environments, also, issues such as anticoagulant and use of the head height and things like that are found to be important for example in leukocyte reduction and we have asked the companies to propose surrogate markers or process monitoring which is perhaps one of the most challenging issues, but clearly it is not going to be possible on a day-by-day basis to measure infectivity in the blood that we are prion reducing. So, we are looking for surrogate markers. We need to be able to demonstrate parallelism of infectivity in a

surrogate marker if at all possible.

Then where possible surrogates present themselves PrPc is an obvious choice but the residual plasma in the red cell concentrate is minimal and therefore the gross levels of prion protein,normal prion protein are very small and it looks as if this might not be possible and so the discussion we are having at the moment is around a variety of different coagulation factors as potential surrogate markers.

Just to comment on the component policy specification we are advising all the various issues that of course were mentioned 5 minutes ago as to the Payee(?) Redbook guidelines which will be very similar to the guidelines here. We are looking at volunteer red cell survival studies. The only point I would draw out is that we have also patterned more detail than we normally would at changes in red cell membrane physiology and red cells do appear to express normal prion protein albeit low levels and probably in a truncated form and that is just a little matter of concern for those who operate by removing prion protein itself and so we are looking at the expression of protein on the surface of red cells before and after filtration and at the other factors which might lead to a reduced red cell survival as well as remember that these red cells have to be cross matched in hospital blood banks

up and down the country. So, any alteration in antigenicity would be of concern. I have to say for those that we have evaluated so far we have not really come up with any problems using any of this preclinical evaluation and I guess our main concern at the moment would be that this does take 14, 15 mls of blood out of the pack in addition to the leukodepletion filter which is kind of unavoidable and probably doesn't matter for most patients but for some patients who are receiving large volumes of blood or frequent blood transfusions may have a consequence of them receiving more units at the end of the day.

Now, the UK, SEAC and MSBTO and UK Blood Service chiefs have asked us to carry out an independent evaluation study and the purpose is really twofold. One is to provide some independent replication of some of the key data that is being provided to use by the manufacturers and where possible to extend that data into a more clinically relevant or clinically informative model and certainly I think we would be keen to look at more than one model. Data generated on a particular strain and animal species is generalizable. What we proposed in the first instance is that we will start very close to some of the data that has been provided by the manufacturers. We will introduce it in 3K in hamster homogenate for the exclusion homogenate for microsomal fraction and sonicated fraction assessed by

immunoblot and bioassay and in parallel with 301B BSE stedic(?) spike assessed by Western blot if possible and by bioassay and those two studies have now gone out. Because of the size of the studies they have to go out through a formal procurement process. So, the process is being instigated and we expect that to be completed by the end half of the new year. We are expecting data to be available within about 6 months of the initiation of the study. Bioassays are going to take up to 2 years. So, we are hoping that we will have initial data available by the third quarter perhaps of 2006. We obviously also very mindful that we would like to see endogenous infectivity studies probably in a rodent model and in a sheep study. We haven't planned these at the moment. We decided to focus on these initial studies in the first instance as a kind of gatekeeper and we will be putting further thought into these in due course. These will take an even longer period of time to create a sheep study which takes many years and I will come back to this theme if I may at the end. There is clearly a trade-off here certainly from our point of view between the comprehensiveness of the evaluation and the kind of time lines needed to implement these technologies in a timely way if they are effective.

Finally we are running a series of clinical studies and the primary aim here obviously is to look for

the incidence of adverse events and adverse immune responses. Study 0 has been commenced or is imminently going to commence in Ireland where we will be putting in the very first instance a model there that is full use of blood from the patients through the prion reduction filter. So, 20 single units will go into patients that are being transfused and 20 double units as it were in trying to keep the patients that are receiving 3 units which may take perhaps to the end of this year, the end of 2005.

Early in 2006 we will be initiating two much larger studies one in patients undergoing complex cardiac surgery, 300 patients in the UK all of whom are to receive the prion reduction filter treated with concentrates. Study 2 is a randomized blinded study in chronic transfusion dependent patients probably MDS patients rather than thalassemic patients because we want to note particularly these kind of patients who could be vulnerable to allo-antibody development and we want to look at that issue specifically and the statistical estimates are that these two studies have around 90 percent power of picking up one additional adverse event in these study populations.

So, that is really all I wanted to say ladies and gentlemen. The baseline assumptions and these proposals will go to the UK SEAC probably at the end of November for their consideration and commentary and the only other

comment I wanted to make is that I think that clearly the situation in the UK is an order of magnitude more grave than it is perhaps here in the US and I think that we are going to be faced with very difficult decisions and as to this balance between wanting to ensure a comprehensive evaluation on the other hand and wanting to move forward on the precautionary principle and implement these kind of technologies as soon as is reasonably possible.

Thank you very much.

DR. PRIOLA: Okay, thank you, Dr. Turner. I am going to put a question to the Committee. We have another hour's worth of talks on removal of prions using various filters. We can take a 10-minute break or we can plow through. If we take a 10-minute break we are going to be later. We are about one-half hour behind.

So, do the Committee members want to break or do you want to plow through?

We will take a short break for 10 minutes to sort of re-energize people and then we will come back at ten to four.

(Brief recess.)

DR. PRIOLA: Take your seats so we can get going. Our next speaker will be Dr. Sam Coker from Pall Corporation.

Agenda Item: Performance of Pall Corporation Leukoreduction Filters on TSE Infectivity of Blood Components: Experimental Studies and European Experience - Dr. Sam Coker, Pall Corporation

DR. COKER: Thank you very much. What I am going to share with you today are some of the results of the validation work that we have done on this particular filter which is currently being licensed for use in Europe.

Some of the topics that I will be going over today include how we validated the particular product. I will talk a little bit about the process that we use and then I will give you some of the results that we obtained using this particular Western blot.

I will go to part three very quickly just to give you a brief update in terms of the ongoing validation work that we are currently doing.

In part 4 of my discussion I will also give you some of the work that is also ongoing at Pall to identify a particular surrogate that can be used as a QC for looking at the efficient removal of infectivity using this particular product and I will give you a brief rundown of some of the red cell quality that we have already done and finally I will just give you an understanding of some of the work that we are currently doing in Europe.

Some of the tests that we had done to validate

this particular product including Western blot which is mainly an exogenous spiking study to kind of give us an idea of how efficiently this particular product is working and then we also did a bioassay to give us a much more realistic indication of the log removal and finally which we think is also the most important aspect of this is to demonstrate the removal of the infectivity from blood that had been infected endogenously using hamster model. So, these are the three main approaches that we use to validate this particular product.

The type of materials that we used in the validation included brain homogenates in this particular case using the hamster model. We also have data using mouse adapted variant CJD which is the closest we can get to variant CJD itself and we also did additional work with sporadic CJD but the main validation data that we used for CE map of this particular product was based on the hamster model.

The Western blot assay that we used is based on the publication from Wadsworth and his group in Edinburgh and we also validated assay using an outside contractor BioReliance which is an FDA licensed contract lab. I will skip some of this but these are just the dilutions that we used to validate the Western blot assay. So, you have an indication of how to measure the infectivity level or the

level of the residual amount of PrPres that is present in the blood.

So, the spiking study we did especially we used brain homogenates from hamster. We homogenized it. We added it to the red cells and we simply just filtered. We measured the level of infectivity before and after filtration using the Western blot and we evaluated several different processing conditions, the effects of different anticoagulant filtration heights, effects of leukocytes, the effects of different filter lots.

This is mainly to kind of study the filter that we developed. This is just an example of what a typical Western blot looks like. This is before filtration. This is the proteinase K resistant form which is believed to be the infectious form of the prion. This is before filtration and this is what we obtain after filtration and looking at this over a whole lot of processing conditions, anticoagulants, we saw no significant difference especially between the CPD and the SAGM. However, there is a slight increase or improvement in removal efficiency using CPDA-1 but a critical aspect of this is that the filter is effective in removing infectivity using different anticoagulants.

We also looked at the leukocyte reduced and non-leukocyte reduced blood that had been spiked with infectious prions.

Again, we didn't see any significant difference between that. We looked at different filter lots and again t he level of removal is consistent which again indicates that the manufacturing process that is being used to produce the filter is very consistent and we looked at the effect of filtration heights. We identified that the lower the filtrational height the more effective the removal process. So, that allows that to kind of identify the particulate filtrational height that would be used by this particular product.

We, also, identified filtrational temperature as well as the contribution to improve it in prion removal. Just to summarize this initial aspect of this particular filter it has already been CE Mark in Europe. So, it is available in Europe for use and the CE Mark is based on the hamster model. We have about 2.9 plus or minus .7 logs and we have demonstrated that this filter is effective across all processing conditions and most importantly we saw no significant difference between different lots of filters which indicates consistency in the production of this particular filter.

So, what I am going to share with you now are some of the additional tests that we did to kind of confirm that the filter is able to remove not only the hamster prion but also prions from sporadic CJD and most

importantly from mouse adapted variant CJD which I think is very critical and relevant to discussion here.

Again, when you think of the same experiments again, but here using brain homogenates with red cell measured in levels before and after using the Western blot assay and typically for most of the sporadic CJD you can see very clearly the three bands or the three different forms of the PrPres,the diglycosylate and monoglycosylate and this is very important because this shows that the sporadic CJD the amount of these different bands can be used as a way of identifying different strains of sporadic CJD.

Over here in type 4 which is believed to be very typical of variant CJD the main impact of this particular one is to demonstrate the effectiveness of the filter in removing different strains of prion.

So, we collected brain homogenates from different groups of patients that have various forms of neurological disease. Some of them have sporadic CJD and some of them have Alzheimer's disease and this was done in a double-blind study with the New York Medical School and the results show very clearly that the fourth filtration will identify those three bands that are present and identify what particular group has type 1 or type 2. The fourth filtration will identify the presence of those bands after

filtration, The filter completely removed the level of infectivity that is present.

The next part of the study was to now go forward and repeat this same experiment using mouse adapted form of infectious prion.

Again, this is before filtration using mouse adapted prion and after filtration again there is removal below the limit of detection of the current assay that we have.

This is just to summarize what we have done to date with this particular product. This is the result that we used for the CEMAC(?) using the scrapie infected hamster and you can see the fourth filtration. So, we are removing about 2.87 plus or minus .7 logs and this is using mouse-adapted variant CJD and there is about 2.2 plus or minus .32 logs and again we will find significant removal with sporadic CJD.

The next aspect of our work is to now demonstrate that the endogenous infectivity can be moved on to similarly. We can see additional confirmation of the exogenous work using a bioassay to determine the log reduction.

We, also, have an experiment going on using endogenous infectivity study and essentially this is just a simple endpoint titration assay to kind of give us an

indication of whatever we had with the Western blot correlates with the bioassay. These experiments are currently ongoing and the results should be available early next year and this is the endogenous infectivity again. This we believe is probably one of the most critical aspects of the validation program because as we have discussed earlier today the use of the brain homogenates has its limitations and the best way to avoid the controversy regarding brain homogeneity is to actually use endogenously infected blood samples. So, we have blood collected from about 100 to 200 hamsters and these are then processed as you normally process with the red cell and plasma and the red cell that is endogenously infected is then filtered with the filter. The filtered blood is transfused or intracerebrally injected into about 400 hamsters. Two hundred of them receive the pre-filtration sample and these particular experiments are finally ongoing and by the middle of next year to early part of next year we should be able to get some indication as to what the results are.

So, in summary some of the validation work that we used to study to see clearly were based on the Western blot. We are currently doing the endogenous infectivity study to obtain the actual log reduction that will complement what we have with Western blot.

We, also, have the endogenous infectivity study that is also currently ongoing. So, we will be able to get an indication of how effective the filter is in preventing the transmission of prion disease.

The next part of my talk actually relates to what Marc Turner mentioned earlier about identifying a particular surrogate that can be used to monitor the effectiveness of removal of infectious prions from blood.

We have done quite a lot of work in looking at several different plasma proteins that are present and we have currently identified a couple of proteins instead of the PrPc because of this limitation that we can use to monitor how effective the filter is in removing prions from blood. So, this can be easily incorporated into any blood bank and we feel that this should be a very good way of performing a QC.

The next aspect from my work, I mean this we have already gone through is to give an indication of the red cell quality. We talked about how we validated the product. We have also talked about the effectiveness of the filter being able to remove different strains. The next aspect is to see what is the quality of the red cell after going through the filter.

We did a whole series of studies including hemolysis study looking at the membrane integrity and the

neurologic properties in the survivor and by all accounts of what we have done to date this is just an indication of the results. We didn't see any particular change at all and when we look at the hemolysis at the end of a 42-day study especially for sagendazen(?) it is still well below the Council of Europe guidelines as well as the standard from the FDA.

In addition to looking at the quality we also look at the safety of the product itself and all of this is really according to the regulations from the FDA that had been established for leukocyte reduction filters and so today all of this has been passed and we have not seen any particular concern.

So, in summary the red cell quality is very well maintained and we did not see any concerns about the safety of the product or of any of the parameters that we measured to kind of look at the safety.

Overall the filter that I have just described to you has been able to demonstrate that we can remove at least a significant level of infectious prions from blood using brain homogenate. We have demonstrated it can remove different strains of prions, sporadic as well as mouse adapted and overall the cell quality was very well maintained throughout the whole process and most importantly we have also identified a series of proteins or

a couple of proteins that can be used as you see of the prion removal efficiency of the filter.

The European experience is that currently we have a series of studies that are currently going on in Europe just to try to validate some of the work that we have done. So, we expect some of these results to come back sometime in 2006.

Thank you very much.

DR. PRIOLA: Thank you, Dr. Coker.

Our next speaker will be Dr. Bob Rohwer who will talk about selection and performance of resin-bound ligands for removal of TSE infectivity.

Agenda Item: Selection and Performance of Resin-Bound Ligands for Removal of TSE Infectivity From Plasma - Robert Rohwer, PhD, PRDT (with Prometic and ARC)

DR. ROHWER: Thank you very much. I am wearing a little different hat than I usually do here because I am representing Pathogen Removal and Diagnostic Technologies who is the company which I helped found and which is developing this removal technology.

I am going to concentrate mostly on the infectivity studies because that has been my contribution to this effort and I will summarize the work of the other partners in this.

As we heard earlier in the day we do have now a

confirmation that there is a transfusion risk associated with variant CJD. I won't go over this anymore and Dave Asher very nicely summarized this data for me earlier, that at least in the hamster model that we have characterized in our laboratory we get a median value of about 10 infectious doses per ml in blood and that doesn't seem like very much of a risk unless you consider it in terms of a unit. For example, at the same time in this disease when we have 10 infectious doses per ml in blood we have got 10 billion infectious doses in the brain per gram of brain in the hamster model.

So, it really is a very, very small effect compared to what is going on in this animal at that stage of the disease.

On the other hand if we consider the way in which we actually use blood it is not on a per ml basis but a per unit basis. In a 500 ml unit we might have as much as 3-1/2 logs of infectivity.

The other important piece of data is our studies on the appearance of infectivity in blood. Dave Asher showed this earlier and the main point here is that we first saw the infectivity in this part of the infection but because we took points along the way if we extrapolate this back to here it is about one-third of the way through the infection that we first start seeing infectivity.

This would be nothing on the basis of infectious doses per ml, but if we put it in terms of infectious doses per unit even at these very early times we have significant amounts of infectivity probably plenty to cause an infection if a whole unit was given.

How do we deal with this? This is the usual triad of, triad because usually these are lumped, of approaches to controlling TSE pathogens and these particular diseases this group up here are all problematical in various ways and therefore we decided to or I have had been advocating this approach to controlling the risk from these particular pathogens for some time and there are some other real advantages to this in my opinion.

First, it removes infectivity that can't be detected with diagnostics. Every diagnostic has a limit of detection and a perfectly working which we may never get to, but in theory at least a removal device would be able to remove infectivity that could not be detected, i.e., infectivity that was below the window period limit of detection for any pathogen, not just these pathogens.

In the case of TSE diseases this would be even clinical disease for blood because we still don't have a convincing assay for detection of the infectivity in blood or the POP signal in blood from the infection, but it also applies to preclinical disease from brain or other tissues

where if you go back early enough in the infection you still may have a risk for example for tissue donation or something like that and still be up against this same limit of detection.

The other advantage of this method is a very big advantage. There is no reason necessarily to need to discriminate between the abnormal from the normal form of PrP.

In the case of blood we can remove both and there is actually even some advantage to doing so because there is measurable PrPc in blood and so far there has been no demonstration of PrPres in blood or we don't have methods sensitive enough to detect it.

So, we can use the removal of PrPc as a assay for the removal of both as long as we have a device that will use both and in fact we selected on purpose for resins that do both and because it can actually access this area of the infection that is below the limit of detection of diagnostics it may be more comprehensive than a diagnostic and in the end it could even be less costly to deploy than diagnostics.

PRDT, Pathogen Removal and Diagnostics Technologies was a company that was put together by Dave Hammond and Rubin Carbonell, a couple of combinatorial chemists. Dave Hammond has, also, had a lot of experience

in the plasma industry. Rubin is an engineer at North Carolina State and myself providing the prion expertise and we interested the Red Cross Informetic(?) in investing in this and it has become a joint venture of these two corporations and we now have Macropharma(?) as a major blood bank manufacturer in Europe as the partner for marketing and production of devices.

The way these things came into being is we screened several libraries of various compounds. They represented over 64 million combinations in total. We looked at 8 million beads in the course of doing this, doing a selection assay based on protein methods, a blotting method and the Western blot. Once we picked out the first 200 candidates we decoded the beads and then made larger quantities of these materials so we could go through a secondary characterization based on protein and then out of that we got another group from which we selected seven for infectivity characterization, first by spiked TSE experiments and then an endogenous experiment and I am going to spend the rest of the talk talking about these experiments here.

These are the kinds of things you get out of these screenings. What you are looking at here is the binding in duplicate. These are duplicate samples which is why you see pairs here without protein PHK and with protein

PHK in plasma as a couple of resins. So, this particular resin binds well in buffer and in plasma. Here is one that binds well in buffer but not in plasma. Obviously we are interested in this type of resin for further development. That doesn't mean that this particular compound, this particular resin wouldn't be useful but it won't be useful for this particular application and we have in our pocket about 200 of these.

The infectivity experiments, this has been discussed before but I want to go over it once more just to emphasize the difference of what we can get out of the various modalities.

If we spike brain-derived infectivity into red blood cells we have the advantage of high titer, high levels of removal that can be demonstrated but will have uncertain relevance because we don't know how well this spike regardless of how we may manipulate it before spiking represents the infectivity in blood.

This is a somewhat earlier readout. It is a fairly crude measurement and it is less costly. If we do an endogenous experiment relevance is not an issue. It is relevant. It is blood-borne infectivity but the titer is very low about 10 infectious doses per ml in whole blood and even lower for our red blood cells where the plasma concentration is lower and the white blood cells have been

removed and the most we can hope to demonstrate inoculating 5 mls of this product into hamsters, into 100 hamsters is about 1-1/2 logs of removal.

It is a long experiment. We have to take the animals to the end of their life essentially but the measurement is very precise by the limiting dilution method which Dave Asher referred to earlier and I really don't have time to explain right here and it is quite a bit more costly for those reasons.

Typically in this type of experiment to measure the infectivity in these models we do endpoint dilution titration. We do serial 10-fold dilutions, inoculate into groups of animals. They get sick and at some point you run out of infectivity and you can calculate a titer from that.

There is also a dose-response associated with this. These animals come down quicker than these and the dose response as Dave showed earlier disappears in this group right here. We are going to make one set of measurements using the dose response in this part of the curve and the endogenous measurements using the infection at the limiting dilution using the Poisson distribution of infectivity into animals at the end.

Now, the dose-response measurements I have always had a problem with this. I have always resisted it but we had a lot of samples we wanted to screen this way and as a

consequence we needed something that we could afford to do basically and the main problem I have had with it, one of the problems I have had with it is that the endpoint is hard to define because there is a progression of symptoms especially in the hamster model but saying exactly when one stage of the disease ends and another starts there is a lot of interobserver variation in that.

We developed this method of just weighing the animals. They gain weight throughout their life and as they get sick their weight falls and taking this cutoff at 80 percent of maximum weight as an endpoint.

So, from this we get an empirical determination. It is observer independent. We developed our dose-response curves from duplicate measurements, two completely independent measurements. They are indicated here. Each animal is indicated by a circle here and the means by these triangles and they are displaced around these values. So, you can see the data actually and it is actually much more tightly clustered than I ever would have thought.

Here we are getting at limiting dilution where some of the animals do not get sick and so how does this assay work? We took a large pool of human red blood cell concentrate and then divided it into one unit quantities after spiking it and mixing it. So, all of these challenges were identical and then passed it through our device. This

was a prototype device at this time in the development and then collected the unit here, looked at the PrP scrapie signal that is retained on the device here and measured the incubation time of the infectivity that remains in this bag in a test group here.

So here is a case where we removed the infectivity. We can see it here. There is not enough infectivity left. There is not enough PrPres signal left in this bag to measure it by Western blot. So we have to go through the infectivity measurement. Here is our standard curve again. Here is our test group. Here are the incubation times for the test group. They are at this concentration but they are displaced off the curve to this level.

We carry this down to where it belongs on the dose-response curve and we see that we have got 4.33 log of dilution between here and here and we presume that we have removed around 4 logs of infectivity.

We did that for a number of samples. These are all different resins here. These are controls here. They are all at 10 to the minus 3. They all belong on this line but I displaced then so you can see them. This is the data I just showed you. Here is the next best one and here is the worse one right here.

So, the clustered in this fashion. Here is the

data summarized in a bar graph, log removal on this side and log reduction on this side. These are our controls again and these are all probably pretty much equivalent within the error associated with this type of measurement.

Just to remind you we challenge with a million infectious doses per ml. The actual blood will contain about 10 infectious doses per ml, but if you will remember we did get infections and that worked out to about 20 infectious doses per ml. Not all of the infectivity was removed by this filter and this infectivity right here when passed through subsequent resins of the same type was not removable. This is in some form that is not recognized by the resin.

So, what we have is we have a spike at this level, a residua at this level and in blood if the proportion is the same we will start with 10 infectious per ml with a residual way out here at .0002 infectious doses per ml. This would not be significant and we would still have quite a significant margin of safety.

Nevertheless we can't be sure of this. We don't know that this distribution is the same because of this question that has been discussed throughout the day. We don't know what the form actually is in blood. Therefore it behooves us to measure this to the best that we can that we can actually remove the endogenous infectivity from blood.

What if it is all in this form, for example?

There is a form that would not be removed by our device and that is cell associated infectivity. Everything that we will have to do looking at endogenous infectivity will have to be done with leukoreduced blood because we don't claim that the resin would remove cell-associated infectivity and we know that there is a significant amount of cell-associated infectivity from this leukoreduction experiment that we did a couple of years ago in our laboratory where we took a unit of whole blood prepared from hamsters, that was 500 mls of blood, passed it through a leukoreduction filter and titered the infectivity before and after leukoreduction and got this type of data. This is the leukoreduced whole blood. These are the incubation times down here. These are the animals that did not come down and there is about 40 percent removal here of the starting infectivity by leukoreduction.

This, also, gave us a way to do the experiment because we had this as a precedent and we knew what to expect in terms of the amount of infectivity we could in the leukoreduced blood in order to challenge the device with endogenous infectivity. So, we expected about six infectious doses per ml in whole blood. We knew that if we had made red blood cell concentrate from this and ended up with 20 or 30 mls of plasma our expectation was that we

would be down to less than a single infectious dose per ml in that material and this would not be, we didn't consider this to be even though this is the target of the device and the target we were going for we didn't think we could do a meaningful experiment with this material.

So, we actually have done this with whole blood even though we consider it to be a, leukofiltered whole blood even though we consider it to be a worst case. It gives sufficient titer from measurable effect and we have the preceding experiment to inform us.

We are currently at about 420 days, well, at 420 days when I made this slide which was about 30 days ago and this is where we are in this experiment. What I have plotted here is this is incubation time on this axis and the number of animals on this axis. This is all of the limiting dilution titrations we have done to date in our laboratory that are summarized in that first slide that I showed you showing you 10 infectious doses per ml where this is a distribution of about 500 animals from blood infections that have come down over the course of those experiments and this is the distribution of those infections and the point I want to make here and this is, if we add these all up and say, "What proportion of the infections have occurred by 215 days for example?" it is about 50 percent of them, and that is indicated on this red

curve here. This is the cumulative number of infections that have occurred at any given time. You can see that they go all the way out to 550 days. So, you can get infections out at this level but at 420 days when I summarized the data that I am going to show you next we are about two-thirds of the way through the infection but we are about 94 percent, we have seen 94 percent of the infections we are going to see. That is the main point. We are very close to seeing everything we are going to see in this experiment. So, here is the data. In this case we took our non-leukoreduced whole blood before leukoreduction and put it into 50 animals instead of 100 and so you need to multiply these numbers by two to get a direct comparison with these values over here.

Each dot here is an animal. Each S means an animal that has come down with scrapie. Here we are in the challenge. This is the titer in the leukoreduced blood. One of our disappointments is we are seeing a lot less infectivity in the leukoreduced blood than we saw in our first experiment, the experiment that was published in the Lancet last year.

Here is the final flow through the device and thank God we haven't seen any infections yet though every time I get this update on this data I get a little heart flutter because we are getting so close to the end of this.

We are 94 percent complete. This is the data in terms of infected over total number of animals inoculated and this is what if we project what it should look like at 100 percent completion we will get another infection here, another infection here, another infection here and hopefully we will see no more infections here.

This titer is coming out to exactly what we always get, about 10 infectious doses per ml or what we usually get but on the other hand we are seeing a lot less infectivity in the challenge. We were expecting about 6.2 infectious doses. Here we are only seeing 2.6, about half that and what has happened here is that the leukoreduction was much more efficient this time than it was the first time around where you lose 75 percent of the infectivity in the leukoreduction instead of 40 percent.

Nevertheless if this relationship remains because we have inoculated 5 mls of this we will have 13 infectious doses in that 5 mls of blood and we will be able to demonstrate a log. We have already demonstrated a log of removal.

Now, just two more comments on what is going on in this leukoreduction. We have done another experiment during the last year and that is we have spent a lot of time over the last 8 years or so trying to figure out what white blood cell type actually harbors the infectivity and

every time we purify the white cells we seem to lose the infectivity. So, we just did a simple experiment. We collected the white blood cells from a buffy coat and measured the infectivity before and after a simple centrifugal wash in PBS and that centrifugal wash removed 80 percent of the infectivity.

So, in a typical leukoreduction we have been thinking about this in terms of 50 percent of the infectivity in plasma, 50 percent in the white blood cell fraction because we have shown in other experiments that it is not in the red blood cells intrinsically associated with red blood cells or platelets at least in this model and 80 percent of this plus this leads us to believe that we really should be thinking about the infectivity as plasma associated and it is not that tightly associated white blood cells at that and as a consequence there may be some variability here in the leukofiltration results just based on things like flow rate or pretty mild parameters that we don't have a understanding of yet.

So, that is just summarizing that in words. So, where are we now with this? We have this resin which we have now characterized in an endogenous experiment. It has a very high affinity for the prion protein, 10 to the minus 9 KD. This is mysterious to me and I think it is indicating that there is some cooperativity in this binding. It

removes PrP from rodent brain and human brain. We have looked at scrapie, sporadic CJD, familial CJD and variant CJD using the WHO standards and it binds to all of these. It works in red blood cell concentrates, whole blood and plasma. The plasma work has only been done in vitro so far. We get 4 logs of removal of brain derived infectivity greater than 1 log from endogenous infectivity to date and the human compatibility studies have all been done by the Red Cross and of course they have done them very well and so far we have seen no impact on red blood cells, plasma proteins or platelet activation.

By this I mean plasma proteins that are important for therapeutic development. We have a partnership now with Macropharma and they will manufacture and supply this and it is in the latter stages of development.

I wanted to acknowledge Louisa Grigoria in my laboratory and her staff who have spearheaded this effort on its day-to-day basis and I will conclude there.

DR. PRIOLA: Thank you, Dr. Rohwer. We will move on to our final presentation and that is Dr. Ralph Zahn from Alicon.

Agenda Item: Other Industry/Academic Filter Chromatography Develper - Dr. Ralph Zahn, CEO, Alicon AG

DR. ZAHN: Good afternoon and thank you very much to the Committee for inviting me here to talk which is a

big honor for a small Swiss company actually. So, when we started with our company at the beginning of last year we decided to work on BSE diagnostics and also on biochemicals which are somehow related to BSE but then we found that our technologies can actually also be applied for other diagnostics like scrapie or CJD and it also works with prion filtration and most probably also for Alzheimer's diagnostics and so we currently have 35 products to supply which are 33 different recombinant prion proteins and two monoclonal antibodies and so two of these prion proteins are probably important for diagnostics and also for prion filtration, PrP pure and also PrP beta.

So, why are they important? I think they are important for TSE diagnostics and prion filtration because they can be used for research and development. They can be used as positive and negative controls and they can also be used for checking the quality control for assays and filters and so Alicon PrP pure corresponds to the natural prion protein found in healthy humans and animals or in other words PrPc and this is available for different species including bovine, deer, hamster, human, mouse and sheep.

So, we not only have different species available but all the different lengths of different constructs in particular for the human protein. We have seven different

constructs. Also for sheep we have three different polymorphisms and then on the other hand we have PrP beta which corresponds to the natural form of the prion protein found in infected humans and animals or in other words the PrPsc and again we have different species available for this protein.

So, PrP beta is produced starting from PrP pure, the same principle in a three-step procedure which should somehow mimic the production of PrPsc in nature. So, we start with PrP pure and then in the first step we have a conversion from PrP pure to PrP beta star where PrP beta star is the better suited protein. It is oligomeric and it is completely water soluble, and in the second step we have a conformational transition in 2 PrP beta fibrils. So, we think that these three forms of recombinant prion proteins resemble very much the three forms in vivo, so, PrPc, oligomeric PrPsc and also PrPsc fibrils and I also should mention that this procedure has been worked out at the ETH in Zurich in the lab of Professor Retrich and the main work was done by Atoss and Vias and this is some of the biophysical data just to show the conformational transition from PrP pure into PrP beta. You see PrP pure before conversion there is an alpha helical secondary structure and after conversion there was a better secondary structure as indicated by the single minimolar to 15

approximately and this also works for a different construct as is shown here. Then this is the PrP beta fibril which is typically formed off of filaments which are elegantly wound as shown here and where the single protofilaments(?) show this beta substructure and exactly the same morphology has been described for PrPsc, for natural PrPsc and similar to PrPsc the PrP beta also binds Congo red and shows this typical Congo red bifringence(?) and it is also, PrP beta is also more proteinase K resistant compared to the normal prion protein PrP pure. There is at least a factor of 10 difference and there is also an accumulation of this typical 16 kieregard(?) fragment which has been ultimately described for PrPsc and PrP beta binds also to PrPsc conformation specific antibody which is the 15 P3 antibody from another Swiss company. So, this binds PrP beta not PrP pure.

Now, we also have done the biosafety checks because we wanted to know whether this protein, PrP beta is also infectious. So, this was actually what we wanted to show at the beginning but we are trying this since a very long time but we never could really show infectivity. So, we did infectivity checks in TG20 mice but also in wild-type mice. We inoculated about 18 micrograms of mouse PrP beta into these mice. We, also did a serial transmission experiment but we never got clinical signs or pathological

signs or proteinase K resistance in the brain homogenate.

Of course, this also has some advantages because if you want to work under less stringent biosafety conditions then the PrP beta has some advantages over natural PrPsc.

So, the main project we are working on or we have worked on is the matrix which has a very high affinity to all kind of prion proteins and so why is this so? Because this matrix does not have only one binding site for PrP but it has three different binding sites indicated here by different colors, so making this contact between PrP and the matrix very efficient and does really very tight binding and another interesting feature of this matrix is that the specificity for the PrPsc conformation can be modulated. In the absence of aligning with X, so zero concentration the matrix binds to PrP pure. So, corresponding to PrPc and to PrP beta corresponding to PrPsc and it also binds to dimeric forms of those proteins and there is only a very low amount of unspecific binding of these averages here in 1000-fold excess over the prion proteins, but if you increase the concentration of this ligand X let us say for example to four then there is only PrPsc bound but not PrPc anymore. So, the specificity of this matrix can be modulated.

So, the applications of this matrix technology,

the name of this we call Octapetform(?) for more for historical reasons so that there are two main applications. One is PrP enrichments which can be used for prion detection and the other application is PrP removal which is of course necessary for prion filtration.

So, I would like to show you some examples for PrP enrichment. So, this is an enrichment experiment where we started with 4 mls of plasma and we did an 800-fold enrichment of PrPc in a lot of healthy cows which are not infected with BSE and as you can see here we have a nice signal of PrPc which is 800-fold more sensitive compared to the normal Western blot assay and so we mostly observed the diglycosylated form of PrPc in the one blood in this case in plasma and if we add some proteinase K then of course the protein is degraded.

A similar picture we see also in interface cells or in white blood cells. Again we have a strong signal here after enrichment corresponding to diglycosylated PrPc. This is our standard protein and this is a dimer of the standard and again if you add proteinase K then we get an intermediate fragment first and then at 5 micrograms per ml the PrPc is completely degraded and the same also works for platelets, again this strong signal here for the platelets and this also works for PrPsc from scrapie brain homogenate which was added to bovine plasma in this case here. So, we

started with 1.5 mls of plasma. So, we have a 300-fold enrichment and we can show how we can measure very low amounts of proteins for example here 800, 200 and 40 picograms of PrPsc using this enrichment procedure.

Now, so to summarize using this technology we are able to at least 5000-fold increase the signal for example in Western blot assays using this matrix and I am sure we can go even to a higher concentration if we would try. We didn't try so far and in terms of protein concentration we even have a more than 50,000-fold enrichment process going on here.

Now, so we have applied this technology for a BSE live test for cattle and so I would like to show you just one result here. So, on the left side you see a cow which has been experimentally infected with BSE prions about two twenty months ago and this blood was sent us from Germany and on the right hand side you see a control. There is no proteinase K resistant protein seen and this pattern of four bands we observed for, as well as we observed experimentally infected cows as well as natural BSE cows.

This is also an important figure which shows different variants of our matrix which has been used for treatment with BSE infected cows again but which will also spike with PrPsc and as you can see here only one matrix bound to this typical four band pattern which indicates

endogenous PrP from an infected cow but the other matrices only recognize PRSE after spiking. So, this means that this is quite important in my opinion because this shows if you have a matrix which binds to PrPsc from brain homogenate this doesn't mean that it also hinds at the same time endogenous PrP.

Now, I am now changing to prion filtration. As we heard this morning there are some important applications like plasma fractionation, plasma banks and also for pharmaceutical industry. This is an example where we have completely removed PrPc from bovine plasma of a healthy cow. So, we did this experiment similarly like before. So, we started with 20 mls of plasma, treated this plasma with our matrix and then we diluted the bound protein and loaded this on a Western blot here for two different cows, A and B. You can see one cow has a little bit more PrPc than the other cow and this is the recombinant protein again and this is done after recombinant protein and if you do this experiment a second time then after the second time there is no PrP left anymore in the plasma. So, this means that we have completely removed the PrPc from the plasma using our matrix and with a detection limit of less than 1 picogram per 20 mls which is about 50 femtograms per ml which corresponds to approximately 0.5 infectious units per ml of blood plasma.

Now, this is a similar experiment with human plasma which was spiked with PrPsc. Again, after the first treatment we see a lot of PrPc and PrPsc before and after proteinase K digest on the Western blot and after the second treatment we only see recombinant prion protein which we used as a marker to show that the matrix works actually but after proteinase K there is no protein left anymore. So, again we have completely removed PrPsc in this case at the concentration of lower than 1 picogram per 20 mls and of course we wanted to know whether our matrix has some effect on blood coagulation. So, we did some different, we did various tests on the different variants of matrices and the results are summarized here. So, there is some effect for some matrices for example here in this global factor but there is a small difference compared to the control. There is also some effect of matrix on the two. There is a slight increase in Factor 7 and there is also a small decrease of von Willebrand's factor of measures 1 and 3 but all the other parameters for example, fibrinogen Factor 5, Factor 8, Factor 9, the fibrin dimers, the three inhibitors of the protein concentration are not changed at all and most importantly there is one matrix where we didn't observe any change in these parameters. So, this matrix is probably quite useful for that transfusion.

So, to summarize the advantage of our technology

in our opinion at a really high affinity matrix first and second we can completely remove all prion proteins including PrPc and PrPsc and also endogenous PrPsc as we have seen from this in cows and this matrix also has a high compatibility to blood plasma.

These are the people who did the work finally and thank you very much.

Agenda Item: Open Public Hearing

DR. PRIOLA: Thank you, Dr. Zahn.

Okay, I think we will move on to the open public hearing portion of the afternoon.

DR. FREAS: Dr. Priola, at this time we have not received any request to speak in the open public hearing in the afternoon. Is there anyone in the audience at this time who would like to address the Committee on this topic?

I see none. So, we will move on.

Agenda Item: Committee Discussion and Recommendations

DR. PRIOLA: Okay, so I think we should address the two questions that the FDA has posed to us based upon topic 2.

So, the first question is are the FDA's proposed minimal criteria for validation of TSE infectivity reduction by filtration adequate and appropriate and I think if you will put the slide up there this is a voting

question but before we vote they would like us to go through and comment on some of the points behind this question.

So, if you look in the handout from Dr. Vostal on the last page you will see those comments, the points they would like us to comment on.

The first is the rationale for the use of specific animal models to study the properties of blood-borne TSE infectivity. Specifically are experiments in rodents sufficient or should experiments also be done in sheep or any other sort of TSE model?

So, I would like to open that for comments from the Committee.

One model, two models?

DR. TELLING: I had a comment talking about using sheep but another large animal model that springs to mind is infected cervines because obviously there are large amounts of blood available from such models, and it would appear that the lymphoreticular distribution of infectivity to the extent it has been looked at may mimic variant CJD.

DR. BROWN: Just as a practical matter I don't think the FDA can require companies to use small ruminants if they are going to require bioassays. I think bioassays and small ruminants or large ruminants or any ruminants simply won't get done in time to be of any use. We won't

have a problem anymore when the titers are finally in.

If they were to use Western blot as a marker for infectivity then I think it is possible to require larger animals. My inclination would be to go with two strains in an appropriate rodent model and I think one of those strains would have to be 301V mouse adapted variant and the other model could be sort of anything you want, 263K in a hamster but I think maybe two species, two models, that is a strain in a mouse and a strain in a hamster and the mouse strain clearly should be 301V as the closest thing that is in a rodent to variant CJD.

DR. PRIOLA: One of the speakers brought up earlier that the size of the blood cells for example differs in blood from different species. What about trying to address that sort of issue? That might be one of the things the FDA is thinking of with using different animal models and using rodents, either mice or hamsters wouldn't necessarily address that unless you just want to stick with Western blot for ruminant models.

David?

DR. BOLTON: What if the infected blood came from naturally infected larger ruminants but the bioassays were done in transgenic mice? Then you have a chance of getting the data back in some sort of reasonable time but you are actually studying the natural product which is closer to

what you are looking for.

DR. BRACEY: Are transgenic mice of this sort readily available? I am not a transgenic person. If they are not that kind of throws a wrench into practicality, but if they are --

DR. BOLTON: I don't have them either but I understand that they are in development. Glenn, ovinized transgenic mice, are they --

DR. TELLING: Yes, the ovinized mice have certainly been published on by the French group in particular. As to whether or not they are available, you know, there are cervinized mice.

DR.BOLTON: We produce cervinized mice as you guys have produced them and the group at Case Western has and Stan's group has and we are certainly committed to making all of our transgenic mouse models available and those include not only cervinized but also ovinized and bovinized. So, yes, as far as we are concerned they are available.

DR.ALLEN: I am certainly not expert in this area but I will make the general comment that we have heard an update today and certainly been given enough background information to suggest that there is a lot of variation here both in terms of the host animals as well as the prions themselves and I would suggest as the former

speakers have said that looking at multiple models makes a lot of sense and not going with just a single model.

In addition assuming that this is to be used on human blood and plasma certainly one wants to make certain that there is no damage to any of the cellular components or to the end product from the use of the filtration.

So, one looks at it, needs to look at it from both the safety as well as the efficacy points.

DR. PRIOLA: So, from a practical point of view where would this blood come from if it were, I mean would manufacturers be required to have scrapie-positive sheep and CWD positive deer and elk or are there other sources for that available?

DR. BOLTON: They can just go to Colorado and Wyoming, can't they?

DR. PRIOLA: It is possible I guess. That is a practical point but if that blood were available then it would provide a basis for that sort of test. It would also sort of negate the second comment there, is it necessary that each experiment should be done at two separate laboratory sites and that has to do with the contamination issue from people not being careful enough when injecting their animals and if you had common source blood and the same sort of transgenic model systems it would be easier for independent labs to do that.

Comments on any of those first two points? I think it is a really good idea, actually taking blood and using the transgenic mice to test.

DR. ROHWER; I could say a lot about this but I will try to keep my remarks fairly brief. Obviously the best of all possible worlds would be to use human blood inoculated into a transgenic animal that was sensitive enough to assay it and I think that has always been the dream behind the transgenic work was to make that possible but as far as I know it is not possible or not possible yet and certainly there have been a lot of people trying.

The next best thing would be to have a large animal model like the sheep assayable in a transgenic and I think there are a number of us who are trying to do this and we have been talking to each other and hopefully that will come to fruition but we have no idea whether it works now. You know we know you can infect transgenics with brain-derived sheep infectivity but whether that will work for blood and at what efficiency who knows, and the final thing I want to say is just you should consider the following aspects. There is a reason that we use the hamster and we stumbled on it but it turned out to be a pretty ideal model. It produces enough blood that we can actually obtain the blood in quantity. Using 120 hamsters we can make a unit of blood and that is a doable thing. It

takes a morning, etc., and we can quantitate it because the animal is small enough we can put it into a large number of animals to do that.

Doing the same types of experiments in mice and we have done this a couple of times now in the 301V mouse it takes eight times as many animals to get the same volume of blood and it takes twice as many animals to do the titration because you can only put half as much inoculum in the brain of a mouse compared to a hamster. So, the cost and logistics go way up, plus it takes a lot longer for the infections to develop in mice.

So, they are also much more sensitive to blood. Blood is toxic when it is inoculated IC. The hamster can tolerate it if you do it right. In the mouse it is much harder to do and so there are lots of things working against the mouse and for that reason we have recently passaged through OMV into the hamster just so we will have access to it but of course by putting it in the hamster that doesn't give us access to transgenics because so far no one has made a transgenic hamster though we have been watching that very carefully and then the other thing I think you should consider is in the sheep model besides these, there are some serious differences in the behavior of sheep blood and plasma compared to humans and hamsters. Actually we find the hamster to be a better model for human

blood than sheep even though we use the sheep a lot.

Nevertheless it is going to be hard to quantitate it unless we have a transgenic and if try to do the Fiona Houston type of experiment back into sheep when would we ever know whether the experiment is over? What we are looking for is a negative and a negative result. We don't want the animals to get infected. Well, do you wait until they die? Is that 10, 12, 15 years, something like that? They have a fairly long life span in captivity anyway and so anyway these are some other things that I think have to go into the planning of this and I think it would be important for what I would like to see the FDA do which is voice their concerns in a more general way and give people as much flexibility as possible in meeting the requirements that you want to see them meet so that as animal models develop they may converge on this need or not but basically what we want to do is the best possible experiments we can whatever they happen to be at a given time.

In terms of two sites I think you are going to have trouble doing that with sheep. There aren't that many places you can do this. We have a sheep flock that we couldn't use for this particular application because they are all infected.

DR. BROWN: That recapitulates the notion that

several of us have already expressed which is the need for two strains, two hosts. Yes, give people enough flexibility to make their own decisions. As information comes in one host strain combination may turn out to be the ideal. Mice I think are certainly as Bob said in some ways, many ways less practical than hamsters. On the other hand you can get around most of that by doing a spiking experiment with 301V in the mouse and do an endogenous infectivity experiment in the hamster and you don't have to wait 2 years when you use high input infectivity in the mouse and you don't have to collect a unit of blood to necessarily do the spiking experiment.

So, that would be a sort of reversal that might be practical.

DR. EPSTEIN: I just wanted to ask the question how important is it to do an actual transfusion experiment because the advantage of the large animal model is you can actually transfuse an intact unit into a whole animal with volume relationships comparable to human transfusion and I know you certainly can study infectivity with IC inoculations in readout animals but I think part of the idea which embedded suggesting a model in sheep is the actual transfusion experiment and also you know looking forward to question 2 we are sort of suggesting that one might stratify efficacy labeling according to whether an

actual transfusion experiment had or had not been done. So, you know the idea of waiting forever for the result you might be able to approve products with more limited labels pending a more definitive experiment.

So, I would like to hear opinion from the Committee specifically on the question of whether an experiment needs to be done with actual transfusion in a large animal model in order to mimic human transfusion.

DR. BROWN: The goal is to detect infectivity in blood before and after a process and so you want to use the technique which is optimum for detecting infectivity. If that turns out to be a transfusion then yes you would want to use a transfusion experiment but I think there probably are more optimal ways, more sensitive, right, sensitivity and one is the transgenic mouse and it may well be that intracerebral inoculation of smaller volumes in the proper host strain model will be more sensitive than transfusion where you know the transfusion is a very sensible method to detect infectivity in sheep. It is if I am not mistaken, Bob, much less sensitive in hamsters, that is when you inoculate it in blood intracerebrally you got a far greater number of takes than when you transfused blood even in larger volume.

So, in that sense transfusion would be less sensitive than intracerebral even if the volume was

smaller.

DR.ROHWER: That is true except that we don't know what the actual titer was in the sheep blood. That same effect may be present there. It is just that by giving 500 mls instead of 2 mls you overwhelm it and you don't see it. So, I don't think that question can be resolved by that comparison.

DR. BROWN: At least not yet. We just don't know yet, but the point, the principle is what you want is the most sensitive method using more than a single strain.

DR. PRIOLA: Any other comments from the Committee on the comment?

So, if we go on to B is it necessary that each experiment should be done at two separate laboratory sites I think we sort of addressed that to ensure reproducibility and accuracy of clearance. Any comments on that? I know that this is always an issue in any scrapie lab where you are looking at low levels of infectivity. Is it a practical issue in this instance?

DR. BROWN: The difficulty is actually in the wrong direction that is to say if you do get cross contamination you are out of the business. So, it behooves anybody who does such an experiment in a single laboratory to be extremely careful and so there is an enormous motivation to avoid cross contamination if you do it in a

single place.

DR. PRIOLA: And with appropriate and rigorous controls that is completely doable. I am not so concerned about the two laboratory sites anyway as long as the experiments are appropriately controlled.

DR. GESCHWIND: Particularly with that issue of large animals making it really just impractical.

DR.VOSTAL; I would just like to point out the fact that when we evaluate things like leukoreduction filters we always ask for two laboratories to minimize laboratory differences and practices and such.

DR. PRIOLA: It is a bit more difficult situation in the TSE field because of the specialized nature of the infectivity and there are very few labs that can do it. So, it might not be as practical to do that but I see your point.

Any other comments?

Now, C, is general description of informative scaled down processes for reducing TSE infectivity in blood and I have got to admit I am not exactly sure what that means.

DR. VOSTAL; I think we are trying to ask whether scaled down experiments are acceptable or whether it would be better to do a full-scale transfusion like in the sheep.

DR. PRIOLA: Anybody want to hazard a comment?

Dr. Bolton?

DR.BOLTON: Paul actually beat me to it. I guess at some point if you were trying to certify a particular filter you are going to have to have that particular device and geometry to run the full unit of blood through it or some configuration. I don't know how you would do 500 microliters of infected mouse blood through this thing and get any kind of meaningful answer but as Paul said there is a lot of experiments that you can do sort of in the preliminary stage to get a foundation to say that yes, we should go on, but I think eventually you would have to run the particular geometry that is going to be approved.

DR. ALLEN: I think that there are two components to that. As Dr. Bolton said you are going to want to make sure that the process runs on a whole unit of blood.

On the other hand how you analyze it doesn't mean that you have to then infuse that whole unit of blood in a large animal and follow it for X amount of time. If you have got another more sensitive or equally sensitive method of detection and a residual infectivity that should be perfectly adequate. You do it to assess the process itself in full volume.

DR.BOLTON: And the demonstration of the filter, the product coming through the filter still has all the appropriate biological specifications, wouldn't even need

to be done on an infected unit. At least in my opinion you could run a parallel unit on normal uninfected blood that we would then define the parameters in terms of its blood qualities and separately test in infected units for the removal of infectivity. Does that make sense?

DR. PRIOLA: Any other comments?

Let us move on to D which is what are the levels of clearance acceptable for claims of reduced TSE infectivity in blood components as used in clinical settings. So, this harks back a bit to what we talked about this morning. I think Dr. Rohwer showed with his filter he can remove so far at least as far as he can tell all the massive infectivity he has in his blood model using an endogenous sample as well as a good chunk of something in a spiked sample but what would be the clearance that would be acceptable?

DR. BOLTON: Paul, I thought you would jump in here with the two species, two strains. I am reading your mind but I think you would say that it depends on the, if you are doing a spiking study you are going to get one potential level of clearance. If you are doing an endogenous study you are not going to be able to achieve that clearance. So, Bob, you have got what 1 log. You could demonstrate 1 log endogenous. You can't do more than that at least right now.

DR.ROHWER: It is a matter of how many animals you inoculate but there is a point of diminishing return because the returns go down as a factor of two actually, but to inoculate a whole unit would need 10,000 hamsters and I don't think anyone is prepared to do an experiment like that. So, we do 5 mls. It is something we can do and occasionally we have gone to 10 mls but generally you can get an idea of what you are going to get from that kind of data. For example, you don't get another log for that. You get another fraction of a log.

DR. BROWN; The other interesting thing from one of the presentations is it is conceivable that a methodology such as we saw from Alicon could concentrate infectivity in assay experiments so that you could in fact by inoculating, by using a concentrating device you could get the equivalent of a whole unit of blood in a couple of milliliters. That is something that might be considered.

DR. PRIOLA: Yes, especially in combination with the two-mouse model or two-rodent model.

DR. ROHWER: All these devices by definition are concentrating infectivity in the device and in our particular case we haven't been able to figure out how to get this stuff back off without killing it because it sticks so tight, but we are still working on that and if we can figure that out that is definitely a way you could go.

DR. ALLEN: As I read the question it is what are the levels of clearance that should be acceptable for clearance of induced TSE infectivity in blood components as used in clinical settings. Obviously in clinical settings you are not going to have anything above endogenous infectivity. So, you know if using spiked samples you can show a level of clearance is well above what would normally be found in endogenous infectivity and in the endogenous experiments that are done you don't get any evidence of transmissibility it would seem to me that you have satisfied the claim and I understand that this is all hypothetical. It is a statistical process. Nonetheless given what we know now I will be reluctant to accept any evidence that suggested that there could be a breakthrough with or likely be a breakthrough with endogenous level of infectivity. I would like to see it well below that.

DR. TELLING: So, the answer is a log?

DR. PRIOLA: You mean for the endogenous.

Paul?

DR. BROWN: I don't think anybody would be happy with that and I think your question, well, I said, anybody would be happy; you know, you are looking for 5 and historically 6 logs of with HIV and a few other things. Certainly you have to sterilize endogenous infectivity whatever it is, if it is a log, 1 log, 1-1/2, 2 logs. We

can't have a single particle left. There can be no transmission. Any transmission from an experiment on endogenous infectivity is a failure. That is one criterion. I think you can set your own criteria for spiking. We learned from Marc that according to his model it only requires about 3 logs. I think that is more than you need but that is what he got. So, he is the expert.

DR. PRIOLA: Also, this morning we came up with an upper limit of 2 logs. So, from a limit of 2 up to 100. So, perhaps for a validation study 2 logs would be the upper limit in this case as well because that should clear everything in the blood based upon what work has been presented.

DR. BROWN: And that would be my feeling but I know that that makes other people uncomfortable because they like more margin for error and there is always this issue of transferring the exquisite care that goes into the laboratory experiments to the manufacturing scene and so it is not possible just to say, "Okay, in the laboratory here we get 2 logs and say that that is good enough for the manufacturer," So, partly for that reason I think that people want a margin of error. So, I think 3 logs in the spiking experiment might be appropriate.

DR. ALLEN: Yes, one would like to see a reasonable significant margin of error. I think it is

easier to get rid of that first 99 percent than it is to get rid of that last 1 percent or the last 1/10 percent or 1/100 percent. So, to get to the point that you have sterilized, you know one of my mentors when I was much younger always, "Sterility is actually a theoretical concept. You can never guarantee it," and you try to set up a process that goes well beyond whatever would be detectable in a clinical situation and I think that is what we would like to do here.

DR.BROWN: And the other thing that I would emphasize is that almost simply as important and possibly more important than defining a minimum with a margin of error is requiring absolute guaranteed reproducibility in test after test after test and only in that way can you get a feel for whether or not your margin of error is satisfactory.

DR. LEITMAN: So, this whole process tends to put a lot of responsibility on the manufacturer. For leukoreduction filters the blood center validates and does quality control very easily because the readout is so simple. It is a flow white cell count or something like that but the blood center here, that is where this will be used and the surface service won't have the tools to do the correct readout. So, they can't validate their process really. So, I had a little difficulty with that because

everything you perform as a manufacturing step we can do a quality control on but not this.

DR. PRIOLA: Perhaps that is another reason for having it done at two independent laboratories, the manufacturer and somebody else to basically back the manufacturer up or not by doing the same studies. Would that be better? You would never be able to do it at the local level. There is no way unless someone comes up with a surrogate easily detectable marker and even then it might be questionable.

Dr. Weinstein? Oh, I am sorry, Dr. Vostal?

DR.VOSTAL: I just want to ask when you do an endogenous infectivity experiment do you do a leukoreduction on that product first and then process your product through your device or does leukoreduction become part of that, the accounting of the infectivity from start to finish?

DR. COKER: From what we are doing at Pall there was no leukoreduction prior to doing the filtration. So, the whole blood is actually not leukoreduced at all.

DR. ROHWER: In terms of PRDT device it is not a leukofilter. It is a light and it binds PrP specifically and it will only remove from plasma. There is no claim that it would remove cell-associated infectivity. So, this device would be docked below the leukofilter in a

collection scheme and will be an add on and in terms of evaluating what these things do it is important I think to get an idea of whether it removes from plasma, whether these devices remove from plasma or not because basically I think that is the residual risk we are trying to get rid of. We already know the leukofilter will remove white blood cells and we can get rid of that risk with the leukofilter and so in terms of figuring out what these things actually do and whether they actually work I think it is important to test it against the residual plasma component.

DR. PRIOLA: So, D and E sort of go together in a way. It is all about levels of clearance, the first in blood components in clinical settings and the second to conclude that blood filters have effects with related infectivity from those blood components. So, does the Committee have kind of a consensus as to what that range of clearance is then? Are we talking as we did earlier this morning about spike being a really good way to show high clearance and then doing it with endogenous infectivity as well?

DR. BROWN: Yes, I think there is a consensus. I think everybody agrees that sterility of an endogenous infectious sample is mandatory and I don't know we might leave it up to the FDA folks in attendance to make their own decision about what kind of level of concentration of

infectivity represents something that they are comfortable with. We certainly had a lot of discussion about it this morning and whether it is 3 logs or 2 logs or 4 logs maybe it is best that they decide. I doubt that we are going to be able to make the decision here.

DR. PRIOLA: I think that is exactly right. We are not going to come up with a bottom line here for this.

So, I think the final point that they would like us to comment on is the methodology appropriate to use in evaluating TSE agent clearance and we heard this afternoon that they start with Western blot and then move to bioassay and I know my opinion is always the bioassay has to be in there before anything is approved because that is the most sensitive technique so far.

Dr. Brown?

DR.BROWN: In terms of reproducibility you can design a spiking experiment and do 100 spiking experiments and use a Western blot. That is okay. You can't do 100 bioassays. That is unreasonable but you could do one or two bioassays to complement and you could use Westerns for reproducibility and the bioassay as the most appropriate test for what you are looking for which is transmissibility.

DR. PRIOLA: As long as your Western blots are reproducible, right.

Dr. Telling?

DR. TELLING: Along those lines we heard about almost 3 log reductions based on Western blot and what is the dynamic range of that assay?

DR. COKER: It is between about 1 log and 3 logs. So, 3 logs is about the maximum.

DR. TELLING: So, how do you know that you are going to get 3 log reduction if you are at the limit of your --

DR.COKER: I don't understand. Do you mean for the endogenous?

DR. TELLING: Three logs is your limit. You are saying that that is what you are achieving. How do you know that you are not --

DR. BROWN: The best Westerns that I know of were done by Bayer and on a good day they could detect close to 2 logs of infectivity. So the 3 log minimum threshold is sort of your everyday best. So, you certainly have to if you want to demonstrate 3 log reduction of infectivity using a Western blot as a marker you have to start with 6 logs and then if you get nothing in the filtrate you know you have got at least 3 logs. You might have more but you know you have got that.

DR.ROHWER: Considering this is another complication and that is that doing a Western blot out of

plasma is almost the most difficult challenge you can present a Western blot with, so you have to do some sort of preprocessing in order to get a signal in the first place.

DR. PRIOLA: Yes, maybe the Western blot would only really work well with the spiking experiments.

DR. GESCHWIND: I just had maybe a point of clarification. Are we leaving open the option of tests other than a Western blot just checking that there are tests that are currently out there and tests that have just been presented in Dusseldorf that are certainly more sensitive than the standard Western blot; so, I want to make sure that we are not restricting it to the Western blot.

DR. PRIOLA: You mean like CDI or PMCA or something? Yes, I am sure we are not restricting it.

Dr. Creekmore?

DR. CREEKMORE: It actually says, "Or other assay for serum proteins."

DR. GESCHWIND: And we are also in the bioassay. Is there another question about biomarkers or is that a separate question?

DR. PRIOLA: I don't think that, but go ahead and comment on the biomarkers.

DR. GESCHWIND: Just from the clinical side and seeing the biomarkers that have been touted for the

diagnosis I am very worried about the use of biomarkers when we actually have the actual protein itself or the disease, the bioassay. So, I just would say that I am against the idea right now of biomarkers particularly because of the difficulty with reproducibility between lab to lab. I think we should really go down that path with great caution.

DR. PRIOLA: I think it was Dr. Turner who presented some data that they might have a couple of proteins in plasma, but I agree that really has to be validated and studied very hard.

Dr. Allen, did you have anything you wanted to say?

Bob?

DR. ROHWER: Marc, please correct me but my understanding is that the idea of using the biomarker is simply to get a way of routinely testing whether the thing is working at all, you know, are there holes in it, that kind of thing and if you have a biomarker protein that you know also binds to the device you can at least assay for whether it is being removed. If it is easier to assay for it then the PrP protein and the idea is to do that on a routine basis to make sure that the device is working.

DR. PRIOLA: Just for reproducibility, yes, although I still think it would be nice to someday have

another biomarker for TOC here but I would like a lot of things.

Any other comments from the Committee?

Should we vote on this issue? Are the FDA's proposed minimal criteria -- we are not voting?

DR. FREAS: We are not voting on this issue.

DR. PRIOLA: We are not voting on this issue. So, we have had a discussion. Would the FDA like us to have any other thing they would like to mention or point out or have us discuss?

DR. EPSTEIN: I guess we would like a vote overall whether we have the right set of criteria.

DR. PRIOLA: Okay, so we will vote on do they have the right set of criteria given what has been discussed this morning and this afternoon. Are the FDA's proposed minimal criteria for validation of TSE infectivity reduction by filtration adequate and appropriate?

Do we have a slide of that?

So, again, these are the minimal criteria.

I don't know if they have to delete the sheep but I think the point is just to have two animal models, or one that might be applicable to transfusion experiments and that would be sheep if they can do it.

DR. BROWN: Susan, yes, I think rodent and sheep should probably be excluded.

DR. PRIOLA: Just two animal models in general.

DR. BROWN: Two animal models and I would in that first one demonstrate elimination not reduction. We were just talking about that. If you can only reduce a log and one-half you are not in business, okay? So, it is the elimination of endogenous TSE infectivity and then reduction of spiked infectivity.

DR. CREEKMORE: I agree with that and then also in our discussion about the two separate sites how does the Committee feel about that? It seemed like if there were adequate controls that the two separate sites weren't that critical.

DR. PRIOLA: That is what I would think. I mean I agree with that but apparently with other infectious organisms the FDA requires that. So, it might be nice and then Dr. Leitman brought up that it would be another way to validate the manufacturer's claims to have it done independently but I agree. I don't think it is essential, but Glenn, did you have a comment?

DR. TELLING: Basically only to underscore your earlier comments that if these are adequately controlled and from reputable laboratories then I wouldn't have any problems from one location.

DR. BOLTON; I recall going through this once before some years ago but it seems to me that we are in