FOOD AND DRUG ADMINISTRATION

DEPARTMENT OF HEALTH AND HUMAN SERVICES

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Meeting of:

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES ADVISORY COMMITTEE

September 19, 2006

Holiday Inn Gaithersburg
Gaithersburg, Maryland

Reported By:

CASET Associates
10201 Lee Highway, Suite 180
Fairfax, Virginia 22030

(703) 352-0091

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COMMITTEE MEMBERS:
GLENN C. TELLING, PhD, Chair. University of Kentucky, Lexington, Kentucky

VAL D. BIAS, National Hemophilia Foundation, Oakland, CA

LYNN H. CREEKMORE, DVM, USDA, Fort Collins, Colorado

R. NICK HOGAN, MD, PhD, University of Texas Southwestern Medical Center, Dallas, Texas

MO D. SALMAN, DVM, PhD, Colorado State University, Ft. Collins, Colorado

JAMES J. SEJVAR, MD, CDC, Atlanta, Georgia

RONALD S. BROOKMEYER, PhD, Johns Hopkins University, Baltimore, Maryland

BERNADINO GHETTI, MD, Indiana University School of Medicine, Indianapolis, Indiana

SUSAN F. LEITMAN, MD, Department of Transfusion Medicine, NIH, Bethesda, Maryland

JAMES W. LILLARD, Jr., PhD, University of Louisville, Louisville, Kentucky

MICHAEL D. GESCHWIND, MD, PhD, University of California, San Francisco

JAMES MASTRIANNI, MD, PhD, University of Chicago, Chicago IL

MARK R. POWELL, PhD, USDA, Washington, D.C.

LAURA K. MANUELIDIS, MD, Yale University School of Medicine, New Haven, Connecticut

CONSUMER REPRESENTATIVE:
FLORENCE J. KRANITZ, Creutzfeldt-Jakob Disease Foundation, Akron, Ohio

NON-VOTING INDUSTRY REPRESENTATIVE:
TARYN ROGALSKI-SALTER, PhD, Merck & Company, Inc., PA

EXECUTIVE SECRETARY:
WILLIAM FREAS, PhD, CBER, FDA, Rockville, MD

COMMITTEE MANAGEMENT SPECIALIST:
ROSANNA L. HARVEY, CBER, FDA, Rockville, Maryland

P R O C E E D I N G S (8:00 a.m.)

Agenda Item: Topic II. Possible Criteria for Approval of a Donor Screening Test for vCJD.

DR. FREAS: Good morning. I would like to welcome you to this our second day, of the transmissible spongiform encephalopathy advisory committee. I have to read the brief conflict of interest statement for today, September 19.

This brief statement is in addition to the conflict of interest statement read at the beginning of the meeting on September 18, and will be part of the public record for the transmissible spongiform encephalopathies advisory committee on September 19, 2006.

This announcement addresses conflict of interest for the discussion of topic two, possible criteria for approval of donor screening tests for vCJD.

Waivers have been issued for the following committee members: Drs. Bernardino Ghetti, Laura Manuelidis, James Mastrianni.

Waivers were previously granted to Mr. Val Bias, Dr. Lynn Creekmore, Dr. Nick Hogan, Ms. Florence Kranitz, Dr. Glenn Telling and Dr. Mo Salman.

Dr. Taryn Rogeski-Salter is sitting as an industry representative acting on behalf of all related

industry, and she is employed by the Merck Research Laboratories. Industry representatives are not special government employees and they do not vote.

With regard to FDA's guest speakers, the agency has determined that the information provided by these speakers is essential.

the following information is being made public to allow the audience to objectively evaluate any presentation and/or comments bade by the speakers on topic two.

Dr. Phil Minor is the head of the division of virology, National Institutes for Biological Standards and Control, England.

Dr. Marc Turner is clinical director, Scottish National Board Transfusion Service, Edinburgh Scotland.

Dr. Claudio Soto is director, center for Alzheimer's Disease, University of Texas Medical Branch in Galveston. He is founder and chief scientific officer of a firm that focuses on the development of tests for prion diseases.

Dr. Jiri Safar is associate professor, University of California, San Francisco. He has a financial interest in a company that is developing prion diagnostic products. As guest speakers, they will not participate in committee deliberations, nor will they vote.

In addition, there are several speakers invited

from regulated industry and other outside organizations to make presentations.

These speakers will have financial interests associated with their employer and other regulated firms. These individuals were not screened by FDA for those conflicts of interest.

This conflict of interest statement will be made available for review at the registration table. We would like to remind members and consultants that, if discussions involve any products or firms not already on the agenda, for which they have a personal or imputed financial interest, they need to exclude themselves from such an involvement, and their exclusion will be noted in the public record.

FDA encourages all meeting participants to advise the committee or nay financial relationships that they might have with sponsors, products, competitors and firms that could be affected by today's discussions.

Also, I request that committee members, if you need a taxi at the end of the meeting, please during a break go to the outside table and check with Rosanna Harvey, and she will arrange cabs so there won't be any delays. It is a long trip to the airport from here, and we will do the best we can to get you there on time.

Also, at this time, if you have a cell phone, if

you would please put it in the silent mode so that it will not be disruptive? Dr. Telling, I turn the meeting over to you.

DR. TELLING: Thank you, Bill. Good morning, everybody. We are going to move on to topic two on the agenda today, possible criteria for approval of a donor screening test for variant Creutzfeldt Jakob disease.

There are three presentations before the break. The first presentation is from Dr. Piccardo from the FDA, who will discuss donor screening test issues, sensitivity, specificity and confirmatory testing.

Agenda Item: Donor Screening Test Issues.

DR. PICCARDO: Thank you. Topic two refers to potential screening assays to detect blood and plasma donors infected with TSE agents, possible criteria for validation.

There is no common policy on this topic, which is presented today for discussion. The issue before us is the following:

FDA seeks advice from this committee regarding potential approaches and issues to consider when validating and evaluating candidate screening tests for vCJD and other TSE infections in donors of blood, plasma and human cells and tissues.

Regarding the background, there are some concerns

which are the following. There have been three presumptive transfusion transmitted cases of vCJD infections in the United Kingdom, reported in a small group of individuals receiving non-leukoreduced red blood cell concentrates from three separate donors who later developed vCJD.

Therefore, red blood cell concentrates transmitted vCJD efficiently. It is possible that other blood components might also pose a risk.

There are unknown factors, for example, the concentration of vCJD infectivity in human blood is unknown. The time during in the incubation period when infectivity appears in blood before the onset of vCJD is also unknown. The prevalence of vCJD in blood and plasma donors is unknown.

So, the aim is to discuss desirable performance characteristics and a potential approach to validation of a candidate test for the detection of PRPTSE in blood.

A note regarding the nomenclature, as Dr. Asher mentioned yesterday, consultants to the WHO recently recommended designating all abnormally folded forms of the prion protein associated with TSEs as PRPTSE. Therefore, PRPTSE would equate the PRPSC and PRPRES.

Now, as a form of background, given the lats TSEAC meeting in October of 2005, it was discussed the validation criteria for devices to remove TSE infectivity

from blood components.

It was discussed that it would be useful to use at least two animal models an two strains of the BSE agent, and at least one agent strain to be derived from cow with BSE or with human vCJD.

It would be important to determine the reproducibility of the results and, for logistical reasons, most tests will be developed using small animal models.

Regrading the analytical studies, it is important regarding the potential use of exogenous TSE spiked materials using animal derived or human derived material, it would be important to demonstrate proof of principle, determine the limit of detection of the test, determine the intrinsic variability of the method, and perform blinded studies.

Regarding animal derived exogenous spiked material, it would be important to demonstrate that the TSE spiking material contains both infectivity and PRPTSE and, of course, the controls did not.

The assay can discriminate between samples of normal human blood, despite the suspensions of cellular tissues obtained from TSE infected animals, for example, brain tissues and blood spiked with similar tissue suspensions from large infected control animals.

What are the potential animal models? They

include, for example, rodents infected with scrapie, rodents infected with human vCJD or other BSEs, sheep infected with scrapie or BSE, and cattle with BSE.

Regarding human derived exogenous spike material, it would be important to discriminate between samples of normal human blood, despite the suspension of cellular tissues from human BSE infected in match controls. The tissues you can see once again include blood, brain, spleen and other tissues.

Regarding endogenous infections, in animal derived endogenous TSE infected blood, we have demonstrated that blood from animals with TSE contain both infectivity and PRPTSE and the matched controls did not.

We did the same to discriminate between blood samples from TSE infected and matched uninfected control animals.

Once again, the potential animal models to consider include rodents infected with scrapie and infected with vCJD or other TSE, sheep infected with scrapie and BSE, primates infected with BSE and vCJD -- if and when available -- and of course using two models would be better than using one. The FDA acknowledges that there are logistical difficulties in using non-rodent models.

Regarding endogenous infections and, in general, in the use of animal derived endogenous TSE infected blood,

what are the periods of concern?

The periods of concern include the analysis of material obtained during clinical illness and during the incubation period.

Therefore it would be important to test samples during overt illness and in the latter part of the incubation period.

Confirmation of infectivity would be required for forming bioassays. It would be important to determine the limit of detection and the intrinsic variability of the assay. Once again, blinded studies should be performed.

Now, regarding potential clinical validation studies, the use of the following blood samples should be considered:

Blood obtained from patients with vCJD, blood obtained from patients with other TSEs if the detection is to be claimed, and blood from control patients.

What we mean by that is patients with other neurological diseases and age matched individuals not having neurological disorders.

It is important that the diagnosis on those patients is confirmed by biopsy or autopsy, and it would be very informative if infectivity was in the incubation period. Now, this is ideal but probably not logistically feasible.

therefore, cadaveric blood samples could be useful, but ideally you need the comparison between ante-mortem samples in test performance, including false positive and false negative results and end point dilutions, et cetera.

As for other donor screening tests, the possible effects of potentially interfering substances in blood, including hemolysis, bile,lipemia, et cetera, should be evaluated. Coded, replicated, randomized samples tested in multiple runs should be carried out.

At this time, the FDA is attempting to assemble or obtain access to collections of TSE biological and control reference materials.

The number of TSE blood samples -- meaning from patients with sporadic CJD or from familial CJD that is available to investigate clinical sensitivity is probably limited. Smaller numbers of samples might be available from patients with variant CJD.

Controls should be obtained from patients, as I said before, with other neurological diseases or non-neurological diseases in numbers similar or above the ones obtained from patients.

Now, regarding sensitivity, there is a caveat. The caveat is the following, regarding failure to detect TSE other than vCJD.

Unlike those with vCJD, donors who later become ill with other forms of Creutzfeldt Jakob disease have not been implicated as a source of transfusion transmitted TSE.

Thus, it is possible that only in persons with variant CJD does infectivity reach levels in blood sufficient to transmit a TSE infection.

Therefore, failure of a candidate test to identify patients with other TSEs would not necessarily rule out the possible value of a test to screen donors for vCJD.

What are the FDA precedents? Clinical evaluation and validation of screening methods suitable to identify donors with infections require detecting samples from persons with known infections.

Screening tests for blood and tissue donors provide additional safety, but generally have not replaced deferral policies.

Regarding specificity, the specificity of an FDA regulated donor screening test has generally been determined by testing a large number of samples, usually 10,000, at least 10,000, from healthy suitable donors presumed to be at low risk for the infection of concern. A target specificity of 99.5 percent or greater has generally been sought.

Should the validated PSE tests have a lower

specificity, its suitability for donor screening would be determined based on the evaluation of probable overall risk versus benefit.

What about confirmation? False positive results are especially problematic for TSE screening tests because the incubation period for TSEs are very long, the intervention during the TSE incubation period is not available, the prognosis of TSEs after the onset of clinical disease i dire, and treatments for TSEs are ineffective.

Notification of donors with unconfirmed repeat reactive screening test results would have predictable adverse consequences for notified donors.

The expected adverse public health consequences of notifying a large number of persons having repeatedly reactive false positive results is obviously problematic.

A reliable confirmatory test detecting PRPTSE or other markers would be desirable, possibly obligatory, for every donor with a repeatedly reactive initial screening test.

The ideal confirmatory test may be a sensitive bioassay for infectivity. However, bioassays for TSE infectivity in human blood are not currently feasible in a blood donor setting.

A possible confirmatory TSE assay might be a

supplemental test for PRPTSE based on a principle somewhat different from that of the original screening test, but at least as sensitive and specific.

What about counseling? The predictable adverse consequences of informing deferred donors whose screening tests for TSE infection were repeatedly reactive, with or without reactive confirmatory assays, should be carefully considered in advance.

This leads to the questions for the committee. Question number one is, please comment on pre-clinical analytical studies needed to validate candidate donor screening tests for vCJD and other TSEs.

Comment on clinical studies needed to validate the candidate donor screening tests for vCJD and other TSEs.

Please discuss the relative merits of feasible technical options to confirm screening test results for vCJD and other TSEs, such as bioassays, alternative immunoassays, PRP amplification techniques, or others.

Following this presentation, Dr. Marc Turner from the University of Edinburgh will present an algorithm for approval of human TSE testing in Europe, followed by Dr. Phil Minor from the National Institute for Biological Standards and Controls in the United Kingdom, will present on available reference materials.

Then there will be an open public hearing followed by seven speakers who will present an update on TSE developments.

DR. TELLING: Thank you. Are there any questions from the committee for Dr. Piccardo?

You mentioned that FDA is attempting to assemble samples that would be useful in this process. Can you tell us a little bit more about that?

DR. PICCARDO: That is why Dr. Phil Minor is here. We are doing an attempt, obviously, to gather material, but definitely we are in communication with people in the United Kingdom and with other people in the United States. It is a very difficult task. There is a lot of discussion going on. I hope that in the next six months you will hear more about it.

DR. TELLING: Any other questions? If not, then we will move on. The next speaker is Dr. Turner from the University of Edinburgh, who will speak about an algorithm for approval of human TSE tests in Europe.

Agenda Item: Algorithm for Approval of Human TSE Tests in Europe.

DR. TURNER: Thank you very much, Dr. Piccardo, and thank you, ladies and gentlemen. I have somewhat modified the slides again, overnight, to make them a bit more succinct.

So, they will be updated from what you have in your packs, but the updated slides will be on the FDA web site if you should wish to download them later.

I am going to just discuss briefly with you some of the issues, some of our thinking in the United Kingdom and Europe on how to evaluate potential human variant CJD assays.

I thought I would start by just commenting on the current regulatory position in Europe. As you may or may not be aware, assays of this kind, like other in vitro devices, need to be approved by the European Union through a process known as CE marking. These assays would be considered under the remit of the in vitro diagnostic medical devices directive.

There are a small number of assays within the general directive which are on what we call annex 2A. These are assays which we consider to be critical in terms of wrong assay results would have very significant impact either on donors or patients or both, for example, HIV assays, HBV, HCV assays and blood grouping assays.

Variant CJD assays, as a category, are not currently on annex 2A, probably because none have yet come forward for the CE marking process.

To put them into that annex requires a request from a member state and the United Kingdom, the Department

of Health Committee, on the microbiological safety of blood, tissues and organs, is now working with the medicines and health products regulatory agency in the United Kingdom in submitting such a request to the European Commission.

It is, however, likely that that process will take at least a couple of years. All member states will need to agree to that categorization of variant CJD assays.

I think our concern has been that first generation assays are coming forward to the clinical arena, and there is very likely to be public and political pressure to consider implementation of these, and we need to put in place an evaluation process.

With that in mind, the United Kingdom and Irish blood services have established a collective current assay working group under the aegis of what we call the joint professional advisory committee.

Remember, in the United Kingdom the various countries of the United Kingdom and Ireland will have their own blood services so that, operationally, and in governance terms, are completely separate, but we do work together collectively on a whole range of professional issues as you might expect, under the JPAC structure.

The remit of this particular group is to act as a primary point of contact with the UK and Irish Blood

Services for research groups and manufacturers who are developing prion assays.

We have put in place a very similar group for working with manufacturers of prion reduction filters. We have found that there was a lot of complexity when manufacturers were approaching various different individuals and various different blood services, and we found that having a single point of contact was helpful, both to ourselves and to those colleagues that were working on the development of both filters and, we hope, prion assays.

The objective also is to provide expertise and advice to manufacturers on our laboratory and clinical development requirements for prion assays, and to liaise with individual UK national services on matter relating to prion assays, to ensure that appropriate UK national decision making bodies are kept appraised of the state of play of the UK spongiform encephalopathy advisory committee and NIBSC and to prepare ourselves a technical specification and a route to evaluation, and to make that open and transparent so that manufacturers have very clear understanding of the routes that we are trying to develop.

I want to just pick up one or two of the issues around sensitivity and specificity, discussed by Dr. Piccardo, and really just remind colleagues of some of

the difficulties in PRPTSE detection and sensitivity. I am grateful to Professor Paul Brown for these thoughts.

If one assumes, as I think we are in our risk assessments at the moment, that the level of infectivity in human blood is likely to be on the order of magnitude of one to 10 infections per ml during the incubation period, and one looks at studies of infected hamster brain, this suggests a ratio of about 10^5 molecules of PRPTSE per infectious unit.

One could be looking for a level of infectivity of only around a million molecules per ml. So, it is a very significant challenge for the sensitivity of the assays.

In addition, I think there are some very important caveats that we need to put around that apparently very simplistic point estimate.

For example, colleagues on the committee will be aware of recent studies suggesting, I think in hamster brains, that specific infectivity was most highly associated with polymeric aggregates of a relatively small particle size in the range of 300 to 600 kilodaltons, or up to perhaps up to about 28 molecules of PRPTSE.

That would suggest that, in fact, if the infectivity in plasma is of a similar nature -- and of course that is an open question -- but one ml of blood containing, say, 10 infectious doses, could actually

contain a far smaller number of molecules up to several hundred molecules, perhaps, only of PRPTSE, which is a very different challenge, clearly.

The other issue that I think we need to keep in mind is that I think it is not yet clearly known whether abnormal, but proteinase sensitive forms of PRPTSE would be infectious, in which case the population of the molecules might underestimate the actual infectivity present in them.

So, I think there are still some core issues for us relating to the physical chemical nature of PRPTSE and infectivity in blood, and the relationship between those, and those are key issues that we need to continue to try to address.

The previous speaker spoke about specificity. I just want to perhaps develop those thoughts and give some indication of the likely impact of core specificity on a normal population.

If you will bear with me, in this small horse experiment, per a million donors or donations tested, we are currently working in the United Kingdom with point estimates all around one in 10,000 donors with subclinical variant CJD.

Clearly, there are wide confidence intervals around that kind of estimate, but that is the point estimate that we are currently working with.

If one assumes one has an assay which is 99 percent sensitive and 99 percent specific, then that breaks down into four boxes.

There are the true positives, those who are infected with the condition and will test positive, and you get around 99 of those.

There are false negatives, those who have the condition but test negative, with a 99 percent sensitive assay. So, there would only be one of those. That is a reasonably favorable outcome.

The true negatives, those who don't have disease and test negative, are clearly the majority of individuals. The real problem is the false positives, those who are not infected but are testing positive. That is, for every million donors, with a 99 percent specific assay, you would get about 10,000 donors in that category.

So, in the United Kingdom, for example, where we test about 2.5 to three million individuals per annum, we would be looking at 30,000 positive individuals per annum, the vast majority of whom would be false positives. Of course we wouldn't know which -- the negative predictive value of the assay would be very, very good, better than 99 percent. The positive predictive value would be less than one percent.

Just to remind you, of course, that in a country

without cases of clinical variant CJD, like the United States, for example, it seems like to me that the prevalence of subclinical variant CJD is at least an order or two of magnitude less than that which we are likely to have in the United Kingdom.

So, the true positive rates, of course, would be extremely low, but the false positive rates would be no different.

You would still be looking at the same rates of false positives, and obviously a much lower positive predictive value. So, that is a problem for any country which wishes to implement such an assay.

Then we need, of course, to think therefore of the impact on the blood donors of such an assay and, indeed, of the blood supply.

What we are talking about here is trying to balance the public health, the overriding imperative to try to protect public health and protect patients from transmission of clinical variant CJD against the potential negative impact on blood donors and the potential or possibly catastrophic impact on the blood supply.

Those issues around how would we manage these test positive individuals, with apologies for split infinities, to tell or not to tell, to bleed or not to bleed. In Europe that is not an issue.

We would not be allowed to bleed people and discard their blood without informing them of that, but continuing to bleed them.

They would have to be deferred, but not just being deferred, they would clearly need an explanation why they were being deferred.

So, these individuals would have to be told. The previous speaker has touched on the issue of how we are going to find truth in the false positives. Clearly, the number of false positives would have a direct impact on the blood supply.

Then there is the issue of, even if we have a group of individuals we think are truly positive, what does that mean?

The current mathematical modeling suggests that maybe 95 percent of individuals could have long term subclinical disease.

So, it is not necessarily the case that all of those individuals are going to have clinical variant CJD, but of course how do we know who is going to develop clinical disease and who isn't. So, the implications of true positivity.

Then, the psychological and social impact on the donor and the indirect impact on donor recruitment and retention, for those of you in the audience who are -- the

donors would need to ask themselves, would I want to have such an assay undertaken.

Just really to inform that and perhaps give it a slightly human face, I came across this data which actually is data which is developed on the impact of presymptomatic testing of individuals who were considered at risk for Huntington's disease, which as you may or may not be aware, is a familial genetic condition.

In that condition there is, in fact, a very, very well developed and formalized international protocol for prescreen counseling of those people who seem to be at risk. They are not just briefly informed by their doctor of what a test might mean, but they have the opportunity to explore what a positive result might actually mean for them and for their families. This is not a curable condition. So, there is no way of averting the onset of this disease at the present time.

Interestingly, studies in Wales suggested actually the majority of people -- remember, these people are at familial risk -- chose not to be tested, chose not to have that test.

International data suggests that those people who do choose to have the test and do test positive -- and these are many true test positives -- do experience what neurologists call catastrophic events, two percent have

what they call a catastrophic attempt, by which they mean suicide, attempted suicide or serious psychiatric breakdown requiring hospitalization.

Of course, a larger proportion are all right. A number of people go on to also suffer significant levels of personal psychological stress, social problems, family breakdowns, difficulty with insurance and so on and so forth.

Indeed, those proportions in this setting are thought to be relatively low, relatively conservative, because of the effort that is put into prescreening counseling.

Certainly the time one can devote to that in the setting of a relatively smallish number of individuals considered to be at risk is likely to be much greater than if one were trying to prescreen counsel three million blood donors per annum, for example.

So, if anything, the impact on the blood donor population would be higher than those figures that you see there.

So, we have tried to develop an evaluation algorithm, and this in simple terms is the approach that we feel we would like to take.

Entry of a clinical assay -- really what I mean by that is, because this is an evaluation strategy, we are

not talking about research collaborations. What we would be looking for is an assay which manufacturers feel is in their final format, or in a format that is ready to go into the clinical environment, not a prototypic assay.

The starting point, I think, for us in this kind of an evaluation would be human brain and spleen as spiked materials in the peripheral blood.

I am not going to discuss that in any further detail because my colleague, Dr. Phil Minor, will be talking about that in his presentation about the resources in NIBSC.

We would have to give consideration to the histochemical format of a spike, for example, as a crude homogenate, microsomal format or so on and so forth.

We would expect assays to go through that in both the underlying data and then a blinded panel of the dilution series, so we would have a first look, as it were, independently at the probable likely sensitivity.

I think we will then want to move on to TSE infected animals for blood samples. Again, the previous speaker touched on these

There are, in fact, many blood samples already available, but they tend to be scattered amongst a variety of different institutions and research groups, and of course they are from a variety of different animals with a

variety of different TSEs, and pattern the variety in different ways.

I think for us there is a pressing need to establish a systematic library of samples, which include whole blood, red cells, platelets, leukocytes, plasma, separated to treatment to a standardized protocol and well characterized, both its infectivity and the nature of those samples.

There is a piece of work that I think could be done around that, and we will move on to certainly BSE samples from a variety of TSE infected and control animals, for example, scrapie or BSE infected and normal control rodents, scrapie and BSE infected sheep, perhaps infected cattle, perhaps CWD infected deer and elk and TSE infected primates.

Probably not all of those, but certainly a collection of those kinds of materials I think is going to be essential for us.

I think that would be the critical gateway, from our point of view, to access the issue of peripheral blood samples from patients.

Really, this has proved an extremely difficult ethical issue in the United Kingdom. The starting point would really be peripheral blood samples from patients bearing CJD.

Of course, at any one time, fortunately there are very few patients with this particular clinical condition available.

They are rarely in a clinical condition where they could give written informed consent. Obviously, there is a moral issue, obviously, of taking large amounts of blood from an individual who is already extremely ill.

Although there are smallish aliquots of peripheral blood held by the units and by the CJD patient units. These amount to 40 to 50 ml aliquots, which have been separated according to standard protocols.

Certainly not either the volumes or the spread of samples that one would normally acquire to evaluate an assay such as an HIV assay, for example.

Of course, a key issue is that we are not trying to detect variant CJD in patients with clinical disease. We are really trying to detect it in patients with preclinical disease.

There are a group of patients who are considered presumed infected by the CJD incidence panel. These are the 25 or so individuals who have received blood components from donors who themselves went on to develop clinical variant CJD.

There is a lot of discussion around how these patients should be followed up from a clinical perspective

and whether it is ethical to approach them to donate large amounts of blood for these kinds of purposes. I think those issues have not yet been resolved.

Thirdly, there is a broad spectrum of people who are considered at risk of variant CJD for public health purposes, again by the CJD incidence panel.

These tend to be individuals, for example, who have received plasma products from a pool to which a donor who donated went on to have clinical variant CJD, or patients who have been exposed to peripheral surgical implementation.

So, there is a larger number of such individuals, but clearly there is less confidence that any of these individuals are actually infected. Those would clearly be key samples which we need to try to collate in some way.

Finally, we need to look at peripheral blood samples from blood donors in order to try to establish specificity, and the UK blood services have established what they call the test assessment facility, the purpose of which is to collect 10,000 whole blood units from normal donors.

These have been aliquoted in multiple aliquots, separating the red cells, buffy coat and plasma, and multiple aliquots are prepared.

The aim is to develop or provide a comparative

specificity panel. We have chosen -- clearly, the panel is not sized -- this is a misunderstanding -- to give accurate epidemiological data, even in the United Kingdom if the subclinical prevalence is one in 10,000. We might not expect to pick up a true positive in this panel. So, it is very much a specificity panel.

The typical view is that it would be prudent, given that we don't know the prevalence of subclinical variant CJD in the United Kingdom, to use at least half the samples from a country where the prevalence is likely to be extremely low or negligible. So, we have 5,000 U.S. samples and 5,000 United Kingdom. If I may, chairman, I will stop at that point. Thank you.

DR. TELLING: Thank you, Dr. Turner. Are there any questions from the panel for Dr. Turner at this point?

DR. MANUELIDIS: I do have a sort of general question. If it is so difficult to get vCJD blood, why not do something, especially in England, with just sheep, which are endemic flocks, before they are clinical and clinically ill sheep, versus flocks that don't have scrapie or haven't been exposed to scrapie.

There you have huge numbers of samples, you have endogenous material. You can really see if something works in a huge number of samples blindly.

DR. TURNER: Yes, and we do have a piece of work

that we are undertaking with the Institute of Animal Health with both scrapie and BSE infected sheep.

We clearly know the point of infection and the intent is to collect sequential samples during the incubation period and, of course, in due course we will know the outcome of which sheep were actually infected or not. So, that is part of the intent of that study.

DR. BROOKMEYER: We are dealing with a very long incubation period, as you pointed out. The key is that we have high sensitivity during that window period.

You spoke a little bit about our ability to determine if we have sensitivity during that incubation period.

I was wondering, the kind of data that we have that could speak to that, and how difficult that is going to be, to determine whether or not you could, with good sensitivity, detect during that long window period.

I am wondering -- in your last slide you mentioned 5,000 UK and 5,000 US samples. If you assume that the prevalence, say, of silent infection is higher in the United Kingdom than in the United States, then if one were to do a test and found higher prevalence in the United Kingdom than the United States, would that provide some indirect evidence that you are detecting -- you have some sensitivity for detecting the silent infection, if you see

in two populations, one where you are assuming epidemiologically there is more silent infection going on than in another.

Then if you apply these tests and actually see you are detecting more in one population than the other, would that be a way of indirectly providing some evidence that you can actually detect during that window period?

DR. TURNER: Part of the thinking when we adopted that strategy, as I said earlier, and as you remarked, if the prevalence really is around one in 10,000, then we wouldn't expect to maybe pick up more than one true positive in the UK sample.

Say, for the sake of discussion that we were to pick up, say, 10 UK positives. There would be two issues. One, having the US samples would allow us to establish whether they were really true positives, or were they more likely to be false positives.

Clearly, also, if we were to find 10 true positives out of 5,000 samples, that would be an extremely worrying finding.

DR. TELLING: I have a question. You made what, to me, was a rather surprising comment. You referenced modeling studies that suggested that only a portion of true positives would develop variant CJD. Could you talk a little more about that and the assumptions that were made

to arrive at that conclusion?

DR. TURNER: Sure. The mathematical modeling studies built around the current clinical incidence of variant CJD in the United Kingdom currently suggest, with wide confidence intervals, obviously, that we might now expect to see around another 70 to 100 clinical cases.

Clearly, there are a lot of caveats around that, but the clinical incidence in the United Kingdom is currently diminishing.

In sharp contrast to that, is the study that was published I think a couple of years ago now, that was a retrospective study of tonsils and appendices in the United Kingdom, if you remember that, where fully positive samples -- positive, I think, on western blot of immunohistochemistry -- were found amongst 12,500 samples.

If one mathematically models that, that suggests a prevalence of disease in the UK population, at least in that age bracket of 10 to 30 year olds -- when most people have tonsils and appendices out -- of more than 3,500, something like that.

So, these are mathematical models which are built around that admittedly scanty data, as it were, but it was the best they could have at the moment.

This is mainly work of Dr. Ajudani(?) and colleagues, although I think Dr. Sheila Burt has also done

some similar work.

The mathematical models at the present time which best fit that data would suggest that perhaps up to 90 or 95 percent of individuals might have long term preclinical disease or truly subclinical disease, which comes out in a kind of limited context.

I guess, to some extent, that is at least consistent with some of the animal data that we are now beginning to see, which suggests that the homozygotes and the heterozygotes do have much longer incubation periods, not so, perhaps, on some of the data from other conditions, like Culoo and iatrogenic CJD, which suggests that those genotypes have much, much longer incubation periods. So, that was the nature for that comment, but there are a lot of caveats around it, quite obviously.

DR. TELLING: Actually, Dr. Johnson, Dr. Turner mentioned studies from Byron Coughey's group that spoke to your question directly that you raised yesterday about the size range of aggregates of PRP scrapie that were associated with infectivity. It is in the range of 300 to 600 KD and 14 to 28 molecules of PRP scrapie. The most infectious, I have forgotten about those.

DR. JOHNSON: Sorry, I couldn't hear you.

DR. TELLING: So, yesterday you were asking a question about the state of aggregation and the size range

of the infectious agent. Actually Byron Coughey's studies speak to this directly. This was published in Science a couple of years ago.

Dr. Turner alluded to these studies this morning, suggesting that aggregates in the range of 14 to 28 molecules of PRP scrapie were the most infectious.

DR. MANUELIDIS: Actually, I think the first studies that were done were done from our laboratory and published in 1996, and showed that the infectious agent by fractionation was 25 nanometers, and probably about slightly more than 600, but in that range, KD 600 to 1,000 KD.

The difference is the interpretation. We first isolated infectious fractions and got rid of most PRP. I think that until somebody shows that those particles contain PRP, there is an assumption that they are made of PRP molecules. We found the opposite. Antibodies did not bind those particular particles.

DR. KRANITZ: Dr. Turner, I have a question. I am not exactly clear how to form it, so bear with me. On those tonsil and appendices that were positive, did you also do comparative blood studies on those patients to see what the level of infectivity was in those patients?

DR. TURNER: It wasn't me, but the answer to your question is no, and the reason it is no is that these were

tonsils and appendices which had been removed -- they were archived samples, effectively, from people who had these taken out for routine clinical purposes.

My understanding is that they were all anonymized and unlinked before the assays were done. So, the data just stand as they are. There was no linkage back to those individuals.

DR. SCOTT: I just had a question about the 5,000 UK and the 5,000 US blood samples that are planned. If you really want to show the difference, if there is a difference in terms of positivity, would you be selecting perhaps age group 20 to 29 for the UK samples and perhaps samples or units from donors who have never traveled from the United States as sort of your US cohort?

DR. TURNER: Yes, I think that would be a fair point. Just to come back to what I said earlier, it is really -- the purpose is simply a specificity panel, so that we know what the positivity or false positive rates are likely to be.

It hasn't been structured in other ways. There are clearly a number of other ways in which it could be structured which might give more information. That maybe is something that needs to be thought about and discussed with funders and so on.

DR. BROOKMEYER: Just to follow that last point

up, if you could structure it where you could see some of the subpopulations, identify them epidemiologically to be at the highest risk, and then a more modest risk, and then a US group which you would think to be extremely low risk.

You could actually then see -- if you see a gradient in positive and actually see some sort of dose response, if you will, in terms of prevalence of positivity, that would be, I would think, reasonable evidence that you are detecting something.

DR. TURNER: I should say I think there is some demographic information which will be collected on these individuals.

Again, although it will be anonymized, unlinked, things like age, the part of the United Kingdom or the Untied States, and basic data like that will be available.

I suspect that if we start to see that kind of pattern that you are describing, then that would be a very strong imperative to go out and collect a large number of other samples to try and address some of those issues.

DR. TELLING: Thank you, Dr. Turner. I would like to move on now and call Dr. Minor from NIBSC, who will talk to us about available reference materials.

Agenda Item: Available Reference Materials.

DR. MINOR: Thank you very much. Obviously, this is a ferociously difficult area to actually be discussing,

and there are a number of particular things that make it almost impossible.

One is the availability of what you might call relevant samples. I think if we are looking at most blood borne infections, it is reasonably easy to determine when somebody has actually got the infection or not got the infection. I am thinking about hepatitis B for example, things like that.

In this case, that is not the case. There aren't very many cases any, and that is a major constraint on what happens next.

There are actually process constraints on the kind of assay methods which would be useful in the blood transfusion service, and the whole area is of such horrific complexity that what ends to happen is that the discussions tend to focus on the ethics of false positives, rather than actual technical aspects of how you know whether you have got a test or not.

So, I am going to be slightly repetitive of some of the stuff that Mark was talking about. I think the process by which you develop this thing actually influences the kind of reference materials that you might actually want. So, I am going to go through it and if it is repetitive, I apologize for that, but I don't think it can be helped.

So, the objective really is to try to define a process such that, if you have an assay method which feeds into the top of it and successfully completes it, you have some justification for turning it loose on the innocent public. That is the argument.

Many developers are attempting to produce these kinds of tests. The concentration is always on the abnormal form of PRP, and you can call it PRPTSE if you like.

It is a fact, I think, that nobody knows quite what it is. We just had a brief discussion about the nature of the infectious particle.

Even if you assume that it really is PRPTSE, the infectious particle, quite clearly not all PRPTSE is equally infectious. The oligomers of 14 to 28 are more infectious than the larger oligomers or less.

So, even if you believe, which most people do now days, that the abnormal form of PRP is the infectious agent, it is still a cause of infection.

It may well be that the tests that we are going to be talking about will be making different versions of PRPTSE.

For example, Jiri Safar yesterday talked about proteinase K sensitive PRP, which may be infectious. Many people were using proteinase K to define their PRPTSE. They would clearly be measuring something different.

So, you have to bear in mind it is a cause of infection. in my view, not all of the assays are necessarily proven to be detecting the same thing. So, that is problem number one, but this is the marker that they are looking for.

The analites, again, a certain amount of thought went into this, about what could be the possible analite. Really, the only reasonable conclusion is that it has to be blood or something like it.

There are other easily available fluids, but I am not sure you could provide them in the context of a blood transfusion donor session.

So, blood is basically the analite everybody has to look at. I think there is no real argument about that. It could be whole blood, plasma, serum or leukocytes. Most people, I think, are going for plasma because that is what other blood tests tend to be performed on.

Bear in mind that infectivity is low and the form of PRPTSE present in blood is not known. Many people say it is different from what you would find in brain, but I don't know how they know that, apart from the fact, of course, that it is obvious that it has to be different. There is no evidence right now.

This is a trivial technical issue, but it is not so trivial. When a test will be rolled out to the blood

transfusion service, there would need to be some form of run control included, to ensure that the tests are being done properly.

You can't really hand out homogenized human infected brain under those circumstances. The supply of it would be quite limited and there would be safety issues associated with it.

This actually puts constraints on the kinds of tests that you have to be looking at. They would have to be just not applicable solely, perhaps, to the human situation.

So, there are constraints that arise from the technical issues of what you are trying to look at, and these actually influence the kind of tests that you might do and the kinds of reagents that you would have to use to control them.

Whenever how tests are going to be developed gets discussed, it tends to be a very, very skittery kind of discussion, because there are so many difficulties and issues to do with it that people tend to move on to the next issue because they recognize it as the next issue.

So, what I have tried to do here is split this thing into three levels of questions and I am just going to deal with the first one, which is here.

The real question which I am going to deal with,

which the reference materials are supposed to deal with is, how good is the assay at detecting the marker that you think you are detecting.

So, this is, how good is the assay at picking up PRPTSE, never mind what it means, for the moment. How good is it at actually detecting that. What is its sensitivity and what is its specificity. Most of the samples that I am going to be talking about are addressed to that question.

Question two is a question of how good is it at measuring what you actually want it to measure, which of course is the infectivity of the unit that you are taking.

It is quite possible, it seems to me, that a test could turn up 90 percent of your donors actually positive, although only like one in 10 million of them is actually infectious.

This is quite conceivable. The relevance of the marker to the thing that you are really interested in, which is infectivity, is something which needs to be proven.

That is very difficult, but I think there may be a way to actually begin to address that, perhaps which I will try and remember to discuss later on. I am not going to talk about this very much. Question one is the main thing.

Question three, which is the one that people talk

about an awful lot, is what is the relevance of the marker to predicting eventual disease in the donor that you get it from.

I think that reflects on Glenn's previous question about the number of subclinical infections that you might have, what do you actually say to the donor in terms of whether you are positive, you are basically going to die of this disease.

I think it is not clear that would be the case for any of the tests that you are actually talking about, that necessarily they would predict disease in the patient in the end.

Like I said, mostly what I am going to talk about is the technical aspects, which is question one, how sensitive and how specific is the assay.

So, this is the initial process which Marc referred to and which we sort of grinned up, and I am fully prepared to take criticism on this, although I am not sure what else you do.

The idea is that somewhere out there, there will be a laboratory which will be working on an assay to measure PRPTSE in some way.

What I have to say is that there is a certain amount of skepticism in the community about whether these things are sensitive enough to pick up any signal in blood

at all.

At the WHO meeting which David Asher reviewed yesterday, the conclusion was pretty ambiguous about whether there was definitive proof of the presence of PRPTSE in blood or not, although infectivity was accepted. So, there is skepticism about how good they are at doing what people say they can do.

So, the idea is, then, that there must be some independent blinded way of actually looking at these things, to provide some assurance that they are doing what they all claim to be doing.

So, firstly, we look at assays on human brain and spleen which can be provided by NIBSC. We have these kinds of materials available and you would get those unblinded, basically, just to work up your assay and adapt it to the appropriate sample assay material which you would be looking at later.

At such a time, when the developer has decided that he has got a satisfactory way of looking at these things with a suitable sensitivity and specificity, then you will get sent blinded samples by us.

So, they won't know what they are getting, but there will be a dilution series of brain and spleen in plasma and then they would be assayed, the results would return to us and then we can see how good it is, what the

false positivity rate is in that particular context.

The criticism of this, of course, is that everybody will say that, well, what is present in brain, what is present in spleen is not what is present in blood, which is a fair comment.

There are assays out there which don't appear to detect things in brain. The immunocapillary electrophoresis method of some years ago clearly could pick up something in blood but it really wasn't picking up brain too well.

So, there may well be instances where brain samples are not actually detected by the methods which are being developed.

That doesn't mean, then, that if they don't pick it up, that it is a deal breaker, but I think it does mean that you have some explaining to do about why you can't pick up something which is highly infectious, whereas something that is not that infectious you can pick up. Nonetheless, I will show you some data on that in a minute.

The next step will be to look at animal blood samples. I just specified positive and negative scrapie negative animals.

There are panels of this available from the veterinary laboratory agency in London and from other source as well, I think.

What would be quite nice would be if everybody

got the same replicate panels. Again, you will see that we have some of that at NIBSC already, and the idea would be that everybody should get the same answer, perhaps.

Finally, this will show that the assay measures something in tissues which are known to be infectious and it establishes the sensitivity compared to other methods.

The gold standard method that everybody uses is an immunoblot, which is known to be not sensitive enough to pick up something in blood. So, that gives you a bench mark for where you actually have to shift it on.

It shows that it will or will not consistently record a signal in samples which you think maybe have PRPTSE in them, a scrapie infected blood or whatever.

There are obviously other sources of blood that you can actually look at as well, which again, I will maybe discuss later on.

In your handout, this has come out completely blank. It is not deliberate. The data are not actually presented there. Nonetheless, it has come out completely blank, which I apologize for.

This is the results from seven labs. If you are sitting on that side of the room, you probably can't see the relevant data, which is at the bottom here.

There are seven labs listed here now. That is the lab number. The first two columns represent uninfected

spleen, diluted into human plasma.

This is a one to 10 dilution of a 10 percent homogenate of uninfected spleen. So, this should have no signal in it at all.

This is a one to 100 dilution of a 10 percent homogenate of uninfected spleen. This is infected spleen at one to 10, 10 percent, one to 100, one to 1,000 and one in 10^4.

So, the argument is that these laboratories have very bravely said they can actually do this stuff, and that therefore they will go out and look and see how it comes out.

They get these samples blinded. So, they don't know what the results are. In some cases, at least -- most cases, actually -- it makes sense.

If you look at laboratory number one here, there were no signals from the uninfected spleen here. There is a bright red, that is to say, unambiguously positive signal, from the one in 10 at the 10 percent, the one in 100 of the 10 percent. There is a green, which is the ambiguous signal, from the one in 1,000 dilution of 10 percent.

So, this makes perfect sense in terms of saying the assay is sensible and it is actually acting as you would expect it to.

Laboratory two down here is also making sense,

because the most concentrated version of the uninfected spleen gives a false positive. So, it is a way of actually detecting is it consistent, was it not consistent.

One thing which I would like to put out to you which you again won't be able to see on that side of the room is that laboratory number seven is showing an ambiguous positive with the one in 10 suspension of the infected spleen. It is about two orders of magnitude less than laboratory one in terms of its sensitivity.

Then we start looking at the infected brains and the uninfected brains. You get exactly the same kind of picture.

So, there are problems with background, perhaps, up here with some of these particular assays, although again it makes perfect sense. So, everything that we have had back more or less makes perfect sense in terms of what is picked up and what is not picked up and what high dilutions are picked up and what aren't.

Again, I would like to point out to you that laboratory number one does very well with all of these negatives up here, and this is the infected brain across the side here.

You can see that it comes out ambiguous at one in 10 to the 10 percent infected brain. This is actually very, very sensitive, if you will.

Down at the bottom here, laboratory number seven, it is again about two orders of magnitude less sensitive than laboratory one.

Laboratory seven is probably using something which is about western blot equivalent, if you will. So, this is very, very much better than a western blot.

The fact that it is two orders of magnitude more sensitive on the brain and on the spleen I find very, very encouraging because that is, roughly speaking, what you would hope if they were measuring the same thing in the two preparations and, if they were doing that, maybe they would be measuring the same thing in blood.

I think that something like that is actually looking very promising in terms of blood. In fact, I think they will be looking at blood specimens and turn up certain positives and certain negatives.

This is a reasonable way of screening our assay to see whether it is sensitive or is not sensitive, and these panels are freely available. You can get hold of these very, very easily if you want them.

So, one of the difficulties, then, is to know whether the assay is actually measuring something which is relevant or even significant.

I think that the way we are thinking about it is that, if you have only got one assay, you probably haven't

got an assay.

There are possibilities that bloods are not positive, even though the animal is scrapie is infected, for example.

So, really, it is a good idea to have precisely the same samples tested by everybody, because then you get cross confirmation, if you like, of what the results are, and that would actually be quite good.

So, the assays have to work equally well on a variety of species, and I think that is another issue to do with how the thing would actually be rolled out in real life.

The key point down at the bottom here, which I mentioned yesterday, is that the number of samples from variant CJD patients is really trivial.

There are possibly 200 samples which are at least potentially accessible and most of those will be resent in one to two ml volumes. There really is very, very little of this material actually available.

In other words, the validation of these assays in practical terms is almost certainly going to have to be made on the animal data that we can try to get hold of.

I think that is unprecedented, actually, for a human diagnostic. So, here are some suggestions about what you can do.

There was the mention earlier about testing samples from sheep from a scrapie endemic flock. That is a really good idea, I think.

It doesn't follow that any of those are going to turn up positive, but what you should get is a consistent story. That is to say, if you test the same sample from the same sheep on the same day, you should actually get that positive more than once.

So, what you need to do is send out blinded panels of this to show that you can actually get the same answer twice running.

If you then are going to send that out to another developer who has also got a test which may work and they get the same samples positive, I think we are beginning to look quite good. It begins to look as though you are measuring something that is real, I think, more than anything else.

You could also look at samples from sheep from a scrapie free flock. Really, they should be at least consistent and possibly all negative, depending upon what the last positivity might be.

We can look at samples from UK blood donors which Marc referred to. I think the samples that he was talking about are being collected by the blood transfusion service for their own purposes, for actually validating tests as

they come on the market.

What I am talking about here is how you get to the stage of getting the test to the point where the UK Blood Transfusion Service could actually use it, if you like.

Again, that is potentially possible. You may find some positives like that. Then again, you may not. Again, the same samples should be consistently positive.

You could look at samples from other blood donors, such as the United States, where it should be negative. It doesn't necessarily follow that it will be negative.

There may be differences in diet, for example, or habit or whatever which would give you false positives in one but not in the other, for example. So, you can't necessarily draw a hard conclusion about that.

Finally, there are samples from vCJD patients. It doesn't follow that all of those are going to be positive either, but again, it should be consistent. There are not very many of those, as I keep saying.

What you could also do is look at, if you pardon the expression, seroconversion panels. You could take mice and you could shoot them up with scrapie and then bleed out mice at regular intervals.

I mean you could say, at what point in the

incubation period did it become positive. This is something that many laboratories are actually already doing, in terms of how things become positive.

It would be a very good idea to have such a panel available for everybody to have a look at if they need to do that, and we are actually working on trying to develop that sort of panel. It takes a lot of mice to make a decent sized panel.

The bunch that Marc referred to is those who are actually at risk for variant CJD. There is a cohort of people who have received units of blood from patients who later went on to get variant CJD.

There have been discussions for really quite a long time about trying to go back to them and get blood from them to develop a panel of this kind to say when do they actually seroconvert, if at all.

I have found that process extremely frustrating. I think it is very, very slow moving at the moment and, the longer you leave it, the less valuable it is going to be.

Finally, we have some samples from hemophiliacs. This turns up just as a bit of serendipity, if you like. There was a study done at the Royal Free Hospital in London over the period of the late 1970s to the late 1990s.

Samples were taken from hemophiliacs over the whole of that period, mainly to look at things like

hepatitis seroconversions and HIV seroconversions. So, a lot of these things would actually be HIV and hepatitis C positive.

Clearly, this is a period of great interest from the point of view of variant CJD as well. So, we have those, but again, there are not much of them. So, these are just tiny little icing on the cake sort of validation efforts, if you like.

I think it would be very well worth looking at. It would be very nice to know if any of those turn up positive at all, frankly.

I think it might calm the discussions about plasma proteins down enormously. Those are actually available, and we can actually get into those.

Finally, this is the key question for me. If you actually got a test which detects brain and spleen to high sensitivity, scores appropriate animal and human blood samples consistently positive, scores seroconverting samples consistently positive, scores three of 10,000 UK donations positive, none of 10,000 US donations positive, gets the same results for the samples as another test working on a different principle, and if it is easy to implement it, is that when you can turn it loose on the blood transfusion service. I am not saying anything.

If it not, what do you do next? I think there are

experimental ways of actually approaching this.

This is a slide which actually is in your handout in a comprehensible form, and I am not going really going to run through this in any great detail.

This is the kind of samples which are actually available to NIBSC, which can be accessed. So, there are human brains, there are human spleens from variant CJD and normal spleens.

There are scrapie infected brains from a couple of sheep cases which have been homogenized. There are some whole blood buffy coat plasma preparations from the same sheep. There are a couple of BSE suspects and one BSE genuine case, if you like, which we have homogenized, stashed away and aliquoted.

So, in principle, at least, if you want these materials, you can all get the same kind of materials. The animal material have to go through the Veterinary Laboratory Agency because we hold it on their behalf, basically, although we make it basically, but the human materials are freely available if you want them.

There will be other materials which are soon available, blood donor specimens from the United Kingdom, which we are working with Roger Akley(?) of the Blood Transfusion Service to get hold of, and again, to aliquot it to produce panels of a decent size reproducibly, which

people can actually get hold of.

There is sequential sera from UK hemophiliacs, which we will have soon on the premises. There are sequential sera from infected sheep. This is a study that Marc referred to just a moment ago, where the sheep have been fed BSE and then at some stage, during the incubation period, a unit of blood will be taken from them and transfused into recipient sheep, and you follow the recipient sheep to see when they actually become infectious or not.

I think one thing that is absolutely crucial is to take a sample of the transfused unit and actually keep it away somewhere so that if you find out that it is infectious, you can see does your test pick it up as infectious and does it pick it up anyway, even if it is not infectious.

I think that is possibly only the easy way that I can think of to determine whether the markers you are looking at are actually relevant to infectivity of the unit as opposed to just saying, yes, there is PRPSC present, and sequential sera from infected mice.

I also have down here at the bottom is the web site address of the resource center, NIBSC, where you can find what is actually available there, and the full details of the actual materials that are actually available.

So, what we are doing is, we are trying to produce reference materials which can actually be used for test development, if you like.

It is quite likely that in the near future we will get variant CJD samples down from the CJD surveillance unit in Edinburgh. So, we will actually have the whole lot. It will be a one stop shop, to a degree.

It doesn't follow that you can just tap into these things and get the variant CJD samples just like that, but some of these materials you clearly can.

Things which are under our control, I think we are very, very keen that people can actually look at them and test them and see how they are doing. So, that is the state of play. Thank you.

DR. TellING: Thank you, Dr. Minor, for that summary. Questions from the panel?

DR. SALMAN: This is a question related to the use of scrapie blood. What do you predict, that there would be consistency in the performance of the test on scrapie in the blood in blood from human patients?

DR. MINOR: That is under question, clearly. The answer is no, not necessarily. The way I would figure this working is that you would validate your assays on the animal models, if you like. So, the scrapie in sheep, with BSE in cattle, primates, anything that you can get hold of,

basically.

Then having established perhaps that, at the end of the incubation period, 80 percent of your blood samples are infectious or 100 percent are infectious.

You can then try and validate the human samples in those terms. The amount of variant CJD blood you will have from actual symptomatic patients is teensy weensy tiny.

So, one thing you can say is, well, I expect them all to be positive based on the animal models, in which case my test should score everything positive, or a small fraction will be positive based on animal models, but it will be a correlation.

You are right to bring the question up. I think it is a major issue, actually, but the assumption is that the animals will, in some way, behave the same way as the humans will.

DR. SALMAN: This is maybe a follow up question. Have you thought of using other tests that are used for scrapie, such as the rectal biopsy? I don't know if that is possible to be done in human patients.

DR. MINOR: This was to do with the original discussion about what the analite was, and there was a certain amount of thought put into this.

You could do lymph node biopsies, which I don't

think would work on blood donors very well. You could do brain biopsies. I think you would only get one.

You could look at urine specimens, you could look at fecal specimens, you could look at saliva specimens or tear drops or something like that.

When you actually go through and consider the practicalities of this, I am not sure that blood donors would like to present a fecal specimen at a blood donor center.

Maybe they would. Some of them are quite highly motivated people. I think in terms of practicality the only thing that will work, in my opinion, at least, from a practical point of view, is a blood specimen.

DR. SOTO: I have a couple of questions and comments. I think the procedure you outline makes a lot of sense, but you have to be aware of at least two important issues.

One is that the validation based on animal samples requires the test to be performed on animal samples and some of the tests may not do that.

In our own experience, it looks like in different animal models the peripheral phase is quite different, both in terms of extent of peripheral involvement and timing. So, the extrapolation of findings in animals to humans may be very, very difficult.

DR. MINOR: I don't disagree. I think that the first comment, that the test may not work on animals, it is entirely to the point.

Clearly, what you would really like is to have a test which works on humans which you validate on humans. The difficulty with doing that is that there are no human samples available which will do what you want them to do.

The best way of doing this clearly would be to take a human, expose them to variant CJD and then follow what happens to them.

It is very, very difficult to actually get samples which are relevant to that kind of set up. So, my conclusion really has been that the major problem with validation is going to be on animals, like it or not.

That actually puts constraints on the kinds of tests that will actually come to fruition, if you will, which I regret, I have to say, but I am not sure there is a way out of that. If there is, I really want to know what the way out is.

DR. SOTO: The other point is, you kind of hinted in your last slide that the ideal test should give three positives in 10,000 cases of the United Kingdom and none in the United States. This is based on what?

DR. MINOR: I tried to hint at that by saying it may give -- it is based on the appendix studies that Marc

referred to.

It may be that if you screen through 10,000 blood donors you will find three people who are incubating variant CJD. If you do it in the United States, you shouldn't find any.

DR. SOTO: That is what I thought, that it was based on the appendix. You have to realize that the tests that people are developing today are much more sensitive than the system used to detect in appendix. So, you could expect much higher numbers.

DR. EPSTEIN: Thank you, and thank you very much, Dr. Minor. I ask this question with trepidation. What is the thinking in the United Kingdom regarding obtaining post mortem blood samples from people who have expired with vCJD.

One would think that the ethical questions are at least different and that that might be a potential source of human infectious material.

DR. MINOR: The answer to your question is, I don't know. I am, thank God, a PhD, not an MD. So, I know nothing about ethics at all.

I have discussed this with the guys up at the CJD surveillance unit about getting blood at all. They seem to be quite reluctant to do this.

I mean, I am not quite sure why. I guess you

could say that they would have to get permission from the next of kin, and the next of kin would be suffering enough at the time and they don't want to add to the suffering, I suppose. I am really not quite sure what the ethical position actually is. I am really not sure.

DR. CERVENAKOVA: My question is a comment as well, is about capillary electrophoresis which you mentioned here, without mentioning any other test.

Could you please elaborate on the data which you have to say that this test was working for blood and not working for brain.

If we start with that, is something working for blood and not working for brain, what was presented here is that you have to prove that your test works for brain, it works for spleen and it works for blood, is just he opposite of what you were saying.

DR. MINOR: Thank you for the question because it gives me a chance to clarify, I think. The process that I have described, I think, is rationale.

That is to say that what you would expect is that if you have an assay which will detect stuff in brain it might have half a chance of detecting stuff in blood.

You know brain is positive, you know blood is less positive. So, the increment would be a degradation of how much you look at these things.

Most assays, I would hope, would go through that process reasonably. The point was that if the fall at the brain fence, if you like, that may indeed be a deal breaker.

The immunocapillary electrophoresis thing, as you know, was a fairly elaborate and difficult kind of assay to do.

At one stage it looked reasonably promising, and there is a paper in press from the CJD surveillance unit talking about trying to do this immunocapillary electrophoresis on variant CJD bloods where they have said categorically that this doesn't work on brain samples from the NIBSC collection.

Now, that doesn't necessarily mean it works in blood either, but they did get a few positives after the blood.

The point was not to say that immunocapillary electrophoresis should be resurrected necessarily. The point was to say that, if it fails on the brain, it doesn't mean it is going to fail on the blood. I don't want to be quite so definitive about saying that it would actually fail on the blood. Is that good enough?

DR. CERVENAKOVA: I don't know how good it is because in our experience as well -- I never actually discarded that, but when we tested samples from sporadic

CJD patients in my group and we tested samples from chimpanzees infected with sporadic CJD, we didn't find positive samples when we did comparison to control samples.

At this point I believed the test was not optimized and now they claim they optimized it, but I believe we should not discuss this data until you have really a good -- until you have significant good data which would be presented to the public.

I always get annoyed that someone says something, there were a few positive samples. I know that it is possible to get positive samples, but how well are these data.

DR. MINOR: A fair comment. I think the argument could be that in the brain you have got big, big clods of material and maybe an assay system is not well adjusted, if you like, for measuring big clods of material, but it might be better for doing little bits, but I entirely take your point.

DR. SEJVAR: Just a real quick question about the ethical issues with respect to obtaining blood specimens from the variant CJD patients. That is an issue that had been discussed by the ethics advisory committee at the United Kingdom?

DR. MINOR: It is not quite clear who it has been discussed by, to tell you the truth. The specific which I

find very frustrating is the recipients of blood donations have not been followed up.

This was mentioned I think earlier on. I mean they have been followed up. Clinically they have been informed that they have been transfused with this material.

There was certainly a plan to try and get blood from them, which has been into the system somewhere. I am really not quite sure where it has come to rest. It went into the system quite a long time ago and nothing -- I am hoping for a resolution some time in the next few months, to be quite honest, but I am not quite sure who has considered it actually or, indeed, what the ethical issues actually are, to be quite honest but, as I said, I am a PhD.

DR. PRIOLA: Just a couple of questions about the reference samples. I assume the negative samples from the animal models like the sheep are all age matched to the positive samples; right?

DR. MINOR: Yes, up to a point. The BSE -- we have something like five BSE suspects not confirmed and a genuine BSE. So, I guess they are well matched, I would say.

The scrapie negative that we got was actually a sheep that we had wandering around in the field at the back of NIBSC that gave its life for the good of science, not

exactly age matched.

DR. PRIOLA: So, then on the human side, the samples that you have -- I am thinking of this potential that if some of these tests that might claim to be more sensitive and to detect samples as positive that you think are negative, how would you go about trying to confirm that result.

With the human samples, do you have any more information on those banked samples in terms of other tests that have been performed on those samples or the blood tests that have been performed on those, age, PRP level, PRP genotype, anything like that, would that be available to get in the case of a positive result for a sample that is presumably negative?

DR. MINOR: The brain samples were part of a WHO collaborative study that was looking at immunoblotting type sensitivity. That was really setting the baseline, if you like.

So, we have an idea of what the PRPSC content is. Larisa Cervenakova titrated sporadics in humanized mice and also the variant CJD in humanized mice, and the variant CJD, of course, didn't go so well because it doesn't in humanized mice.

Others are actually titrated in humanized mice as well, and we are in the process of titrating the two brains

and the two spleens by intracranial and intraperitoneal routes into the normal mice, C57 blacks. So, there will be some infectivity data that comes out on that. Does that answer your question?

DR. PRIOLA: I was thinking more of the 5,000 UK blood samples and the 5,000 US. Those are the ones where, if you get a positive, those could very likely be false positives, especially if they pop up in the United States. How would you go about trying to determine whether or not that false positive is false or real?

DR. MINOR: I mean, the first thing you do is make sure that it is consistent, that it is not an artifact of the test. I think you then try and pass it around to other people who have other tests and see if they got the same positive answer.

DR. PRIOLA: So, it is variable. You are going to assume it is a false positive.

DR. MINOR: Unless I have a better story to tell, which is that one test is more sensitive than the other.

DR. PRIOLA: That is what is going to be so hard to prove, the sensitivity.

DR. MINOR: Part of the difficulty with this is that the bloods that I am talking about will be anonymized because of ethical considerations, which I don't understand, but they will be seriously anonymized.

If they were not, then you could actually go back to the unit if you want, and you could actually look at the whole unit by some other kind of method, even doing an infectivity assay on it. Maybe we could actually consider doing that, actually, because we could keep the lymphocytes, for example, separate from the plasma, so that we could go back to it actually if we had to. Actually, that is a kind of good thought. I think we will go back and see if that is feasible.

DR. GESCHWINd: I guess maybe more of a comment, and that is just that I know that the politics in the United Kingdom and in the United States are so different around this issue, given the histories.

The fact that we have been able to get serial 200 ml blood draws, I think that if there is some way that model could be implemented in the United Kingdom, realizing that there is a limited number of patients, but working with the patient groups -- I mean, that has been so critical for us -- is having a good relationship with our patients, even those who aren't -- just in terms of the numbers, since we started our treatment study, I think we have had about 85 referrals of sporadic CJD, 25 of whom have come to be enrolled in this study.

Every single one has given at least one blood, 200 cc sample, except for those who we didn't feel were

medically able, and then we either took -- in a few cases we took less.

We only use the blood bags because we don't want to lose blood on the tubes. So, we use the 200 cc blood donation bags.

I really think it is because we were able to establish a relationship with the patients, we had a long diagnostic process to rule out other diseases.

It is because of that, that I think they were willing to, and also work of the CJD Foundation, that patients are willing to participate in research.

Again, I realize the clinical situation is different, but we would be happy to share our protocols of how we do it with whoever wants it, in terms of how we collected, our safety measures.

DR. MINOR: I think that would be very valuable, actually.

DR. TELLING; We are heading toward a break, but there are two other comments or questions I would like to take.

MS. KRANITZ: I was going to pretty much say what Michael said. Also, the National CJD support network possibly could be a resource, because they do work with the families and the patients, and could speak on behalf of that.

DR. MINOR: I think this is very good advice.

DR.TURNER: I am sorry, but just to try and clarify for colleagues the current ethics position in the United Kingdom, and that is that we are taking some samples from patients with clinical variant CJD.

They are 50 ml blood samples which are then separated according to a standardized protocol. That clearly has gone through a research ethics committee process in the United Kingdom. We have been doing that for some time.

I agree with the comments of colleagues around the table that it may well be that we can move on from that now and take sequential samples from such individuals. I think that would be extremely helpful.

Whether the ethics committee would tolerate it or whether, indeed, it is clinically acceptable to think about taking whole blood units from such individuals, I think, is debatable. We would have to have a think about that.

With regard to the 5,000 or the 10,000 blood donor samples, that also clearly has gone through an ethics approval process.

The reason for us blinding and unlinking those is that we think we probably will come up with positives and that would put us in a terribly difficult moral position, of knowing whether we should go back to those individuals,

whether we should defer them as blood donors at what stage.

We come across such problems with HTLV assays, for example. We don't want to particularly go there at this stage. So, that is really the reason for keeping them unlinked.

It means that we would not be able to go back to that individual. Nevertheless, there is a whole blood unit available, albeit that it is aliquoted.

For example, we could go back to those individuals, extract DNA from their leukocytes and look at their codon 129 genotype, for example. Thank you.

DR. TELLING: Thank you, Dr. Turner, for that clarification. I am going to end here because we are running a little bit late.

I would like to thank the presenters. We are going to take a break now and convene back here at about 10 to 10:00, and we are going to hear research updates at that time from the various test developers. So, thank you.

[Brief recess.]

DR. TELLING: So, I would like to reconvene the meeting.

Agenda Item: Research Updates from Test Developers.

DR. TELLING: We are going to move on to hear research updates from seven test developers, who are going

to review the current state of the art in terms of diagnosis of prion infectivity -- well, prions in blood. So, the first speaker is Dr. Alex Raeber from Prionics.

Agenda Item: Prionics.

DR. RAEBER: Well, let me thank the organizer from the FDA and TSE advisory committee for giving us the opportunity to present our approach to development of rapid blood screening tests for variant CJD.

The key issues in the test design -- and we have heard that already from previous speakers -- are that the test needs to be adaptable to multiple species that, because validation will most certainly take place with animal samples, then the platform for a test to be implemented in a blood service needs to be a simple microplate format. It needs to be alternatable for HTS.

Sensitivity-wise, it needs to detect about 10 to 100 infectious units per milliliter of blood. The specificity needs to be higher than 99.8 percent.

Our approach is to use an antibody in an immunoassay, that I will come later to the characterization of the antibody 15B3, and our approach is also to show that the assay has a broad species specificity, and that the antibody is bale to detect the abnormal PRP, which is disease associated.

The platform in our research state is IP western

blotting, immunoprecipitation followed by a western blot for the identification of a molecule.

In a second stage -- and I am going to show you data on that -- is an ELISA platform as well as a FACS platform.

The test principle is a simple antibody capture and enrichment step, because of the low levels of analite in the sample, washing and then detection using a second antibody.

The antibody 15B3 was described in 1997 in this Nature publication, as the first antibody which was able to detect to detect the abnormal disease associated form of PRP without the use of protease digestion.

More recently, we have gone on with the characterization of the antibody using a mouse model of the human form of the prion disease called Gertsmann Straussler Scheinker syndrome.

In this mouse model we could show that the antibody 15B3 is able to capture an abnormal disease associated form which is protease sensitive.

We have used this model to show whether the antibody 15B3 is able to detect infectivity which is associated with this protease sensitive PRP molecule.

This is the experiment. We used an immunoprecipitation with either 15B3 or an IGM antibody.

The immunoprecipitate was either than digested with PK as a control, or left undigested.

These immunoprecipitates were then inoculated into indicator mice, and what we could show is that only from the animals which received immunoprecipitated material without previous digestion, all the animals developed disease around 215 days, whereas the control animals all remained healthy for more than 300 days.

So, this shows that the antibody 15B3 is able to capture an abnormal disease associated form of PRP, which is protease sensitive, and which is associated with infectivity.

We have also cloned the region of the antibody into an IgG framework, and we have shown that the antibody is showing similar infectivity as an IGM or as an IgG molecule.

Originally this antibody was isolated as an IGM and some of the claims were that the affinity of the antibody to the abnormal form of PRP is due to the pentameric structure of the antibody molecule.

So, to summarize, the antibody 15B3 detects PRPTSE of humans, bovines, sheep, rodent and deer, and that was shown in multiple publications back in the Nature publication in 1997.

We could show that the antibody detects protease

sensitive as well as protease resistant PRPTSE, that it detects and captures PRPTSE which is associated with TSE infectivity, and that it binds PRPTSE with high affinity as an IGM or as an IgG subtype.

Our assay principle is shown on this slide. On the left-hand side we have the classical detection assays for post-mortem rapid tests which are currently employed in the European Union for rapid TSE testing.

The tests include a proteinase K digestion step and then detection by the 6H4 antibody using either a western, LIA or strip platform.

With ante-mortem testing on the right hand side of this slide, we don't need any proteinase digestion. The antibody 15B3 is directly incubated with the matrix and then the detection goes by an immunoprecipitation western blot, ELISA or FACs.

The assay format for the ELISA is the antibody is being first coated into the well of a micro titer plate via an anti-IGM antibody.

Then the sample is incubated, washed, PRP scrapie remains bound to the 15B3 antibody. Then detection is by segmented PRP antibody, which is linked to a horseradish peroxidase and detection is then by our chem luminescence detection system.

This shows early proof of principle studies found

with brain material from either cattle or sheep. As you can see, the positive yielded a strong signal which was between one and two orders of magnitude higher than the uninfected control animals.

We did testing of serum panels from clinical scrapie in sheep. In this slide you see a panel which was obtained by a naturally infected sheep flock in Sardinia.

All of the animals were clinical except one animal which, at the time of testing back in 2004, was still in normal and didn't show any clinical signs.

When this animal was later sampled, about six months later, it showed a high signal, and from this still rather small panel we show that the test has, on clinical sheep scrapie, a sensitivity of about 100 percent and a specificity of about 100 percent.

We then went on and tested preclinical panels of sheep again, and the results I am going to share here with you are from a Dutch herd.

This flock is a naturally infected flock and it is being sampled regularly in the preclinical stage by tonsil and testing.

So, what I can show you here is samples which are from sheep which have given a positive immunohistochemistry on tonsil. These are shown in red. Animals that have given a negative immunohistochemistry on tonsil biopsy are

shown in blue.

As you can see, from the preclinical sheep, about 70 to 80 percent yielded a significantly higher signal in terms of relative light units, in this chemoluminescent readout and, from the negative sheep, all of them are clearly below the cut off.

In terms of analytical sensitivity, we have used variant CJD brain homogenate provided by Jillian Cooper and Phil Minor from the NIBSC.

Doing a serial dilution of these brain homogenates into a negative brain homogenate gave a limit of detection of about 250 infectious units per milliliter.

I have to stress here that this is based on an assumption that there are about 10^7 infectious units of variant CJD prions in the brain, but this hasn't actually been titrated.

So, in order to increase our sensitivity and further, we have resorted to FACS technology, and we argue that, with the FACS technology you are not any more limited to the 200 microliters which you can use in a micro titer place, but you can use a larger volume, where we can actually go up to a couple of milliliters of sample in order to enrich further for the analites.

What we have done here, we have the antibody 15B3 captured on a bead, and then we incubate the bead with the

sample, which is either serum or plasma, and then we detect the bound PRP scrapie.

So, this is the antibody 15B3 on a bead, then it is incubated and it is washed, and only in a positive sample we have the secondary antibody which is labeled with a fluorescent dye, and giving a signal here in a positive case.

This is an RML mouse, scrapie brain homogenate, and half a microliter, 99 percent of the events are in this gate here whereas, in the negative control, only five percent are in this gate, and this is no sample at all.

Titration down to eight nanometers still gave a significantly higher read out in this experiment, and we did a limiting dilution experiment with, again, the same mouse brain homogenate which was titrated, and has a titer of about 10^7 to 10^8 infectious units per milliliter.

As you can see here, the limit of detection is around four nanoliter of a 10 percent brain homogenate, which corresponds to about 10 to 50 infectious LD 50 units per milliliter. This is about in the range where you would expect a potential blood screening test to have its sensitivity.

So, how do we see the further steps in the preclinical evaluation? We have already tested the analytical sensitivity panel from the NIBSC.

We are currently in the process of testing the blind panel of clinical scrapie blood samples from the NIBSC.

We will also look at blind panels from mouse and hamster and diagnostic sensitivity on the preclinical blood samples that will be tested in the next step, and these are also the panel from NIBSC.

We will also test seroconversion panels of experimentally BSE infected sheep to determine the earliest stage during the incubation period in which our test yields a positive result.

The clinical validation will include diagnostic specificity panels from normal human subjects, blind panels of other neurological diseases and blind panels of non-neurological diseases, to achieve the desired performance of less than 0.2 initial reactives, and less than 0.1 repeat reactives.

Diagnostic sensitivity will be tested on human clinical variant CJD samples using the blank panels from the NIDSC.

Diagnostic sensitivity on the human preclinical variant CJD samples, and hopefully there will be reference material around for testing that.

As confirmatory tests, we foresee different tests in animal bioassays because those are clearly not feasible,

but testing like amplification tests, or cell based bioassays would clearly offer alternatives as a confirmatory test.

I would like to thank you for your attention and I am available for questions.

DR. TelLING: Thank you, Dr. Raeber. I think I will reserve the time for questions until after all the speakers have completed their presentations. There will be about 15 minutes. What I would like to do now is ask Dr. Soto from the University of Texas to present his findings. Thank you.

Agenda Item: University of Texas.

DR. SOTO: Thank you very much. I am going to try to give you an overview of where we are at and what we are doing with our amplification technology for detection.

As you know, in early diagnostics the sensitive detection of the infectious agent in prion disease is very important.

PRPSC or PRPTSE is so far the most specific marker for the disease, but the level of other fluids and tissues other than brain, it is very hard to be detected.

Many of the people who are presenting today are following the approach of designing the more sensitive tests for detection of this marker.

We have a different angle and say, let's not care

too much about detection. Let's try to amplify the level of this marker present in a sample.

The rationale for amplification is that this is, indeed, what seems to be happening in the disease. If you look at the initial phase of the disease when animals have been infected or humans have been infected, they can be infected with very little, small amounts of PRPSC material, which grows by converting the normal protein, and at the end of the disease you get a very high amount of the mis-folded protein and little is left of the normal protein.

So, we are doing the infection period to the clinical disease. There is a huge amplification of this marker.

So, the reason that -- one of the good ways to approach the goal is to produce a similar mechanism establishing the disease process, obviously in the test field, and then obviously the biggest challenge is to be able to do it in an affiliated way so that we don't have to wait around the years that it takes this process to appear in vivo.

So, a number of years ago we came out with this idea of what we call protein folding amplification, which is ways of mimicking the amplification process in vivo, by doing a cellular mode.

This is again based on what may be happening

during the disease and, from the data from many different groups, what seems to be happening with the molecular mechanism of this process is that the misfolded prion protein is, indeed, a small earlier amount composed of similar units of the protein folded in a way that would fit the pathological form.

The way that this protein transformed the normal protein is by integrating into the aggregate, making this polymer to grow, and the protein is combined into the misfolded form.

This process is kind of limited by the number of nuclei that you started the process with. So, our idea was to say, well, let's combine this step of growing of this polymer within a study in which we multiplied the number of units available.

So, for doing that we used ultrasound authentication to break down these big pieces into smaller pieces. So, then now we multiply, we amplify, the number of free ends so in incubation with the normal protein we now have many more units growing at the same time and we can come again with another round of sonication to multiply the units again, then incubate them again with the normal protein and have each of them growing at the same time.

This is a cyclical process that can be repeated as many times as we want to increase the replication

sufficiently.

So, this model has many applications for understanding the nature of the infectious material and the biology of prions, but also the possibility to be useful for developing a sensitive detection method.

So, we published a proof of concept of this method. I am not going to go into this. It is old data, to show that it not only worked with samples for an experimental model, but then we went ahead and we automated the system, because that was a limitation of the previous experiment, that we were not able to do it on a routine basis to run many cycles.

This was done manually. Now it is done automatically using this mathematic microsonicator where you can put in an ELISA plate and the system works as well as the manual sonication.

So, doing that, we published last year that we can run, for example, 144 cycles of amplification and by comparing the signal intensity before and after amplification we found that we have an increased sensitivity of detection by about 6600-fold.

Obviously, in our estimation of what is needed comparing for what is needed to detect in brain to what is needed to detect in blood is probably like a million-fold more sensitive.

So, the question is whether we can continue doing more cycles of amplification to reach a million fold increase in sensitivity.

However, doing these studies we realized that after a couple of days of incubation like what is required to perform 144 cycles, the efficiency of amplification was going down.

The most likely reason is that some of the components of the mixture were getting older. So, to avoid this problem what we did was to run the serial PMCA procedure where, after the first round of amplification, the negative samples are diluted again to refresh the substrate into normal brain homogenate, and we do another round of amplification.

By doing like these two rounds of serial PMCA, we can amplify the signal by around 10 million-fold, which is rather good, but we wanted to know, what is the minimum quantity of PRPSC that we can detect using this method.

What we did was take a hamster PRPSC brain, spike it into blood, different LD50, about one in 50 -- for your information, it is correspondent to a dilution of about 10^9 from the brain.

So, with one round of PMCA we got two rounds, three, four. We can, of course, continue doing more rounds of amplification.

For example, by running seven rounds of amplification we can detect up to 0.001 LD50. So, much more sensitivity than the infectivity bioassay.

Our mathematical estimation is conceivably how much PRPSC we have in the brain. We are detecting an equivalent quantity of 20 to 50 molecules of the PRP monomer.

So, basically, it is very much similar to what Coughey's result of the single most infectious particle contains around 20 PRP monomers.

So, comparing the sensitivity to other standard methods, like western blot, ELISA, the animal bioassays, it is 330,000 times more sensitive than standard western blot.

One run of PMCA, 2,500, seven rounds of PMCA, we calculate that we are two million times more sensitive than the western blot, and capable to detect what we think is a single molecule of PRPSC. That is in hamster.

We are now trying to adapt the same technology to detect the same sensitive in more relevant samples like human or cattle. I will tell you a few words about that after.

So, I am not going to go into this. This is taken to estimate how much sensitivity we need to detect prions in blood.

I am going to show you a little data that was

published last year in Nature Magazine on the detection of PRPSC in blood.

We took samples from 18 symptomatic hamsters, and 12 controls. One run of PMCA will only detect one, but two will detect many, and six, which is close to what we need to do with the maximum sensitivity, we detected it in 16 of the 18 infected samples, and we did not detect it in any of the controls.

So, we got to around 90 percent sensitivity and 100 percent specificity. What is interesting is that -- that is one of the things you have to have in mind is that, not necessarily all animals that come down with the disease will have PRPSC in their blood.

We have injected the samples of all of these animals and what we get in terms of infectivity results is much lower than what we get with our PMC amplification.

So, we went ahead to see whether we could detect the PRPSC in the blood in the presymptomatic phase. So, we took hamsters again and inoculated them and took blood samples at different times before the clinical disease.

We did not detect any samples in the first time, two weeks after inoculation, indicating that we are not really detecting the material that we had.

At 20 days we get three out of six, 40 days the maximum one in the presymptomatic phase, 60 percent of

detection.

Then surprisingly the presence of prions in RPRSC in blood was going down and it virtually disappeared by around 80 days post-inoculation, to rise again at the symptomatic phase to get around 80 percent sensitivity.

That was published this year in Science. So, we got this kind of sensitivity and specificity. It was 100 percent specificity but the sensitivity was variable depending on the phase of the presymptomatic period.

We found this interesting curve, suggesting that there is an early phase of PRPSC presence in blood which we believe might be due to peripheral prion replication in the spleen or other lymphoid tissue, and then the infectivity goes down when the -- I mean, the PRPSC goes down when infectivity is going to the brain, and it rises again at the later stages, which most likely, to me at least, which suggests that there is leakage from the brain.

What is important to consider is that the specific hamster model that we are using here is one model in which the peripheral phase of replication of prions is rather slow or low, compared to other models like sheep or what we think is the case with variant CJD.

So, the fact that we have this type of curve in the hamster doesn't mean that we would get the same in humans, for example, and the maximum levels of sensitivity

would not necessarily be able to extrapolate to other samples.

We have adapted amplification technology to basically all different samples, experimental samples and natural cases in animals, and also in human samples of different kinds.

We are at this stage trying to get to the same level of sensitivity that we have with the hamster material, we are today very good in hamster, mice, only because we have done much more work, and then human is coming out very well, too. We were able to resolve all the issues of what material to use as a source of PRPSC, et cetera, but the plan is to adapt and optimize the technology for blood detection of PRPSC in natural samples.

We have not yet tried human blood, because I wanted to have the methodology very well optimized using spiked samples. In a couple of months we will be ready to try to detect it in human blood.

Once we have a reliable detection in human blood, our plan for scientific purposes -- this is beyond development -- is to do in correlation with some people, to do large scale studies for the detection of PRPSC in blood of healthy donors in countries with high risk of variant CJD like the United Kingdom or France, to have an idea of the potential number of cases of clinically silent human

variant CJD. In my opinion, I believe that it is underestimated today.

Understanding which PRPSC can be detectable in human and cattle experimental model, I think primate is also a good model. It has not been mentioned too much here.

Perhaps familial cases of -- just for example, the modeling as you know, people who -- we don't know, of course, the distribution of PRPSC in blood in those cases, but we are going to develop that as a potential for presymptomatic detection.

Also, what about other efforts to maximize the method for other samples? We have done a lot of advances on working on plasma.

Consistently in our experience plasma is like maybe 10 times -- has 10 times less PRPSC than buffy coat, but it has certainly PRPSC and it is certainly possible to detect that. Recently, we have been able to detect PRPSC in urine of samples. We are also interested in detecting there.

We plan to develop better technology in practical tests. What I showed you here was mainly our effort from the scientific point of view. There are many things that need to be done to develop this as a commercially available or viable test, but we are confident that it is possible.

The people who have participated, this is mainly

Castilla, Balazar, Morales, and in correlation with -- these are my former lab members, and in collaboration with several people around the world who have provided samples or materials for this study. Thank you very much.

DR. TELLING: Thank you very much, Dr. Soto. I would like to move on now to ask Dr. Wilson from Microsens Biotechnologies to come to the podium.

Agenda Item: Microsens Biotechnologies.

DR. WILSON: Thank you for inviting me here today. As we have heard, we thought of prions as being in different flavors.

A lot of work has been performed on these big aggregates, and these are the aggregates that were often used for spiking.

These are the aggregates that are protease resistant. We don't know what the form of the molecules that are the rogue prions in blood, although we believe that it is unlikely to be protease resistance.

This presents problems for people that have been developing post mortem assays because they can't easily translate those assays through testing, because a lot of those assays are based on protease digestion.

I just want to introduce you to our separation system, and tell you some details about our post mortem testing, which sets the ground work for our blood

screening.

When we first started out many years ago now, we were looking to replace protease digestion. In the literature there are description of polyenic polymers that actually capture aggregated prion protein.

What we did, we took those polyenic polymers -- we call it seprion -- and found specific conditions under which those polymers only capture the aggregated rogue prion proteins and, in fact, other amyloid proteins, too.

This just shows, this demonstrates the use of seprion on amyloid tissues to pull out atypical amyloid fibrils. This is made from material pulled out by seprion. You can see the typical amyloid-like structures.

You can also look at western blotting. The seprion materials was coated onto paramagnetic beads and used to interrogate uninfected and infected BSE brain.

Material was eluted from the beads and then run on a western blot and probed with antiprion antibody. You can see that you don't get any signal from the uninfected brain and you get the typical prion signal from infected brain that is actually protease resistant. You get a shift in mobility as a little bit of the protein is clipped off.

This material, our seprion system, can be used to detected the rogue prion in lots of different materials, in sporadic CJD, variant CJD, in brain, in spleen, of course

in cows, in BSE and scrapie. We can also use it to detect other amyloid diseases in different tissue types.

This raised the question, when I presented this data before, because we can't use the same ligand to pull out amyloid for Alzheimer's disease patients.

So, the question was, okay, do you get any cross reaction or interference. Well, the detection specificity is based on the antibody you use.

If you want to detect prions, use an anti-prion study. I can tell you we have done the studies to show that we don't get interference, we don't get inhibition of our prion assay by Alzheimer material, and we don't get any lack of specificity by using spiked in Alzheimer material.

Idexx has used our seprion technology to build a very simple post mortem assay. Here is shown the protocol of the Idexx post mortem assay, compared to proteinase K assay.

You can see all the proteinase K steps -- centrifugation, incubation steps -- have been removed in the Idexx assay. Of course, we can carry that same protocol through into the ante mortem testing.

The post mortem assay has been USDA approved for BSE and CWD, and the European Union approved BSE and scrapie.

The post mortem assay has shown 100 percent

sensitivity and specificity compared to immunohistochemistry on Rammel testing. I am not advocating this is a way for screening because, as we have already discussed, I don't think the stocks of blood would fall dangerously low.

So, taking that same technology which is being road tested, if you like, for the post mortem applications, we turn to blood screening.

There are lots of ways that we can try to keep blood safe. I just wanted to show this slide because filtration has been advocated as one way of keeping blood safe.

Even with filtration you still need to detect the prion agent because, if you don't, you can't quality assure that it is filtered and you can't do any type of epidemiological studies. You don't know how much infection is actually out there.

Our assay for the ante mortem testing uses, again, seprion coated magnetic beads, simple capture protocol and washing, and then the captured material is eluted and detected by an antiprion ELISA.

We are actually working on a different format where, instead of eluting and going through an ELISA, you could actually detect the captured material directly on the bead.

We have been involved in blind panel studies with mixed. This just shows one study where we were supplied with a blind panel of spleen spiked into plasma.

We assayed those samples. We did it on two separate days. We assayed those samples and we returned results. We said, this one was positive, these two were borderline, and we found a bunch of negatives.

When we broke the codes we found that we actually picked up the lowest dilutions of spleen in the plasma samples. The sensitivity of the assay equated to about -- if you say that spleen has got about 10^5 infectious units per gram, the sensitivity equated to about 100 to 1,000 infectious units per ml of plasma.

As has been discussed already, we don't know how that equates, how spleen infectivity equates to plasma infectivity.

We have used the assay to test specificity. We screened 236 blood donations, the plasma from human blood donations.

We initial found one we showed positive that did not retest positive. So, we had high specificity of our assay and nice low signals generated from those samples.

We have also been looking at sheep as a model. We looked at 12 samples of what we called suspect animals, those who were later confirmed by immunohistochemistry, 11

animals that had been exposed by the agent by the old routes, and those samples were taken at 11 months post-dose, and some uninfected New Zealand derived animals.

You can see that we get a range of signals from the suspect and the asymptomatic 11 month post dose animals. The animals that were New Zealand derived were all nicely negative.

So, you can understand why we weren't picking up all the 11 month post dose asymptomatic animals, but we are now working on the sensitivity of the assay to detect more of the suspect animals.

In fact, when we did a blind study with the VLA looking at a number of animal plasma samples, blind panel plasma samples, we looked at 29 samples.

We assayed the samples and then we compared our results to the VLA designation by western blotting, the subsequent western blotting.

You can see that there were two positive samples by western blotting in that panel. We picked up both of those animals.

We also picked up an extra animal. So, that could be a potential false positive. It was interesting. We got back the immunohistory of that sample. It was a sample from a suspect from the same farm as three previously confirmed scrapie positives.

Just to go back to the previous slide, just to show you that when we have done some data on asymptomatic animals, we have shown that we can detect infectivity in asymptomatic animals sometimes up to a year before those animals actually went on to develop symptomatic disease.

So, just to summarize, we are using a system that has been extensively validated on post mortem samples. We have used that protocol that we have developed to investigate scrapie in sheep plasma.

We can detect the abnormal prion in the symptomatic and asymptomatic preclinical animals. We are involved in doing blind panel studies, and I think we have just sent a bunch of results back to MBSCOM, their sheep scrapie blind panel.

We are poised, and have been poised for some time, to receive the human plasma panel. Thank you for your attention.

DR. TELLING: Thank you, Dr. Wilson. now we are going to hear from Dr Van Driesische from Biomerieux.

Agenda Item: BioMerieux.

DR. VAN DRIESISCHE: First of all, I would like to apologize for Herve Perron, who actually should have been giving this presentation. However, due to personal reasons, he had to cancel his participation at the very last moment.

I am still glad that the committee allowed me to present the work that has been done by Herve. I am not so directly involved in the work.

So, just to give the credits to the people who really did the work, and they have worked along with the group at AFSSA and in collaboration with the neurological hospital in Bron/Lyon.

Many of you heard that the objective here is to detect PRPres in blood. The project that has been chosen is to have proteinase K prion resistant and detect that by immunoassay in blood. As you know, possibly these oligomers are present in blood. We want to detect them.

As measured already, the prions are present in very low concentrations. So, the first set here is the concentrate and we detect these prions.

This experiment show the different glyco forms of prions present, and so with streptomycin, it shows that these are aggregated.

The next step, of course, is that they have to be able to separate these aggregates in a reasonable way. So, with a normal centrifugation method, it is shown that these aggregates are not present in the supernatant and concentrate in the precipitate, proving that we can concentrate the prions in the precipitate.

This is a slide basically showing the principle

of the streptomycin. We have to revise these with eventually concentrated and forming the aggregates.

This one has been tested on the western blot principle. The samples from non-CJD dementia, as well as from CJD patients.

This western blot basically principle, say, Alzheimers were correctly identified as being positive and negative using centrifugation. So, this is without a centrifugation step.

The next step in the assay, as we have some of these aggregates, we have to detect them. As we are not able to set the conditions to denature the aggregates, they are very strong, which makes it then possible to work with directly antibody coated microplates.

What we found is a solution by using calix-arene ligand that traps these aggregates. So, these calix-arenes are compatible with the denaturing conditions of the assay.

This is the chemical representation of the calix-arene. So, how does the assay then look like? You have probably from the plasma sample you have proteinase treatment, the streptomycin step to form the aggregates, the denaturation buffer, and then the ligand to bind and trap these aggregates, flow down by normal detection and ELISA.

these are some results from human plasma series,

different trials, the use of several antibodies with one antibody to show the negatives and the positives are correctly identified.

So, in a study where we used two different monoclonal antibodies, in that case we find false positives, and all the false positives are correctly identified.

This summarizes this experiment. In this case, of a total of 20 CJD patients, 17 were collected and identified with a false negative, resulting in a sensitivity of 85 percent.

When we used the two monoclonal antibodies, we were able to detect all the of these, and are seeing some non-specific results.

These are some results with VLA samples, a series of 40 samples, and 38 were uninfected and two samples were not detected.

There is some question also of the quality of these samples. That is obviously the negatives were all clear negative in this case.

So, in conclusion, we have shown the feasibility of the detection of PRPres in plasma. We are definitely not really to say that we have a product to go to the market. We definitely need some optimization work in the design and the protocol.

Other steps, of course, to make it accessible to the blood bank community are also that we have to take care of automation and utilization to make it a real practical, workable approach. Thank you.

DR. TELLING: Thank you, Dr. Van Driesische. We are going to move on now and ask Dr. Lohman from Adlyfe to come to the podium, please.

Agenda Item: Adlyfe.

DR. LOHMAN: So, I would like to thank the FDA for inviting Adlyfe to speak. I am speaking in place of Dr. Cindy Orser, who many of you have seen her presentations on this data a number of times, and historical data that she has shown is quite nice.

I am going to take a little bit of a different tack today, in that I would like to talk and address some very specific issues for the FDA in the nature of what are the infectious molecules and how do we identify those, and I would like to talk a little bit to that side.

Let me first describe, amplified misfolded protein diagnostic assay. We are not as mature as some of the other technologies, but we are trying to come on strong.

The basis of the technology is simply to mimic misfolded protein diseases, that is, take a peptide or a protein or a portion of that molecule and mimic it, and

also label it so that, when it meets the infectious molecule that is defined by the bioassay, it folds, much like the misfolded protein activity, from alpha helix to beta sheet, and you get a florescent signal.

Now, this is a unique peptide mimic. We see specificity through the amino acid sequence. So, it is driven by amino acid sequence homology.

We have also designed them so that they will fold and mimic the actual disease process. The sensitivity is through the fluorescence amplification.

Now, amplification is the key .You have heard from Claudio about an amplification process. This, in fact, is similar to an amplification process, in that, as long as the infectious particles are in the well -- and this is a 96-well format -- and there is a sufficient amount of peptide in place, it will continue to fold and increase signal.

So, it is a linear application. It is not an exponential amplification, but it does amplify signal in the presence of the target.

The way we monitor this signal is in the association of a conformational change of fluorescence. We look at the unfolded peptide labeled on either end, in mass spectrum, and in the presence of the target molecule you get a different fluorescent signal.

That shift is what we monitor, and we look at a ratio of the exomer, which is the fluorescent product, versus the monomer which is the baseline unfolded protein.

so, some of the development issues -- this is where I want to kind of diverge a little bit. We have much historical data that I could go through, but in 10 minutes I think we will just skip to what we think are some of the development issues for assays in general that we need to address for the FDA.

What we have been focusing on, in our development efforts, are to develop some positive assay controls. We have noticed in our studies -- and I will mention some of those in a second -- that -- and you have heard this over and over again -- that what is in a hamster brain may not be what is in human blood. So, what are the best controls to use? We will talk a little bit about that.

We are tremendously focused on the physical state of the target. We are identifying the infectious molecules, and we have a fair amount of data to prove that those molecules do change, and they do, despite the fact that they are all PRP, they don't all have the same levels of infectivity.

So, we are looking at and describing the physical state of the target. Much of this is in the process of being published and that is why some of it won't be shown

in public today.

The other aspect is the time course. When does the target appear. You have heard some information, some data, from Claudio about how in the blood -- and we have some data in spleen that we are not going to show today -- that support some of his work, that says there is an early rise of PRP in spleen, and then it gets into the blood and it goes into the brain, but it disappears in certain places. So, there is a transiency that we also believe exists. So, we are trying to establish that based on the conformational structure of the infectious particles.

Then we are in the process of looking at what is the prognostic correlation for that measurement of infectivity versus how much PRP is there. I think those are different answers. Can we apply this technology to broader amyloid disease states.

This is some basic information on a synthetic peptide aggregate control. We are trying to build a control that mimics in vivo aggregate, that is, the infectious molecule.

We have identified some peptides out of the native molecules that react very similarly, and you see some of this in the literature as well.

We have control conditions for creating these aggregates that give us a very similar reactivity as the in

vivo situation.

We have stable production methods for these, and the amplification kinetics are being well defined based on our AMP-D assay.

We are getting amplification across time versus the control. So, it is an amplification process and it does mimic in vivo to a fair degree.

The other is a natural control. This is some anecdotal data that we are going to publish. So, all I want to do is give you a verbal review of this data. Others have spoken about Byron Caughey's fractions and Byron and Jay Silviera have provided us very kindly with a series of samples that have been fractionated, also to compare to hamster and normal brains that were fractionated similarly.

The outcome of those results -- I will synopsize -- are that our assay sensitivity is linked to the infectivity of the molecule and its PRPC converting activity.

Now, Byron's data indicates what he calls a direct infectivity and another one is converting activity, and he has an assay that looks at how much PRPC to the native molecules converted.

So, what is the propensity of any aggregate is to convert more PRPC. That converting activity directly relates to our assay. We actually can show very clearly

that, in our assay, we very sensitively detect the most infectious particles.

Then, in a broader sense, identify larger aggregates, which we do identify but, in terms of the infectivity and the amount of signal that we get per milligram or per microgram of PRP, it is reduced. That is because the conformation is different, although there is converting activity. So, we are looking at both, a mixture of signals in vivo.

There are molecules that are more infectious and recruit PRPC in different ways. So, we have to be very careful when we define what we have to stand up against, which is that you have to have an infectivity number, but we have to be careful how we define that.

That is what I would challenge the group here today is, how do we define that. We are trying very hard to understand that.

The other thing that our assay sensitivity is linked to is conformation, not concentration. We are going to publish this data, but is shows very clearly that, if it is in the right conformation, you can very, very sensitively detect those molecules.

If it is in a more aggregate state, there is much less reactivity to it, although we can detect it. So, there is this continuum.

It is also a transiency, when it shows up in the blood, when it shows up in the spleen, when it shows up in the brain and what form it is. So, you have to be careful in terms of defining what those molecules are.

We have done a great deal of work and much of it is being published. So, I am only going to synopsize. We have looked at both experimental disease and endemic disease.

We have looked at clinical cases in hamsters, squirrel monkey and sporadic CJD in mice. Sheep, bovine and human for endemic diseases, and preclinical hamster and sheep.

I know you have heard this a number of times, but we have looked at a number of different sample preparation methodologies with the end point being putting it into our 96 well plate reaction.

What happens in hamsters doesn't necessarily mean it is what is going on in human blood. That is as best I can put this data. We have a lot of data that we will be showing later on in public.

Unfortunately, the timing of this meeting was that we didn't have this out yet so we could talk about it, but yes, we have tested many, many types of samples, brain, spleen, blood.

There is quite a variety of molecular structures

from species and individuals. So, we have to -- we are urging you to be careful and the community be careful about what we are defining in terms of what we have to prove clinically.

So, how would we define a model system for TSE diagnostics? As I previously showed in the slide before, we have looked at a variety of animals, and those animal models can be diverse.

Running bioassays can be costly. It depends on which animal. If you are doing it in sheep it can be quite expensive. So, looking at how we define bioassays is something that we are after and that we are trying to understand.

Standardization of samples, you have heard from Dr. Minor and a number of other groups and also the FDA is making attempts to put together standardization of samples and controls.

How do you confirm your assay? That is another example. What I am trying to make a point about today is, be careful how we say your confirmation of infectivity. It may not be what we think it is in terms of the infectious molecules.

It also made then the issue of, are there multiple assays or multiple technologies that need to be used to reach the goal that you are asking for, and that is

entirely possible.

Should we pursue other options in terms of bioassay? Dr. Minor did a very, very good job of explaining to you how tough it is, and we know very clearly how hard it is to come up with variant CJD samples.

There aren't very many of them and they are sort of precious samples. I am a firm advocate of, if you are going to develop an assay for blood, it needs to be in blood and it needs to be for human. It needs to be in those samples, and that is difficult to come by.

So, what we really, really need to press for is a model system that does mimic that. I know that the NIBSC has done a tremendous job in trying to present a panel for that, a standardized panel, and to understand as a community what animal models would be used.

The other question is, could we use something like cell culture. I know that has been suggested. Are there other methodologies that we could use that are human based cell culture techniques that might work.

What we would ask is that, again, like NIBSC has done, is to make these samples available to the community if it is possible. I know it is definitely not necessarily simple.

So, what we would suggest for some of the performance requirements, we do know that regulatory

guidelines are needed for sensitivity and specificity, but we would ask caution in how those are defined.

Confirming the TSE assay performance using animal endemic or control diseases are needed. We are trying to define those systems out there and I think everyone is aware of that.

What comes out of those animal systems has to be very carefully characterized and we are working on that to define the infectious particles.

Then the correlation of those findings with multiple tests. Could cell culture based infectivity models work, are there other animal models, or do we need antibody tests as well to confirm.

So, we are looking at infectivity. I think we have an assay that very sensitively gives you an idea of what the infectious molecule levels are in an individual.

The question is, how does that correlate to concentration of PRPTSE, and those may not be identical. I think Byron Coughey's data also supports that, to some degree.

The other aspect is, we do need third party validation site testing. Variant CJD is considered a CAT three, and that is difficult to get in laboratories everywhere to do those tests. So, that is another site issue. Again, the standardized battery of samples is

absolutely necessary.

I know I haven't shown a great deal of data and it has been a bit cryptic, but we are in the process of trying to publish a number of things. We wanted to more address this group.

As a summary, the pronucleon AMP-D assay does exploit the basis of the misfolded protein disease. We have good proof of principle in TSE. The historical data that Cindy would show you is out there.

This is an interesting hyphen. That should be any beta. Our technology is being extended into other amyloid diseases -- Alzheimers, we are looking at a number of other diseases as well, for the use of this particular assay.

We are developing the assay going forward toward blood. We are fractionating blood, we are looking at the normal blood fractures.

These are normal proteins that get subverted, and what are they doing to impact the assay. So, we are breaking down plasma and looking at all the plasma fractions as well, and that is part of our development.

Again, we do need some regulatory guidelines. I think where we might look for some help along the way is looking at CE marking guidelines as those come along, and maybe those could actually help formulate guidelines in other regulatory environments.

Although I didn't show the data, these are the group of people that we collaborated with and that were on that list of experimentation that we dealt with, and we appreciate Paul Brown and Larisa and others that are here today for their help in some of the data that was generated. Thank you.

DR. TELLING: Thanks, Dr. Lohman. I think now I would like to ask Dr. Peretz from Chiron to talk about their efforts in detecting infectivity in blood.

Agenda Item: Chiron.

DR. PERETZ: Good morning. I am here to present Chiron's development efforts into a blood test for vCJD. Chiron is an established and mature company that develops many blood tests.

We partner with different companies to develop blood tests for HIV, SIV, HCV and lastly to west nile virus. We have experience with developing immunoassays and other assays but today I am going to focus, of course, on the vCJD issue.

I am going to start with showing our development progress. I will show a little bit of performance data and I would like to show the committee our proposed validation process and have their comments.

As you all know by now, there are many challenges to developing a blood test for vCJD. The biology is

unknown, we don't know what the prevalence of finding prions in vCJD patients are.

We don't know, we don't have any idea -- I mean, we have some idea but we don't know how confirmed they are. We are not sure how the rodent models are relevant to the human situation, and sample availability is a major hurdle for all the developers.

I mean, we are hearing that we have maybe more than 30 samples now, but the volume is critically low. There is no confirmation test, there is no in vitro confirmation test. So, it is difficult to bench mark your test against another test if we don't have it.

The sensitivity is an issue. This is not nucleic acid where you can amplify it with PCR. We also should be aware that there is a decline in incidence, which makes continuous testing questionable. We would like to address that this is really an atypical disease, and there is no predicate to such a test.

This is our assumption originally going through this development work. The assay was developed to detect the human PRP scrapie. I think it is the marker and the cause of the disease, and it is a major analite to try to detect.

What about the assay sensitivity? In hamster models we know that one LD50 would translate to about 0.1

picogram of PRP scrapie.

What about the human homogenate? We have some data on the sporadic CJD brain homogenate. There was a transmission study earlier with monkeys and primates, and this will come with 10 nanoliters, which is about a 10^6 dilution.

Transmission to transgenic mice by other groups suggests a lower LD50 number. So, there is a range between 0.1 to 10 nanoliters of CJD for one LD50.

We started our experiments with spiking experiments with animals, so that we can detect different species.

Now we are focusing our studies and using human tissue of variant CJD, brain homogenates and spiking into plasma.

I would like to thank the MEPS people that provided and made these samples available to the community, which was a great help.

We are now in the process of detecting and trying to detect and develop assays to detect prions in animals that are infected.

All of these are preclinical studies, and in the clinical studies we would like to establish the sensitivity and specificity against vCJD samples. We would like to have a confirmatory assay that is as sensitive and can

actually improve our results.

This is our assay format. We discovered that the prion peptides can interact with PRP scrapie. In our current system we use a modified peptide which we turn with -- actually it was invented and discovered in panels, so it is a clinical modification of the peptide.

We conjugated the peptide, I should say, to a magnetic bead and, in our spiking experiment, we spiked that homogenate into plasma. We used 80 percent plasma and we mix it and with the presence of the magnet actually we can wash everything out.

We don't use PK. So, there is high specificity to PRP scrapie and we did experiments with plasma, and now the plasma is full of PRPSC. So, you would need to have really high specificity to PRP scrapie, if you are not going to use PK. We have shown and found that we can actually capture all the PRP scrapie in the supernatant.

The next step, after washing all the plasma and other proteins, we dissociate and condition to antibody capture. So, this is ELISA, and we have the second antibody specific to PRP.

So, we have two antibodies. So, we have that specificity and actually we -- this is conjugated to AP conjugate, and we use luminescence which gave us high sensitivity.

This is an assay, there is no centrifugation, and this assay can run in a 96-well format, and it is taking about four and a half hours.

This is some data on our sensitivity. This is our data on our ELISA only. This is a recombinant therapy and we just want to show what is our limit of our sensitivity.

Basically, we can detect even low levels of protein, which is an extremely sensitive ELISA assays. We don't have many ELISA assays that actually can detect less than one picogram of protein.

This is our spiking experiment, spiking into plasma. This is our assay a year ago, more than a year ago. We were able to detect one microliter, which is 10^4 dilution of the brain.

This is a variant CJD brain. Now we are here and we can detect about one nanoliter of brain homogenate, and we are talking about -- it is 10 percent homogenate of a 10^7 of a gram of tissue. So, I think we are approaching the limits that are necessary for detecting prions in blood.

The literature will suggest that 10 nanoliters to 0.1 correlate to one LD50. It really depends on the model. If it is then -- this is our initial specificity studies and this is spiking material.

Using a standard deviation we can clearly define

from the negative plasma samples. Of course, this is a very low scale study.

This is our proposed preclinical process. We have shown that we can detect PRP scrapie and also we usually don't use PK, but we have shown that we can also pull down PK resistant material.

We didn't show that the material pulled down is infectious, but there is not any reason to believe that the material is not infectious. This is just PRP scrapie.

Proof of principle, we have some effort into detecting PRP scrapie in sheep blood. This is 100 samples, 100 negatives. We also have a time course in a hamster. These are ongoing studies.

This is really a challenge for a company to develop a blood test for humans, because you have to develop a whole new animal. You use different antibodies, different sensitivity. So, this is a major hurdle.

This is our proposed clinical validation process. We would like to test 5,000 in the United Kingdom, and this is what is reflected in Turner and Phil Minor's presentation. We would like to test 5,000 UK samples, 5,000 in the United States. This is going to be our clinical specificity.

We would like to test hospitalized patients from non-neurodegenerative diseases. We would like to test 100

neurodegenerative patients, and we also would like to test the plasmas with interfering substances.

Our clinical sensitivity, we would like to get access to the variant CJD. We would like to test the sporadic CJD to do the work in actual sensitivity versus our clinical sensitivity.

We also think that we should compare our results to a commercial validated assay which has a similar sensitivity, if available, or with a research assay that will validate our assay, given that the sensitivity is the same or better.

I would like -- this is a busy slide, but I think it is an important slide. This is a recommendation or a proposal. We don't think that assays should be validated using infectivity studies.

We are coming to that because there was a suggestion that any assay that detects prions in a rodent would have to take this blood and reinfect other animals to show that what you are detecting is infective.

We don't think there is much relevance to that, given that these parameters are fulfilled. We think that there is good evidence there is a transmission from variant CJD blood transfusions, and so it is a given that this blood is infectious.

I think detection of PRP scrapie has been shown

in many samples. It is not only in brain homogenate. It has been shown in spleen and lymph nodes or tonsils. We are not sure how different PRP scrapie actually is in blood. So, PRP scrapie will go with infectivity.

We also think that testing rodents is an issue. The PRP scrapie levels are different within different species. The levels in hamster are different from mouse, different from sheep and different from human.

The level of infectivities vary even more. In the same hamster we give 10^{10} or 10^{11} and if it is mouse we will give it 10^{8} and the ruminant or bovine 10^{7}. So, the levels activity are extremely fluctuating.

In such experiments to confirm the data with infectivity studies from a rodent, this is an issue, because with the low levels of infectivity that you might assume you would find in rodent blood, you might exceed the life span of the rodent, and this is shown by Bruce Cheeseborough.

An assay that can -- there is an assay that can demonstrate PRP scrapie detection in disease progression models, this would add to the value, and diminish the value of testing with rodent infectivity.

We think that the assay which can specifically bind CJD positive from normal PRP, that is basically our aim, and an assay that actually confirms this finding.

I think that if there is a way that a manufacturer can separate the distribution between the variant CJD and the normal CJD population, all of this -- actually that is all that is required to make these obsolete requirements, because this is our target, to discriminate. We don't want this variant CJD blood to get into the transfusion.

We also think that it will be helpful to have an allocation of funding to support generation of surrogate references, and a confirmatory assay.

We would all be in a better position today if we had a confirmatory assay that all the manufacturers could bench mark their findings against this assay. This assay could be western blot PMC or cell culture or any other.

We have today with us Andrew Heaton, we have Alisha with us and Rainer, and they probably might answer some of the questions that you would have, and I would like to thank you for your attention. Thank you.

DR. TELLING: Thank you, Dr. Peretz. Finally, i would like to ask Dr. Safar from UCSF to come to the podium. Thank you.

Agenda Item: University of California, San Francisco.

DR. SAFAR: I would like to thank the committee for inviting me. Second, I am here in the position of

really of the highest regard and appreciation of all the samples we receive from patients, and collaboration with their families.

Without them, we couldn't present the data that I will present today. It is also work of the CJD Foundation and many other organizations who helped us to set up a system, the results of which I will present today.

I think that all those very technical discussions before I translated into a very simple outline. Essentially the test for the CJD and human prion diseases has at least three groups of important criteria.

First, it is to provide definite and reliable diagnostic information for prion prevention. That is what is the category in which variant CJD belongs.

Second, we are developing, and there are many groups now, treatment for the prion diseases. So, we need a prion presymptomatic test which would discriminate those patients who require treatment and those which don't.

So, we need a presymptomatic diagnostic test. Of course, those which will test negative will receive no treatment.

We much meet specifications. I think that, again, I translate those highly technical terms presented before into two very simple criteria.

We need measurement at a level which would really

indicate that what we are developing is truly our target. So, the detection has to be true. The trueness of the measurement, I think, is one of the highest possible requirements in this case because of the expected low concentrations of the target.

The tests will require a level of diagnostic accuracy. That means, required sensitivity and specificity. We have heard assumptions already for those requirements before.

Those have to be in the desired range. I think we have a certain intrinsic conflict between what we actually want to achieve, or we can apply flexible criteria for those final goals.

If you think about the preemptive measure for the variant CJD where you want to prevent the entry of the contaminated blood into the transfusion program, we have to move the sensitivity to the highest possible level, and the specificity has to be practically 100 percent.

For the diagnostic criteria, it is a little bit different. So, the cut off value can be applied differentially for those two different applications, if the test is quantitative and will allow us to do that.

I think that I will not talk in detail more about CDI. There are now more than 20 papers published in either scientific studies or validation studies with that assay.

It is an assay which is now used for many scientific projects, and it is validated for the detection of BSE in cows in Europe and tested for specificity in the field for one of our 10,000 cows.

It is also validated for the detection of scrapie in sheep and for other cases of scrapie. Also we can use it for discrimination of BSE in sheep from scrapie in sheep. So, I will not talk about specifics.

The assay works for both protease resistant and protease sensitive forms of PRPSC, where the surrogate antibody detection which is exposed in the PRPSC, and measure the reactive material the same way in the native and denatured form, we don't see any differences in the readings, or very little.

When you have PRPSC in the mixture, we will have high increase in the signal from the native to denatured state, reflecting the exposure of the antibody in that denatured state. As a result, we see the increase in the signal which is proportional to the concentration of PRPSC.

The sensitivity of the assay is equivalent to about 78 in the absolute sense for testing recombinant PRP in plasma. So, the target in this case, analytically, is in the current range.

The second conformation I presented yesterday was on comparing the bioassay sensitivity directly to CDI. Our

estimates are that the CDI assay is at least 10 to 50-fold more sensitive than the bioassay in the humanized transgenics.

The important issue in plasma or other blood targets is that it is actually a very complex organ. The 60 to 80 milligrams of protein in the plasma has a range of different proteins about 30,000 different proteins, that range from milligrams to picograms.

That is the range of 10^{9} to 10^{12}. So, in any analytical target, it is as difficult a target as is the brain or any other organ.

So, we focused our attention first into the separating and concentrating the ligands for the prions which we allow to target, more specifically to testing and eliminate those components which do not test and actually interpret negatively as the assay.

After many experiments we found that the human LDL and other lipoproteins containing lipoprotein B are binding in AP CJD prions with very high affinity.

The PRP apoB binding for APOB and LDL is 90 to 31. The order of the binding among the different PRP conformers goes from the PRP 2730, human PRP 2730, to denatured recombinant PRP, to alpha helical recombinant PRP.

The different PRP proteins display different

stoichiometries. Lipids in LDL are not essential and the glycolipid of PRP also is not contributing to the binding.

The LDL and alpha B binding the denatured recombinant PRP is sequence specific and we see differential binding with different prion strains.

So, that was the starting point when we applied our analytical project, which essentially was designed to find the carriers of the prions into plasma.

I think that, without knowing where the prions are, in which compartment to which are we looking for the cells or plasma proteins or components of cells, it is very difficult to design any rational test targeting prions.

So, thanks to our clinical group, who are led by Michael Geschwind and Bruce Miller, we are now able to collect up to 200 ml of blood, which is then separated into the white blood cells and plasma.

White blood cells are then separated by first cell activated sorting or magnetic bead sorting into the different subtypes.

Plasma is used in polyoxometalate fractionation or potassium bromide variant fractionation into the VLDL and other lipoproteins plus remaining proteins.

All components in both cell spectrum and plasma spectrum are then tested by CDI, western blood and bioassay, to detect PRP, infectious PRPSC protein.

So, we simply kept all the options open because we don't know anything really about infectivity within the blood.

So, we look at all the bioassay studies quoted earlier before. Both cells and plasma test about the same way. The transmission rate is between five to six percent.

This is the finding from 21 donors and 20 sporadic CJD cases. If you look in the starting plasma, there is no difference in the total PRP concentration or PRPSC concentration. The total value is about one picogram per ml.

If you look at the VLDL fraction obtained from donor and sporadic CJD, there is a statistically significant difference in total PRP, with higher values in the sporadic CJD cases.

If you look at the PRPSC measured by CDI there is are significantly higher levels, very high levels in some cases, for PRPSC in sporadic CJD cases. There is no difference. It is also statistically significant.

Next the question was, are the VLDL differences in reading in PRPSC protein into the LDL reflecting just differences in the cholesterol or lipoprotein B metabolism.

So, we tested starting plasma and VLDL fraction isolated from those samples for cholesterol and ipo B concentrations in donors. This is 15 donors, 15

neurological controls, and 15 sporadic CJD cases.

Those neurological controls were age, sex matched neurological cases which usually display the early stages of dementia. So, they usually are classified clinically as Alzheimer's disease.

In all those separations, we didn't see any difference in the cholesterol levels in plasma and no difference in the apoB concentration in plasma.

That goes very well with the known studies presented before in the literature on Alzheimer's disease and healthy controls.

When you look at the VLDL, the difference is in the concentration of the cholesterol and apoB recurrent in the VLDL fractionated from this plasma. They were not statistically significant.

There is a trend to look for higher apoB concentrations in the sporadic CJD cases compared to the neurological controls and donors, but the trend is just a trend. It is not statistically significant.

When we look at the readings in CDI and look into our calibrated bioassay, calibrated CDI bioassay in transgenics -- it was published in PNAS in 2005 -- you can actually correlate the levels we detected in VLDL with the expected transmission rate for the VLDL fractions.

The readings we got -- and this box is actually

covering those readings in CJD cases -- are corresponding to about zero to 40 percent transmission rate.

That is exactly what you expect from the animal studies done repeatedly by Paul Brown, Bob Brewer and others, indicating that the plasma transmitted with the frequency of about five to 60 percent.

This is the second stage of the study after improving the protocol. We essentially were able to discriminate donors and sporadic CJD with sensitivity about 90 percent and specificity about 100 percent.

The boxes are 50 percent of the values. The line in between is the median, meaning the midpoint of the higher and lower values, and the lines are describing all the samples.

There are two additional samples in the CJD group which tested very high. So, I didn't pull them to keep the scale up.

The ranges of the readings in the sporadic CJD are very broad. I estimated it is between 100, 500-fold. So, the testing in this case raises the question how reproducible actually those readings are.

So, we used two different capture antibodies and two different detection antibodies, the European recombinant antibody, FAVP, which we use for detection also of the BSE, and European monoclonal antibody CF4.

When we looked at the readings of those IP and CJD cases against each other, they are practically in line, indicating that both antibodies, with European, detected similar concentrations of PRP proteins. So, in conclusion, what we detected so far is truly PRPSC protein.

In new data, which we accumulated in the past few months, we tested again donors, 21 donors, 15 neurological controls, age, sex matched, and sporadic CJD.

We have two outliers in the neurological controls, and because in that group most of the patients are still alive, we don't have pathology, we don't have a confirmation.

So, we don't know where those two cases are, and how they would display after re-testing. Looking at the sporadic CJD, 50 cases in the box are above the level of the highest level in the neurological controls or donors.

Most of the cases thus far test truly positive. There are a few cases in the sporadic CJD which tested negative, and we are going back to them and we will analyze the clinical reports.

So, what are the conclusions and new directions? I think they are conformational specific, high affinity binding of native sporadic CJD prions to the LDL in vitro, and finally of PRPSC protein in the same fraction as CJD infected plasma.

It suggests that apoB containing alpha protein transfers sporadic CJD clearance. Whether that is true for the variant CJD prions is yet to be determined

The existence of stable lipoprotein prion complex in the sporadic CJD plasma implies the role of the lipoproteins in the prion clearance from brain and other tissues.

Both conformational specificity and high affinity will lead to the development of new assays for prions. As the data on other prion ligands suggest, they are lipoprotein binding and they impact the infectivity of sporadic CJD prions.

The conformational specificity of apoB binding may lead to the new ways of differentiating human prion strains, including variant CJD. Plasma lipoproteins provide a highly specific ligand for prion concentration or removal from plasma.

I think that this project is responsible only to very diligent and, from the beginning, a very important contribution of Michael Geschwind and Bruce Miller, who are in charge of the memory and aging center at the University of California, San Francisco, predominantly dealing with Alzheimer's disease, but also now with sporadic CJD.

From our group, we have done a lot of microscopy and many people contributed significantly to immunology or

other aspects of the study.

At the University of California, we have a major collaboration in detection of prions, and with the Gladstone Institute we are working on removing LDL and VLDL and other proteins from the plasma.

I showed you the pictures here and yesterday on the LDL and VLDL and HDL. You don't see it very often that someone contributed his own tissue, his own blood for the science. Thank you.

DR. TELLING: Thank you, Dr. Safar. I am now going to open up the session to questions from the panel to all the speakers who have just gone. So, are there any questions? I am sure there are.

Agenda Item: Questions for the Presenters from the Committee.

DR. LILLAND: A couple of questions. I wanted Dr. Raeber to elaborate on the isotype of the detection antibodies using the B53, as well as clarify -- there were some concerns regarding -- you referred to LD 50 versus ID 50 on one of the slides. I just wanted some confirmation.

DR. RAEBER: I am not sure whether I completely understood the question. The first one, you refer to the isotype of the capture antibody, or did you say the detection antibody?

DR. LILLAND: That is just it. I wasn't certain

from your slides. I was a little bit confused on whether the antibody you were describing was IGM or some IgG subclass.

DR. RAEBER: Okay, I understand. Thanks for that question, and I think it is an important question and has been raised many times in the last couple of years when we have presented our approach.

The 15 B3 antibody, which is the capture antibody, was described in the 1997 Nature publication. This antibody was isolated as an IGM antibody.

This antibody we have engineered into an IgG subtype. So, it is in a humanized IgG framework and we have tested that antibody in parallel with the IGM and it performs with the same sensitivity and specificity.

The detection antibody is just another -- I think it is an IgG subtype, but this does not really have any relevance with regard to the question on the capture antibody.

The second question you raised was the LD50 in the slide where we determined analytical sensitivity. So, the Ld50 are basically derived from the inoculum from the RML homogenate, and this was titrated in mice.

Based on that level of infectivity, we did titration studies, dilution studies, and we determined that the detection limit is around 10 to 50, and ID 50 per

milliliter. Does that answer your question sufficiently?

DR. TELLING: Dr. Raeber while you are there, I have a question. So, you mentioned studies in sheep and you mentioned that you had tonsil positive IHC, IHC positive tonsils of these sheep.

Do you have any idea -- I am sure you do, but could you clarify what stage of the incubation period those animals are when they are ICH positive, and how that corresponds to the ability of 15 B3 to detect -- to diagnose disease?

DR. RAEBER: I am glad you asked that question. It is really something which we are going to go a little bit deeper into now.

This is from a naturally infected herd. Therefore, we don't have any indication at what stage the animals were actually getting infected.

So we don't have any knowledge on at what stage in the incubation period those animals are. The only thing we can really take from these studies is, once we have taken a preclinical biopsy sample and taken blood and assayed it, we can go back to the animal after six months and, if the animals is still alive, we can take a second sample. From that point on, we get a sort of a view how long the incubation time is.

DR. GESCHWIND: Also for Dr. Raeber, this may be

a naive question, but why are there different tests for the post mortem and the ante mortem. Why do you use different methodologies, and have you compared both to each other for pre and post?

DR. RAEBER: Well, post mortem test development started many years ago, back in 1997. For post mortem you test a piece of brain, you test on the obex. There you have the highest concentration of the analites.

So, analytical sensitivity was never really a major issue in post mortem testing, whereas it is in ante mortem testing, where we have very, very low levels of the analite.

I think that is the major -- probably the major point. The other point is that in brain you use proteinase digestion, because the proteinase is very resistant to proteinase digestion, whereas in blot it has been shown that PRP scrapie or PRPTSE and infectivity is sensitive to proteinase digestion.

That has been shown in mouse models of CJD. So, we cannot apply the same principle which we use in post mortem testing to ante mortem testing.

DR. HOGAN: Yes, sir, I had a question for you also. I don't know whether it is just the way it is printed on my sheet, but when you look at the differences that you have between your results for clinical scrapie six sheep

and then the preclinical, the cut offs look a little different.

The cut off for the clinical is about 30,000, whereas the preclinical it looks like it is closer to 39,000. I was wondering how you developed that. If it were lower, it looks almost as if it would capture one of the tonsil negative sheep.

DR. RAEBER: Well, cut off determination, I have to say this is a preliminary prototype test. Cut off determinations are made on the same plate based on the negative samples.

We do see some variation. This variation is, in our view, probably due to different locations where the sheep are coming from.

I think you probably have noticed that in our clinical panel our sheep are from Sardinia. In our preclinical it is a Dutch herd. We cannot explain what it really is right now, but there are variations which could also be due to the sampling.

What we so far haven't seen is that, when we sample human blood, I mean, there you have a consistent sampling which is not really dependent on factors.

Then you have a sheep herd which is somewhere out in the pasture and the blood has to be sampled there and then taken back to the lab. So, it is totally different

issues in blood sampling from sheep than with humans.

We believe that this shouldn't be a problem once we have determined a testing algorithm and how we really calculate the cut off.

DR. LILLAND: While you didn't go into this in great detail in your presentation, I wanted you to also comment on the utility, the therapeutic utility of some of the humanized antibodies, briefly.

DR. RAEBER: This is also of course a very interesting question. Originally, when the antibody was developed, we had thought of therapeutic applications.

Since our company is really focused on diagnostics, we haven't made any further approaches to use it in a therapeutic setting. It would be something which would be open for discussion and for licensing with properly interested partners.

DR. PRIOLA: I have a question for Dr. Soto about the time course hamster blood experiment you did where, at 40 days, you had six out of 10 positive, and then a decrease and an increase.

Were all those hamsters? That was the same experiment? Were those the same hamsters serially assayed for blood at 40 days, 60 days, or were they different hamsters? The numbers vary. You start out with 10, then you go down to five.

DR. SOTO: Those were different animals. We used one ml of blood for the test, and obviously you cannot take one ml of hamster blood and keep the animal alive.

That is one of the problems with experimental rodent samples, that you can take very small samples if you want to keep the animals alive.

DR. PRIOLA: Why did you have to take one ml of blood?

DR. SOTO: I don't know if I have to. This is the way we did it. We took one ml, took it for buffy coat. We never did a smaller amount to see what is the minimal quantity.

DR. PRIOLA: So, really, then, it is a little bit tough for me, then, to reconcile the up and down nature of your signal because you are not testing the same hamsters at the same time. It could just be an indication of variability in the assay, an indication of accuracy in the assay.

DR. SOTO: Well, this is indirect evidence that the quantity of PRPSC you expect in different incubation periods could be different, considering that we have handled the variability of the LD assay.

It could be inter individual variability. That is probably correct, but that is what we can say. With different animals you have more chances of detection during

certain parts of the incubation period than others.

I agree with you. I mean, the best way to do it is to have the same animal and detect it within the same animal at different periods during the incubation period.

I think it is probably better to do this type of assay in like sheep or other animals where you can get larger volume samples.

DR. PRIOLA: Just one more quick question. Do you attempt to standardize the different samples that gather from different animals in any way? Do you -- how do you ensure that you use the same amount of sample; just volume?

DR. SOTO: In the case of blood?

DR. PRIOLA: Yes, when you sample your one ml, how do you match up those samples, that you are looking at the same relative amount?

DR. SOTO: It is the same volume and the same protein concentration.

DR. GESCHWIND: Just for Dr. Soto -- is it okay if I ask a series of quick questions?

DR. TELLING: It helps the speaker, I am sure.

DR. GESCHWIND: The first question is, on the slide that you had that was entitled, application of PMCA to different samples, you had negative and positive columns. All of them looked positive to me. I wasn't sure what the negative and positive meant.

Another question is, have you looked at infectivity of the resultant product from the amplification?

Then lastly, this might apply to all the panelists or whomever. You seem to find that the highest level PRPTSE was in the plasma, assuming that it is TSE -- I am sorry, in the buffy coat.

Anyway, it seems to be a contradiction between what others have found, and I am wondering if people can comment about where they think the highest levels of PRPTSE are in the blood.

DR. SOTO: Let me see if I remember all these questions. I don't remember the first one.

DR. GESCHWIND: The first one was the application of PMCA different samples, and the controls looked positive.

DR. SOTO: Yes, that is the problem of speaking in 10 minutes. I couldn't say that. Minus is without amplification, plus with amplification.

The reason we did it in this way is we wanted to very much show the pattern of proteinase system banding in western blot was the same as the inoculum that you obtain after amplification.

That is important, for example, in the case of human, where you have different types of banding in western

blot, to show you that we can distinguish -- not only can we say that it is PRPSC, but also we can say what type of PRPSC is present.

We can obviously -- I can show you a sample experiment in which there was nothing in the negative because it was highly diluted. So, you don't see any signal and you only see a signal after amplification, but only to compare it I put it this way.

Since I was talking mostly about testing, I didn't put any of the slides on the infectivity. This is one of the major processes that we do.

We have now -- we published last year in Cell an article showing that we can replicate the infectivity in the hamster model.

We have done it now in many different species and strains, including mice, and even human. In the samples I showed you with the limit of detection we can detect what we think is a single molecule of PRPSC oligomer. That sample after amplification was detected and this was infectious. So, that is in press in JVC.

DR. MANUELIDIS: Just two very fast questions. One is, did you do any of the samples blind? The second question is, you showed us a slide, and I didn't catch it all -- maybe you could explain it -- you had some other species like mouse.

I was wondering whether these were as sensitive, as robust, let's say, as the hamster 263K, and maybe you could say a little bit more about blood of different species, or the 263K versus some of the other samples.

DR. SOTO: We have defined a process of going very much from the scientific concept to later concentrating more on the development.

So, focusing on the scientific aspect, we have been more -- most of the experiments I showed you were not blind.

We are now working with blind samples provided by others, but for us the most important part is first developing, being confident with the technology, and then we will do all the blind studies.

The efficiency of the test depends on how much experience we have with them or how much organization we have done.

So far I can say that the very best that we have is the hamster 263K, but we have also several other strains of hamster, and we have also amplified mouse.

We have a very, very high efficiency compared to the hamster 263K. We have been able to amplify at least six or seven different strains of mice, also CWD, showing similar levels of efficiency.

The others are a little lower, like sheep or

cattle or human, but it is only because we have really spent much less time on those.

I think it is just a matter of organization. You have to do some of the experimental conditions of strength of sonication, incubation time, et cetera, to reach the maximum efficiency.

DR. CERVENAKOVA: I do have a question, actually, and a comment. My question is, when you showed your proof of presence of PRPres in buffy coat samples, did you try to inoculate these particular samples into the animals to see what the infectivity level was, if there is a correlation between those two.

Your data actually are in some dissonance with data from multiple broader studies, and our studies in mice, when the infectivity rises during the incubation period toward clinical stage.

My second, I would answer the question that you didn't answer. It was the third question about the levels of infectivity not PRP in blood.

If you take per ml of component -- I am talking about buffy coat and plasma -- it is derived that there is approximately 10 times less of infectivity in plasma, per ml of plasma, than in buffy coat. If you take the volume of plasma, it is more infectivity in plasma than in buffy coat.

DR. SOTO: Right, yes, that is the third question. I knew I was forgetting one question. The infectivity studies from the work of Larisa and Bob Rohrer, from PRPSC, I can tell you that we have the same thing, that we have much more, at least 10 times more, infectivity per unit of volume in buffy coat than in plasma.

Larisa's question, no, we did not inject the samples. I was not expecting to get this result. I was expecting to get what you get when you look at infectivity. Now, I regret not to have taken samples and infected it into animals as well.

DR. TELLING: A general question for all the presenters. Am I right in understanding that only Chiron has so far done validation studies with variant CJD, scrapie spiked into human plasma? Is that correct?

DR. PERETZ: That is our plan. We didn't start this validation step.

DR. TELLING: So, what was the curve that you showed, then?

DR. PERETZ: This is spiking experiments.

DR. TELLING: Yes, of what and what.

DR. PERETZ: Of variant CJD into human plasma.

DR. TELLING: That was my question. Okay.

DR. PERETZ: Spiking experiments of brain homogenate from variant CJD WHO standard into plasma, human

plasma.

DR. MINOR: Actually, several of the companies have actually had the blinded panels from us, which is the spiking panel.

DR. TELLING: Okay, thanks.

DR. MINOR: While I am on my feet, might I ask a question?

DR. TELLING: Yes, please do.

DR. MINOR: Bearing in mind the small volumes that the companies are actually putting into their assays, looking at the ones which are not amplification assays, and bearing in mind how low the infectivity is believed to be, can somebody explain to me why these assays actually work?

The tiny amount of protein that they must be picking up would be really quite -- granted the ratios you expect, but would be really, really, very difficult to pick up.

So, has anybody got a satisfactory explanation for why they actually work at all? I have possible hypotheses, but I would like to hear what the companies say?

DR. TELLING: Does anybody have any comment on the mechanism here?

DR. PERETZ: We didn't test any variant or sporadic CJD authentic lot samples. So, I cannot comment,

but the others I guess can comment on that. In sheep scrapie, this is ongoing work and we didn't present it here.

DR. TURNER: I am puzzled by that, too, but I am assuming that you can't necessarily relate levels of protein directly to infectivity, because the rogue prion in plasma is likely to be bound up, as we have heard in fact, in lipids and that sort of thing.

So, we don't know what fraction of the rogue prion in plasma actually can be infective. In the virus world, in virology, it is quite well known that you need quite a large dose of virus or dose of bacteria, in fact, as well, to cause an infection.

One virus doesn't give you a disease. It is probably the same with a rogue prion. You probably need a certain load of rogue prion injected or orally digested, and the vast majority of that probably is bound up with a complex of things that stop it from being infective.

Another portion of it will be sequestered within he body and won't be able to be infected. So, it could be -- actually, we don't know how we can relate protein or picograms of protein to infectivity. You can come up with 10 hypotheses as to why those two are not directly related.

DR. TELLING: Just a final point because then we have to move on to the next item on the agenda. Dr. Soto?

DR. SOTO: I think that is a very valid question. When we started our work, it was unthinkable that we would be able to optimize the sensitivity of a test to detect as little as was thought to be present in blood based on infectivity.

So many of the testing between ours and others rely on the amplification step, but others do not. So, my prediction is that probably there is some kind of PRPSC surrogate marker that is not really necessarily associated with infectivity.

DR. TELLING: One very final quick point.

DR. TELLING: Just one comment. I think it is important to remember all these samples have lots of other stuff in them besides PRPSC.

To prove that you are detecting PRPSC or infectivity, that is why the bioassay confirmation is so important, to prove that you are detecting what you say you are detecting. That could be an easy explanation for why you get positives in samples with extraordinarily low infectivity.

DR. TELLING: Thank you. I would like to move on to the next item on the agenda, which is the open public hearing. So, Bill, who is registered, if anybody at this point, to speak?

Agenda Item: Open Public Hearing.

DR. FREAS: This is the opportunity for any member of the public to make a statement to the committee on issues pending before the committee.

I have received one request following our announcement in the Federal Register. That is from Arllene Carr-Greer, deputy director, regulatory affairs, AABB.

While you come to the podium, Dr. Telling, would you please read the public statement?

DR. TELLING: Both the Food and Drug Administration, the FDA, and the public believe in a transparent process for information gathering and decision making.

To ensure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement, to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of this meeting.

For example, the financial information may include the company's or a group's payment of your travel, lodging or other expenses in connection with your

attendance at the meeting.

Likewise, FDA encourages you, at the beginning of your statement, to advise the committee if you do not have such financial relationships.

If you choose not to address this issue of the financial relationships at the beginning of your statement, it will not preclude you from speaking. So, Dr. Cavenaugh?

Agenda Item: Arllene Carr-Greer.

MS. CARR-GREER: Good morning. My name is Arllene Carr-Greer. I am a full time employee of the American Association of Blood Banks.

This statement this morning is also on behalf of America's Blood Centers and the American Red Cross. i don't have any relationship with any of the companies.

We want to thank the FDA for the opportunity to speak at today's meeting. We are pleased that the FDA is considering ways to deal with the potential threat of transmission of TSEs by transfusion, and agree that it is important to consider ways to manage candidate blood tests for these diseases.

However, we do urge caution in the face of the many unknowns and the ethical concerns associated with the use of such tests.

We share general concerns related to possible approval of donor screening tests for vCJD. Some of these

concerns and comments are based on a presumption of the absence of a confirmatory test being available for approval at the same time as a screening test might be brought forward.

We ask FDA to carefully consider the following issues:

What criteria will FDA use to evaluate the proposed test? Of course, many of these things that I am saying have already been presented this morning as well.

Is there an adequate source of characterized samples to be used in development of these tests? How would clinical trials be conducted? Would there be requirements for follow up of presumptively positive individuals.

What is the prognostic significance of a reactive test for those donors with and without a risk factor? What about the inherent difficulties of specificity and positive predictive value in such a very low risk population.

What is the nature of information to be provided to a donor with a reactive test? We say this keeping in mind that the blood collecting facility may be the primary source of information regarding potential vCJD issues, as practitioners are not likely to be well informed about prion diseases.

Similar issues will arise when considering

product recovery or recall based on reactive test results. Would look back be required for any of these issues? If so, what is the nature of information to be provided to the recipient in those sorts of notifications.

The blood and cellular therapy community does have current experience with the use of screening assays for antibodies in the absence of a licensed confirmatory test.

We look at these issues now with HTLV-1, 2 testing, as well as core testing. There are no clear messages to be provided to the donor and currently there are no testing algorithms that can be used to assess the donor for reentry.

We would not like to see this occur yet again with vCJD donor testing, particularly given the potential severity of a reactive or a positive test result.

We encourage FDA to use tools available to them for critical path initiatives, to bring confirmatory testing methodologies forward at the same time that screening tests are being considered. We do think that is an important part of this whole process.

Then I have provided for the committee's review just a short listing of who our organizations are.

DR. TELLING: Thank you very much for that statement. I think it would be prudent at this point in

time to take a brief break. So, we will adjourn for five minutes and we will reconvene at just after five minutes past noon.

[Brief recess.]

Agenda Item: Open Committee Discussion.

DR. TELLING: So, we are going to move to open committee discussion. I would like Dr. Piccardo to come and summarize the FDA's position, the proposed requirements and the questions for the committee, please. I guess they are up here already.

DR. PICCARDO: We are running late, so I think it is time now to hear from the committee. I have just one brief comment to make, which is, we heard in the research update statements in favor and against straight correlation between PRPSC and infectivity. I think it would be useful to hear from the committee their current thinking on this issue. I will leave it there, I think, and here are the questions.

DR. TELLING: Okay, the questions for the committee are, number one, please comment on preclinical analytical studies needed to evaluate candidate donor screening tests for vCJD and other TSEs.

The second question is, please comment on clinical studies needed to evaluate candidate donor screening tests for vCJD and other TSEs.

Thirdly, please discuss the relative merits of technical options that may be feasible to confirm screening results for cVJD and other TSEs, for example, bioassays, alternative immunoassays, prion protein amplification, et cetera. So, these questions are now open to the committee for discussion.

DR. SALMAN: This is not a question, actually. It is some comments related to these questions. I think we need to be very careful not to use animal samples to represent or predict the disease in humans.

I think it is the pathogenesis and the response in animals may be different from humans, and especially if we are talking about screening or testing for blood in humans. That is one important thing I think we will need to consider.

Saying that, I also believe that we have to be realistic. So, it is possible, like due to the conditions and the lack of samples, like it is possible that maybe tests can be developed and used in animals or using animal samples, but then the test cannot be considered until it will be evaluated into human blood.

The other comments I had, it came through the various speakers this morning and before the break and after the break.

We need to differentiate between analytical

sensitivity and diagnostic sensitivity. I think there are two issues and, I believe, like some of the speakers, discuss these without doing this type of differentiation.

The other comment I want to make is, we are here to talk about a new variant CJD. That doesn't mean that we don't talk about the sporadic CJD.

However, to my knowledge, sporadic CJD has not been proved to be contagious or spread through the agent. So, if we are talking about risk, then we need to focus on the new variant CJD and not to mix it up with the sporadic CJD.

DR. TELLING: Given the rarity of samples and the ethical difficulties associated with obtaining materials from the United Kingdom, in particular, possibly other European countries, how would you imagine getting around that, what seems to be a rather substantial road block? Just some thoughts, not answers.

DR. SALMAN: I wish I had the answer, Glenn. I also feel like we know like the sporadic CJD has a different even way of responses as compared to the new variant CJD.

Yes, maybe we can use sporadic CJD samples, but again, the validation, we are talking about related to the new variant CJD. We need to recognize the limitations of these samples.

DR. TELLING: I, for one, was struck by the fact that Chiron had at least done the spiking experiments with variant CJD in plasma, which I think is, not withstanding the potential biological differences between the scrapie agent in brain and blood, I think it would be a good first step.

How does the panel feel about surrogate animal models as endogenous models for infectivity? For example, BSE infected sheep? Would anybody like to comment on the use and the scientific credibility of those approaches?

DR. MANUELIDIS: Actually, I think vCJD mouse models can be useful, or any vCJD animal in general, simply because at least you have the agent, which tends to breed true and remains the same, even though you keep switching species.

So, the variable that you are going to have is actually the species difference. So, you can take, for example, vCJD or BSE and have different animal models, using that particular strain, which has bred true from the UK studies, actually, and from our own unpublished data as well. I think that is a reasonable animal model to use.

DR. TELLING: So, you are suggesting mouse adapted vCJD?

DR. MANUELIDIS: Yes, or actually any -- one or two other species, with either BSE or vCJD. I think that

the agent, as I say, has been very stable and very constant.

The species express it differently, but basically, for that species, whether it comes from BSE or if it comes from a human vCJD case, it tends to have these same kind of pathology and signature.

DR. TELLING: I think I was struck yesterday with the problems associated with using endogenous models for fractionation studies, which may not be so relevant in this case.

DR. PRIOLA: Actually, I was just going to bring up that same point. Yesterday, endogenous models would be feasible but not particularly scientifically valid.

I think today, starting with animal models is a scientifically valid way to start, particularly since, with some of the tests we heard, species specificity may not be such a big issue.

So, the streptomycin aggregation or these other things, those aren't specific, most likely those aren't specific to any single species. Those are based on aggregated states of the protein.

I am all for animal models, and the variant CJD in mice is a good one. Dr. Minor talked to us about the panels they have available now for sheep and BSE. I think you have to go that route first before using the precious

variant CJD samples.

DR. MASTRIANNI: I would agree completely. I think the animal models are a really long stretch between what happens in animals versus what happens in humans, and even the question of sporadic CJD versus variant CJD. So, I agree completely with Laura that we, at least, the animal models that clearly approximate variant CJD the best, those should be the ones that are used prior to the final validation in humans.

I would also like to extend an idea on an experiment that Dr. Minor alluded to. The best experiment would be to infect humans with variant CJD and then serially take blood from those patients.

I would propose that that experiment has already begun, that the cases of the patients that have received blood transfusions, 25 of those cases, really should not be left to the wayside.

I think the United Kingdom really needs to step up to the plate and get those patients. Those are the most valuable patients in anything we are discussing here to really understand the nature of infectivity in the blood with respect to variant CJD.

If you can track those patients, you can identify who is at risk, who actually does develop variant CJD. That is going to be a great question to answer.

You can also do things prospectively by evaluating patients clinically. You can do neuropsychological assessment on patients serially. You can do brain imaging on patients. You can gather CSF on patients.

Those are the cases that are going to answer the question of how important is blood transmission of variant CJD. That is my statement on that.

DR. TELLING: Thank you for that very valuable statement.

DR. HOGAN: There is another experiment that is ongoing also. It is only three patients, but there are three patients who had corneal or scleral transplants in the United Kingdom from known CJD individuals.

That is the same possible source for blood. Of course, that does bring up the ethical issues, because they essentially have to volunteer to do all of that, and I don't know whether they would do that.

One of the things I wanted to ask Dr. Epstein, because he brought up the very interesting point about cadaveric blood, is there any change in the conformation or aggregation of PRP in cadaveric blood versus non-cadaveric blood? Is there anything known about that?

DR. EPSTEIN: I can't answer that question and probably there are experts around the table who could do a

better job.

The only point I would make is that we would be very concerned about validating any difference in test performance in cadaveric blood versus blood from a living subject because we know there are biochemical differences in cadaveric blood.

Beyond that, I think the point in potentially considering cadaveric blood is that it is likely to contain the agent in a symptomatic -- in a diseased individual who is symptomatic. So, it is a place to start.

You would have to look at potential differences and right now I don't know what they are, both differences in the prion potentially, but also differences just in the sample and how it would interact with the assay.

DR. HOGAN: The reason I asked that is because there are no ethical issues regarding asking for cadaveric blood after death. It is no different than asking for an autopsy. It is difficult to ask the relatives, but it is not ethically problematic, I don't think.

DR. EPSTEIN: I share that point of view, which is why I commented earlier. The patients' families have permitted autopsies, which have included obtaining many tissues from the body -- brain, spleen, lymph node.

You have to wonder why not also blood. I don't know what the answer to that question is, I have not been

privy to the debate, but it is curious.

DR. TELLING: I think Dr. Safar has a comment to make.

DR. SAFAR: We have done experiments, the time course experiments on the preservation of PRPSC in the brain.

Within the first hours, there is about 30 percent lost of PRPSC due to the lost of mostly sPRPSC in the brain that is in the room temperature.

The body with 37 degrees C, starting, will definitely decay PRPSC specifically but also sPRPSC faster. We have done the PRPSC levels in the brains of patients who died from other diseases, and there are definitely time factors in it.

Second, when we freeze dry and we freeze those samples, there is loss of up to 60 percent of PRP protein, detectable PRP protein.

So, any cadaveric source would have to be very thoroughly validated. I think that I concur with Dr. Epstein that there is other data indicating that there are many biochemical changes and invasion of microbial flora from intestine and so on and so on, which would probably make it a very complex source.

DR. TELLING: So, based on that comment and your CDI data, this may be an impossible question to answer but

have you, or maybe any of the other commentators, any idea of whether or not you are looking at s or r PRP scrapie in your analyses?

DR. SAFAR: I think that the dominating species is sPRPSC. The data I showed today with proteinase K, what I showed today was mostly PK resistant PRP, which strengthens the conclusions I made.

The ratio is about one milligram of total protein in LDL and 2.5 micrograms per ml PK, which translates to almost the same condition we observed for the brain. We wanted to know if the PK resistant PRP existed in the VLDL fraction and apparently it does.

DR. MANUELIDIS: Two comments. First, I go back to this one. In terms of -- you know, we used to do a great number of biopsies as well as autopsy material.

In terms of immune histochemistry, resistant PRP, I don't think that anybody could tell a difference between all the cases that we got. They were instantaneously frozen, that we did PRPres in the brain with PK versus autopsies that were as long as 20, 40 days later.

Similarly, the experiments that I have done with some of those frozen brains that were biopsied versus autopsied, there is no difference, at least in sporadic CJD, because that is what we look at in humans, and the amount of PRPres in the human brain in the biopsy versus

the autopsy material.

There may be a difference, but I still think that one of the great valuable resources that we have, and I would like to encourage people to do it is, patients and patients' families are very, very nice about donating blood, about getting a lot of resource material.

The problem has been the lack of autopsies, the lack of facilities at the site in the states to be able to help facilitate this.

People call me up all the time from all sorts of states, and I say, try to contact somebody in that state, or I give them something like the prion surveillance.

Obviously, if it is Connecticut, it is not a problem for me, but then it becomes a problem of who is going to pay for this, who is going to pay for the transportation.

I have gone and drawn blood myself from patients who are terminal, but by the time they get around to getting me there, it is usually impossible.

I think the patients and the patients' families really want to help and I think we should help them facilitate that, because these are valuable samples.

Then an autopsy will come through and say, brain only. I have to take out the brain because everybody is so terrified about it.

I say, why can't I get the spleen or why can't I get blood. It is simply because the families don't know that these are important samples and the autopsy people don't want to do a complete autopsy.

There is a whole series of events that play into us not getting stuff, aside from the fact that the autopsy rate is extremely low compared to when I was a medical student.

DR. MASTRIANNI: I would just add to tha, what I find is, compared to alzheimers disease patients, I get a higher frequency of cases with CJD that the families want to have an autopsy. They just want to know for sure. They want to get a definite answer.

In general they are more willing to be involved in research projects, whether they are premortem or post mortem.

Just one other comment on the blood, cadaveric blood, one of the other concerns about CJD patients is that, at the time of the terminal end point, basically, very commonly they are infected. They have sepsis.

So, bacteremia may be a considerable problem in looking at blood for scrapie, just another interfering factor.

DR. SOTO: One thing that has not been mentioned too much in this panel is that one potential source of

interesting samples are the familial cases.

We don't know too much or anything about the potential presence of blood of the material. We know that it is much less infectious. However, because of the high penetrance of the disease, the people who have the mutation could provide a very interesting collection of presymptomatic samples.

DR. TELLING: Of course, it goes without saying that these are extremely rare diseases in the familial form. I don't know if you would like to comment on that, Dr. Mastrianni.

DR. MASTRIANNI: Yes, they are rare, but as Dr. Soto said, the important thing about those patients is that you can identify them.

If you want a patient to medically identify them or, in the entire family, just have them participate in the study, you know who the carriers are and who aren't, so they are ideal patients to study in that respect.

The genetic forms of the disease typically are slower. So, you do have a longer period of time in which you could collect samples from them also.

Currently I have three families, three patients with genetic disease that I am following and I have been following them for a good five months. So, they are around and I can talk to you as far as blood samples go.

DR. GESCHWIND: Just to follow up on that, we are following about 50 genetic families throughout the United States for about the past six years.

So, I think that is an excellent suggestion by Dr. Soto. Of course, there are all the unanswered questions about when the prion is actually being expressed in these patients and the incubation period as well.

We are doing serial studies both looking at cognitive changes over time, MRI imaging, magnetic encephalography, EEG, on multiple different -- I think we have about -- almost probably a dozen different genotypes.

DR. TELLING: So, are there plans to address issues of infectivity in blood using these patients? Maybe it is not appropriate to ask it in this venue. I don't know.

DR. GESCHWIND: I will let Jiri answer that.

DR. SAFAR: We have collected a limited number of samples. As you know best, there are a lot of issues related to the molecular characteristics of the agents in those patients.

The GSS is one extreme, the is practically not detectable PK resistant PRP, 8,200 K mutations is somewhere in the midway in between.

So, we collected them because they are very important but we don't know really what will be the outcome

of the testing because we don't know exactly the molecular characteristics of the pathogenesis of PRPSC protein in plasma or in cells in general.

DR. TELLING: The optimum would obviously be familial CJD, I think.

DR. SAFAR: Right, so we have quite a few patients and hopefully we will be able to test them. Answering the question why we didn't test variant CJD, we have only one case of variant CJD collected. So, we save it for a final protocol, which we don't feel yet is actually final.

DR. TELLING: But these samples are available from Dr. Minor; right?

DR. SAFAR: No, this was directly in collaboration with Bob Fallale(?) and James Aarons(?).

DR. TELLING: So, let me just be clear what is available, then, from your resources.

DR. MINOR: We have no blood from variant CJD patients.

DR. TELLING: Spiking material.

DR. MINOR: We have variant CJD brain and we have variant CJD spleen and we have normal brain and normal spleen. Any of those are available. We also have some BSE, scrapie in sheep and some associated bloods for those, which you have to get through the VLA, who are holding

those.   Essentially those things are not in short supply and they are actually more or less freely available.

DR. TELLING: I would like to ask the panel to comment on confirmatory testing in particular with respect to bioassay, because this is a subject that has come up a couple of times this morning. Do you have any comments to make about this, Dr. Manuelidis?

DR. MANUELIDIS: To be fair, I think that they are two very different issues. One is the diagnosis using PRPres or PRP in some form that is visible as a surrogate marker of infection.

Since the abnormal form is not found, as far as we know, in any other samples besides infected samples, I think that it is a reasonable marker for infection when it is positive.

I think it is a very different thing to conclude anything about infectivity without testing that particular sample, but I don't see any reason why it can't be used as a surrogate marker without just mentioning the caveat that this is a marker of disease in these particular infections and the caveat that a negative does not mean that the person is not infected.

DR. TELLING: The question is with respect to the requirement for using a bioassay for confirmation.

DR. MANUELIDIS: I think a bioassay is very

important for the material that you are testing, the original material that you are testing, the human material that is, at the current time, not as viable in the immediate future with the current tests that we have.

So, there is a practicality. I mean, I think it is very nice theoretically that everything would be nice if it was going to take three years to test the infectivity of this in that.

I think it is scientifically very interesting and scientifically valid but not absolutely necessary for the first, let's say, test.

DR. SAFAR: I think that, with all the evidence we have the only confirmatory test we have is bioassay in the animal expressing homologous PRP to avoid species barrier effects.

We already have shown a lot of data in bioassays in such transgenic animals showing that, however high the sensitivity, it is very superior to other non-transgenic system. I will not going into the details.

I think that the point in this case is that we want to know, in the preventive aspect of the testing, how safe after such specific testing the blood actually is, and the bioassay will provide such a measure.

We will say that if the test is positive we have such and such level of infectivity. If the test is

negative, there may still be such and such level of infectivity but it is less than whatever will be the correlation.

I think that at least at the beginning with this limited number of samples it is absolutely essential and we actually are going to do this.

DR. TELLING: Let me follow that, actually, Jiri, while you are there. Since you have shown elegantly and persuasively in a number of examples the fact that the CDI is at least as sensitive, if not more sensitive, than bioassay, do you still recommend the use of, if you like, the gold standard assay for these sorts of confirmatory tests?

DR. SAFAR: Yes, I do. We are still not absolutely sure what PRPSC, what kind of PRPSC, we are detecting. Is it infectious or is it not.

So, it will provide another important evidence for future planning or thinking about safety issues and correlations with other issues. We don't know, compared to the brain -- we know practically nothing about the infectivity in the blood.

DR. TELLING: So, a high priority, then, therefore, by extension would be the development of appropriate models for detecting variant CJD by bioassay which, at the moment, well, I wouldn't say they don't

exist, but they are not optimized.

DR. SAFAR: I think that they are not optimized and the best available probably are the bovine transgenics.

DR. SOTO: I think the bioassay is very important mostly for the scientific purposes, and also to make sure for what may be happening with this positive detection.

However, I don't think it is practical, neither for the moment or for practical testing later on. You cannot really wait, once you have a positive result, two years to have a confirmatory result. I mean, you may, but it is not really practical.

DR. TELLING: We are talking about, I think, validation of the tests; right?

DR. SOTO: Right, well, also for the validation. So, you are saying that anyone who has a positive blood test today will have to wait three years to have the full test developed. This is what it takes for the in vivo assay.

Also, the point that you raise before, that many of us are now working with studies that are many times, several lots more sensitive than the bioassay.

DR. EPSTEIN: I want to clarify that it is correct that one needs research assays to validate any candidate screen.

Looking forward to the potential implementation

of a screen, we are talking about the need for confirmatory strategies.

As has been shown by a number of the presenters, almost regardless of how good the specificity is, the use of a test in a largely uninfected population will generate large numbers of false positive screens, and we will need a way to deal with them.

So, the question is, what is the strategy that should be considered here. I think we simply need to consider that question in two compartments, the role of bioassays and other potential confirmatory tests in validation of screens, and then the application of additional assays that could confirm reactive screening tests, given the fact that they might be either true positive or false positive in the use setting. So, two questions, really.

DR. TELLING: Can I ask you a follow up question? Do you feel that the PMC assay would be quantifiable to the extent that it could, at some point, replace an end point titration assay?

DR. SOTO: We are trying to get there. We are trying to be quantitative. I think it is doable. However, in order to validate the PMCA, we have to validate it against the bioassay.

It is the same thing. It will take years to have

the system valuated. That is a problem that we are facing and I don't see how we will resolve it.

Also, the fact that, if we have a positive by the CDI or by PMCA or by anything else and then it becomes negative in the bioassay, what are you going to say, this was a false positive? I wouldn't say that. I would say that the bioassay is probably less sensitive.

DR. MANUELIDIS: I just need a clarification because maybe I asked a different question than was being answered.

I thought the idea was that we were going to have standardized types of materials that were already validated by bioassay.

Those materials, then, including dilutions, or whatever, appropriate dilutions or samples, they were already validated and they were going to be used, then, to see if the tests really correctly diagnosed those things.

So, I was trying to say that the PRP that is isolated doesn't have to be assayed for infectivity. I think that is a scientific question.

I think the other was assuming that there was a bioassay of the material to begin with, that one was using as a standard.

DR TELLING: That is maybe a question for FDA and Dr. Minor. The FDA is suggesting that they are trying to

assemble materials for validation. Would that be titrated and characterized?

DR. ASHER: Yes, within public winching, we just wanted to make the point that we several times submitted proposals to assemble reference materials similar to those that WHO and NIDSC has assembled thus far, unfortunately without success.

We believe that it is unlikely and probably dangerous to attempt, without adequate support, to deal with potentially infectious reference materials and their distribution. That is the only point we wanted to make.

Absent that, I think our best chance for getting these materials would be through international collaborative efforts with organizations like the WHO or NIBSC.

We wanted you to know we are not unaware and have attempted, insofar as we can, but that is life. I think that the point should be made, though, regarding confirmatory tests.

I think it is important to realize that, in practice, the potential societal damage done by an unconfirmed repeat reactive screening test would be predictably very severe.

So, we wanted to discuss the possibilities for actually confirmatory tests, should a screening test be

validated to a level where it looks like it has something of value to offer to the blood programs which, as I think we will agree, at the moment is not the case.

I think they really have to come as a package and the ABB has pointed out that we have already seen problems with screening tests where you have got no confirmatory test available or counseling of a deferred donor.

It is bad enough for diseases that don't have the prognosis that CJD does. I think it might be intolerable -- at least from my personal point of view intolerable -- for a disease like CJD. So, it would have to be, to my mind, a package deal.

DR. TELLING: So, NIBSC first.

DR. MINOR: I think you need to decide what you mean by a validated reference material. We have brains and spleens and the like which are being calibrated and looked like by a lot of different assay methods, including titrations.

I think it is not clear to me that what you find in blood is what you find in brain and spleen. It is not clear to me that the assays that we are hearing about today are actually measuring the same thing in terms of PRPSC.

I mean, I find Claudio Soto's amplification stuff really very interesting in terms of infectivity. It makes you wonder what it is actually measuring there. Something,

but the relationship between the two.

So, I am not quite clear what a validated reference material is. I mean, the key thing for us is that everybody is given the same stuff, so that if it comes up positive in one assay, it should come up positive in another, and that is sort of as validated as it gets.

Actually, just on the funding, I would just like to add my funding winch to that as well, because we are not exactly rolling in cash from the UK government either, you would be surprised to hear. I just thought I would add that one, just to make sure that we are all on the same page here.

DR. CERVENAKOVA: I have a question and I have a comment. My comment is related to the sample discussion. We went through all this working with all different companies and trying to get to them whatever they need.

I think there is a problem even for comparison of different tests. There is no blood panel available for all these different companies to do any comparison, how it was done for the brain from WHO brains, from private CJD patients, from variant CJD cases.

I believe that this is the most important thing, to have these collections which will be available for everybody who needs to use them.

My question is, if you have 50 samples, and you

would be willing to share this material which they have with everybody else, because in my understanding it was that they get funding from NIH from that and they also get help from the CJD Foundation.

I found it very difficult to do any collection of samples because everybody needs samples. It seems like patients, if there is just one patient, to give samples for every organization interested, it is very difficult.

I would like to know if FDA is planning to organize something or who will be organizing it here, not in the United Kingdom. They need to do something there, but we need to do something here as well.

DR. PRIOLA: This is not necessarily a question. It is more of a comment, that this issue is one of false positives, and the horrible consequences that arise if you don't retest, try to confirm that false positive.

So, the question of a confirmatory bioassay, I agree with I think Dr. Soto that it is completely impractical in the screening world to do 30,000 bioassays for false positives.

That means it is incumbent upon the people making the tests to validate their tests. It is going to take years, but they have to be absolutely sure that what they say they are detecting is infectivity. Otherwise, you can't use -- you are going to have to use one of these assays to

confirm the other assay, is what it comes down to. So, you need two assays that are just beautifully controlled in terms of bioassays and sensitivity.

DR. TELLING: Both being cross referenced to the bioassay.

DR. PRIOLA: Yes.

DR. PERETZ: The reality is that we need to have an assay that discriminates between the vCJD and the normal population.

We understand that there is issue with the confirmatory assay which is going to be critical. The reality is that if we are going to start to prepare -- we are going to do some testing in rodent blood. It is going to take a year.

Then you are going to have to collect this blood from the tester because you are going to have to confirm your finding with infectivity studies.

You are going to tag that and you are going to inoculate it into another animal. What is the sensitivity of this bioassay going to be when you are going to inoculate 30 microliters of plasma into mice.

I mean, are these mice going to die in 200 days without showing any symptoms? Are we going to do it with thousands of rodents to actually cover the spectrum of the infectivity?

Then it might take five years working on a rodent model, which we are really not sure how relevant it is to the human samples.

I would like to suggest or recommend an alternative, and I think the alternative is to have some confirmatory tests that can also detect PRP scrapie.

If these assays are developed by different companies or labs and they can show there is some detection in these CJD samples and not in the normal population in controlled specificity and sensitivity, I think this will be a good indication, suggesting that there is PRP scrapie in this variant CJD plasma. I don't know if it is going to be visible.

We know that these samples, I mean, there are only the three cases of transmission from this blood. So, at this stage I don't think it is practical to go to these bioinfectivity studies. We can circumvent this need by using the appropriate detection systems for PRP scrapie.

DR. LEITMAN: I just want to point out to the committee that an alternate manufacturer's screening assay is currently used as confirmation in blood banking.

So, for the two tests for which there is not an immunoblot assay or something like that -- that is the HTLV-1, 2, and the anti-hepatitis B core -- when we get an initial repeat reactive result, we confirm that with an

alternate manufacturer's assay. Often we send it to a different laboratory.

It doesn't make a difference to the unit of blood. That is excluded. It doesn't make that much of a difference to whether the donor is excluded in the future. That depends on a repeat positive test.

It makes a huge amount of difference to the counseling of the donor. So, what you are suggesting, what we have heard suggested here, to have several highly -- terrifically validated, highly sensitive, highly specific screening assays using different techniques is a very reasonable approach to screening the blood supply. For reasons discussed here, the bioassay would not have any practicality in blood banking practice.

DR. CARR-GREER: Dr. Leitman is correct. We are also looking at issues of a high rate of false positivity, and how to get these donors back into our donor pool.

That is the constant ongoing issue with the HTLV and the core. We have many donors who we tell them something to allay their fears at the time of donor counseling. They are still not blood donors any more. So, that is the other issue we are talking about.

DR. PRIOLA: Just to go back to what Dr. leitman said and the argument from Dr. Peretz that we can just use PRPSC as a marker, I don't think you have to go back and

assay 50 microliters of plasma to detect infectivity there.

I think you just have to validate your assay against known levels of infectivity and show that, when you dilute it out by bioassay to 10^{-12}, your assay detects the same amount.

That is all you need. If you can do that and have two tests that do that, then I agree with Dr. Leitman. The issue of false positives, you have a second test to confirm it.

If it confirms it, then you have to deal with the issues of informing the positive donor. I think you have to have more than one test and both of them have to be very well validated. I will just repeat it.

DR. SOTO: I think the problem with the bioassay, in addition to the practicality, is that it is not the gold standard that it was several years ago, especially in the human case.

In the hamster 263K or mice it might still be, but in the human, the studies that we have today, bioassays are not very sensitive.

The question is, if you detect PRPSC and the bioassay comes negative, when you inject 50 microliters or whatever of blood into the hamster brain, are you going to say that this is not potentially infectious material?

I wouldn't say. You are not receiving 50

microliters of sample. You are receiving much more in a blood transfusion.

I don't see an easy answer for this, but I just want to point out that the bioassay for human is not as sensitive as we think.

DR. TELLING: So, you mentioned alternate methods that have gone beyond the sensitivity of the bioassay for human infectivity, such as? You are still optimizing these with PMCA for human infectivity now; right?

DR. SOTO: Right, and the goal is to get to what we have in hamster, which is at least several thousand several times more sensitive than the bioassay, bearing in mind that the hamster bioassay is the most sensitive one, probably, of the bioassays.

So, when we go to humans, it would be pretty easy to go beyond the bioassay sensitivity. The question is, from the ethical point of view, do you want to exclude samples that have PRPSC there detected by a couple of tests, but not necessarily infectivity? I don't know.

DR. SAFAR: I think that in the past eight or maybe nine years with BSE and BSE testing in different states and in Europe, there is a good lesson what happened if you concentrate first only on analytical sensitivity and to disregard the diagnostic sensitivity.

So, the lesson with IRMM and with the EC is that

you can apply -- you have to apply both. You cannot simply substitute with one or the other.

So, for the false positives, you need to test 10,000 samples. That is relatively easy to do by any test. if some test were approved that they have declared specificity by showing one or two positives, then the next step is to look at the analytical sensitivity and the ability to detect infectivity in the blood, because that is what we want to prevent.

Infectivity, in this case, we don't know really how is it distributed, how it would behave. So, the bioassay to characterize standards to calibrate or to measure the sensitivity, analytical sensitivity of a given assay, I think that the consensus now -- with the previous comments -- that we have to have it.

Otherwise, we will have a perfectly specific test which wouldn't detect anything, or some substitute which will be a surrogate marker which will have nothing to do with the disease, and will come out with some other diseases, so it will become a very nonspecific surrogate marker.

We have also experience with proteins in CSF as the best example. So, I think that the consensus in this case, as I feel it, is to apply both analytical tools, and I think that the gold standard is a bioassay, a bioassay

characterized standard material, and a statistical tool, testing a large number of negative samples.

DR. TELLING: I am going to approach the close here, except to ask if the committee members have any more questions or comments that they would like to raise at this time.

I think we have captured a great deal of opinion here. We are not being asked to vote up or down on this, but I hope these comments have been useful for FDA. There appears to be some controversial opinions here, but nonetheless --

DR. EPSTEIN: I just want to thank the committee members. I think the discussion is very useful to the FDA. Obviously, it is difficult to say something conclusory at this point.

I think the conversation helps us a great deal, to understand just what we are dealing with and what the path might be.

DR. TELLING: So, I would like to thank the committee and the speakers today and the members of the public and the meeting is now adjourned. Thank you.

[Whereupon, at 1:00 p.m., the meeting was adjourned.]