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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

ADVISORY COMMITTEE MEETING

Gaithersburg, Maryland

Wednesday Afternoon, June 26, 2002

COMMITTEE MEMBERS PRESENT:

DAVID C. BOLTON, Ph.D., Chair
New York State Institute for Basic Research

JOHN C. BAILAR III, M.D., Ph.D.
University of Chicago

ERMIAS D. BELAY III, M.D., Ph.D.
Centers for Disease Control and Prevention

STEPHEN J. DeARMOND, M.D., Ph.D.
University of California San Francisco

SAMUEL H. DOPPELT, M.D.
The Cambridge Hospital, Cambridge, Massachusetts

LISA A. FERGUSON, D.V.M.
United States Department of Agriculture

PIERLUIGI GAMBETTI
Case Western Reserve University

KATHARINE E. KNOWLES
Health Information Network

JEANNE V. LINDEN, M.D.
New York State Department of Health

JEFFREY J. McCULLOUGH, M.D.
University of Minnesota

STEPHEN R. PETTEWAY JR., Ph.D.
Bayer Corporation

PEDRO PICCARDO, M.D.
Indiana University

SUZETTE A. PRIOLA, Ph.D.
Rocky Mountain Laboratories

COMMITTEE MEMBERS PRESENT (CONT'D):

ELIZABETH S. WILLIAMS, D.V.M., Ph.D.
University of Wyoming

SIDNEY M. WOLFE, M.D.
Public Citizen

ALSO PRESENT:

DAVID ASHER, Ph.D.
Office of Blood Research and Review
FDA Center for Biologics Evaluation and Research

JAY S. EPSTEIN, M.D.
Office of Blood Research and Review
FDA Center for Biologics Evaluation and Research

MAHMOOD FARSHID, Ph.D.
Office of Blood Research and Review
FDA Center for Biologics Evaluation and Research

WILLIAM FREAS, Ph.D.
Committee Executive Secretary

ELLEN HECK
Eye Bank Association of America

RICHARD HURWITZ, M.D., F.A.C.S.
LifeNet

DAVID KORROCH
Lions Medical Eye Bank of Eastern Virginia

C. RANDALL MILLS, Ph.D.
Regeneration Technologies, Inc.

P.J. PARDO
Tutogen Medical, Inc

P. ROBERT RIGNEY JR., J.D.
American Association of Tissue Banks

ALSO PRESENT (CONT'D):

ROBERT ROHWER, Ph.D.
University of Maryland VA Medical Center

RICHARD RUSSO
International Osteotech, Inc.

RUTH SOLOMON, M.D.
Office of Blood Research and Review
FDA Center for Biologics Evaluation and Research

ALAN E. WILLIAMS, Ph.D.
Office of Blood Research and Review
FDA Center for Biologics Evaluation and Research

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C O N T E N T S

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

(2:05 p.m.)

DR. BOLTON: Our first presentation for the afternoon part of the session, which is "Process Validation - Industry Presentations," will be from Dr. Richard Hurwitz. He is the Interim President and CEO and Medical Director of LifeNet.

Dr. Hurwitz.

DR. HURWITZ: Good afternoon. Thank you very much for inviting me to speak. I would like to first give a little bit of an overview of the impact of tissue banking in the United States and talk a little bit about validation but first a little bit of history. LifeNet, which is located in Virginia Beach, was established as a tissue bank in 1982 to provide allograft skin for the local burn center. When it became apparent --

DR. BOLTON: Dr. Hurwitz, let me

just interrupt you for a second. I didn't get a chance to speak with you beforehand but I have asked each of the other industry representatives to please be as brief as possible. I would like to keep your presentation to 10 to 12 minutes so we'll have time for questions.

So anything that is not necessary you can bypass quickly. Thank you.

DR. HURWITZ: How do I decide? LifeNet has grown to be the largest nonprofit full-service tissue bank in the United States. It was one of the first banks to be accredited by the AATB and is ISO 9001-certified.

LifeNet's donors come from many areas of the country largely through relationships with organ procurement organizations which in many states function as the appropriate stewards for donated human organs and tissues. The ethical issues surrounding tissue donation, care of

the donor family, and profit making are separate but important ones.

In addition to musculoskeletal banking LifeNet is the second largest provider of cryo-preserved human heart valves. My comments today, however, will be related to musculoskeletal banking and nothing from the central nervous system.

To show you the overall impact of tissue banking this slide shows the increasing number of tissue donors in the United States to meet the growing demand. In 2000 almost 20,000 tissue donors were processed. LifeNet, for example, accepts donors up to the age of 70.

This slide indicates the number of grafts by type distributed by LifeNet in 2001. Allograft bone is used in the majority of spinal fusion operations. Autografting, which is the alternative to allografting, significantly increases patient morbidity and prolongs operating

times. Demineralized bone particles enhance the outcome in thousands of dental procedures yearly and the ability to use allograft tendons for sports medicine knee construction procedures also greatly facilitates the operative procedure.

The next two slides indicate the present and projected needs for allograft tissue. In 2001 more than 400,000 different procedures involving bone graft were performed and the projections are for continued increase.

For soft tissue grafts, which includes skin, fascia, tendons, and pericardium, the projections are for continued increase over the 90,000 implants or transplants that were performed in 2001. It is important to note that to preserve the functional integrity of tendons a processing methodology somewhat different from the one used for bone grafts is utilized; therefore, it is important that antimicrobial process

validation be done for each separate tissue type application.

This slide illustrates some examples of the application of tissue engineering in the design of bone grafts which better serve the clinical need such as these machined allografts for spinal fusion procedures, demineralized bone powder used in dental applications, and you have already seen pictures of a patella ligament used for sports medicine. Hundreds of individual grafts therefore can be made from a single donor. The actual and predicted clinical utilization of a human tissue is an indicator of beneficial outcome; however, outcome studies are limited and expensive to perform in a controlled fashion, especially in a rapidly changing arena of new innovation.

Historically tissue banking is an extension of organ donation and transplantation. Tissue donors are

screened, tested, and aseptically procured by LifeNet like organ donors. In fact many organ donors are also tissue donors. Properly performed, tissue transplantation should be as safe as organ transplantation. The question still needs to be asked whether the expectation is for a zero infection rate from human tissue. What about for organs or eyelet cells or other human tissue? Clearly the risk-benefit must be considered.

The approach to tissue banking changed in 1991 when LifeNet became aware that a 1985 donor had transmitted HIV to recipients of organs and tissues. The case was reported to and thoroughly investigated by the CDC and subsequently published in the New England Journal of Medicine.

Recipients of tissue which contained residual bone marrow, fresh frozen grafts, became HIV-positive while recipients of processed bone grafts did not; hence, the new era of tissue banking at LifeNet

unfolded with the development of procedures for removing bone marrow and for disinfecting bones and soft tissues. LifeNet was alarmed at the recent reporting of tissue allograft-related infections from clostridia and other pathogens by the CDC. It has been subsequently determined by Dr. Kiner that 12 out of 14 of the clostridia cases were from one bank, which was not AATB accredited.

It also has become apparent from the CDC investigation that grafts which are processed differently to preserve cell viability or graft function such as ---- condyles, tendons, and menisci have more often been implicated in patient infection.

I have summarized in this slide all of the complaints received by LifeNet concerning possible allograft-related infections or graft contamination during the past four years. Each case has been investigated and to date no serious

allograft-related infection documented. Many of these reported positive cultures were done in the operating room prior to implantation. This has been recommended against by the orthopedic societies because of frequent environmental contaminants.

This last case is significant in that tendon tissue from a donor processed by one bank known as tissue processor A in the MMWR resulted in a clostridial infection and other tendon tissue from the same donor processed by LifeNet was used in four recipients who have been followed without evidence for infection.

The HIV case notwithstanding, the risk of disease transmission from allograft tissue has always been considered to be extremely low. Many but not all banks have been inspected by the AATB and those conforming to standards have been accredited.

At last count the CDC has gathered

information on 54 cases of allograft-related infection over the past four and a half years. Twenty-five of these were from one bank. With hundreds of thousands of tissue transplants each year is there a significant public health risk or can the reported allografted infections be explained by deviation from excepted AATB standards?

There are multiple steps to assure safety. This information has been shown to you before. I would like to note that all of the data concerning screening information and the circumstances leading to death and the initiation of procurement must be evaluated by the medical director, who needs to do all of the research to draw a conclusion that any particular donor is safe.

The donor screening is performed to reduce or eliminate the donor who may be at risk for transmitting malignancy, bacterial disease, parasitic disease, viral

disease, and, of course, prion-associated disease. You have already been shown the standard screening questions that are asked which concern high-risk behavior and specifically symptoms, signs, or past history that would suggest CJD-related disease.

Aseptic procurement and processing are essential for infectious disease safety and to preserve the functional intent of the allograft tissue. Following procurement all musculoskeletal tissues are held in quarantine until qualified for release. A swab surface culture of each tissue is performed.

Tissue cultured positive for a list of pathogens is irradiated prior to processing with 15 to 25 kilorad. Tissues cultured positive for clostridia, fungi, or yeast are discarded. In fact 10 percent of tissue is discarded and never enters the clean room for processing.

Many other potential tissue donors are rejected just on the basis of screening and they are not procured. The reasons for discard include not only procurement cultures but positive serologies, medical history, autopsy findings, and hemodilution. Therefore by utilizing strict donor screening, aseptic procurement, procurement culturing, final medical director review, and appropriate pre-processing discard the risk of an infectious agent being present in tissues sent for processing is minimized and the likelihood of significant bio-burden similarly reduced.

LifeNet uses clean room technology to control contamination. Each room is tested to Class 100 at rest and environmentally monitored. Processing suites are decontaminated between procedures using commercially available reagents and all surgical instruments are sterilized following AAMI guidelines.

Based on our own HIV transmission case in other transmissions this MMWR admonition was published in 1993 which said it is prudent to process bone and bone fragments and carefully evacuate all marrow components from whole bone whenever feasible.

The Allowash process implemented in 1995 was developed to facilitate the removal of bone marrow and the introduction of antimicrobial agents into the allograft tissue without altering function. Allowash itself is a combination of biological detergents used in combination with isopropyl alcohol and hydrogen peroxide to remove bone marrow, blood elements, and lipids.

Centrifugation and ultrasonification are also employed to achieve near total cleaning of the bone. LifeNet has always employed single-donor processing and strongly objects to the

pooling of donors per AATB standards.

In addition to bacterial and viral testing a number of other parameters were validated, including measurement of detergent residuals, osteoinductivity, and inflammatory response in a mouse model, measurement of protein residuals, light in electron microscopy. Biomechanical testing included compression strength and tensile strength measurement.

Time kill studies using Allowash solutions for the six USP organisms depicted by the asterisk and others tested as well, including clostridium sordelli, which is the organism that led to the death of the tendon recipient, demonstrate greater than ten to the sixth (10^6) log kill. These studies were done in the presence of bone marrow and cortical bone to mimic the processing environment.

The time kill studies of the allowash components were also performed with

model viruses for HIV, HTLV, and Hepatitis-B and C. LifeNet is completing additional studies looking specifically at measurement of sporicidal activity. A definitive reference lab protocol for quantitating activity against anaerobic as well as aerobic spores has been difficult to find. We believe it is important to have a validation model which incorporates the cleaning process as well as the disinfection process.

We have therefore begun a validation protocol utilizing uniform size cubes of cancellous bone impregnated with bone marrow and known bacteria. Each step of the Allowash process will be examined by an independent lab to determine reagent consumption, protein removal, and bacterial log reduction.

All production equipment is qualified using standard qualification practice. LifeNet continues to evaluate

other methods which might be considered for terminal sterilization such as low temperature irradiation, plasma phase hydrogen peroxide, and decellurization methods.

Post processing cultures are performed using BacTAlert, a very sensitive, automated microbial detection system based on carbon dioxide and other metabolite measurement. A rinse of the final graft or a co-process sample inoculated into media for the BacTAlert system in a seven-day incubation protocol has been validated. If any post-processing culture is positive the entire donor is discarded except when skin organisms are detected, in which case tissue is reprocessed and retested.

AATB inspects and accredits tissue banks and has continued to work with the FDA and CDC to develop and update standards. AATB accreditation should be required with the governmental financial support to help

cover the expense of comprehensive inspection.

Single donor processing should be mandatory to prevent the possibility of cross-contamination and to prevent the need for a greatly expanded recall if required for a possible disease transmission. Dura should not be procured for transplantation as only dura and chorda (?) have been implicated in a TSE transmission. Validation standards should be uniform for all banks and not proprietary and presented in a scientific form for general agreement and they should be specific for each tissue-type process.

Additionally, with regard to TSE tissue donors are not presently screened for travel or residence in Europe or for receipt of bovine insulin. It is unclear how such screening would affect the number of tissue donors. Once would guess that in areas near military bases it would have a significant

effect.

Also recommend investigation of all potential allograft infections with central reporting and investigation to determine more thoroughly the incidence and cause of disease transmission through route cause analysis.

In conclusion human tissue transplantation is widely used and safe. Although there may be a temptation to consider a final sterilization process for human tissue this should not be a recommendation until a process which does not interfere with the biological properties of the tissue can be validated.

The doctor is saying, "No, I wouldn't call you a mad cow exactly. I would say you are a cow with issues."

Thank you.

DR. BOLTON: Thank you, Dr. Hurwitz. Questions from the committee? Dr. Wolfe.

DR. WOLFE: Again, one of our favorite topics, dura mater. The policy that you mentioned in the recommendations, do not procure or distribute dura, is this the policy of LifeNet or is this what you would recommend more generally for all the tissue banks? Is this is a policy of AATB or what? Just what is the origin of this and what are your own views about the use of cadaveric dura in this country now?

DR. HURWITZ: Well, it certainly is a LifeNet policy. I don't know if AATB has a specific stand on dura.

DR. WOLFE: When did LifeNet adopt this? Is it recent in the wake of the alternative tissues or what? When did you come out with it?

DR. HURWITZ: I don't remember. For several years.

DR. BOLTON: Any questions?

DR. DOPPELT: Rich, in terms of the Allowash could you clarify how different

tissues are processed, for example, bone versus the soft tissue, bone, tendon, bone or fascia lata versus the cancellous cubes or struts?

DR. HURWITZ: Bone tissue undergoes processing with all of the Allowash steps, which include the detergents in association with centrifugation or ultra-sonification, isopropyl alcohol, and hydrogen peroxide. Soft tissues do not tolerate the hydrogen peroxide step very well and that is not done with most soft tissues. Fascia is generally irradiated, tendons are not, and they are not treated with the peroxide.

DR. DeARMOND: Is there any residual skeletal muscle attached to any of these tissues?

DR. HURWITZ: No.

DR. DeARMOND: And as far as we know there has never been a case of CJD that could be linked to these grafts. Is that

correct or have I missed something which I do all the time?

DR. BOLTON: I just missed your questions.

DR. DeARMOND: The bone and tendon grafts, has anyone ever reported a case of CJD that could be linked to it?

DR. HURWITZ: Not that I'm aware of.

DR. BELAY: Not as far as I know.

DR. BOLTON: Thank you very much.

Our next presenter is Richard Russo. He is Executive Vice President and General Manager of International Osteotech, Inc., from Eatontown, New Jersey, which is near where I live, actually.

MR. RUSSO: We are going to keep this moving along quickly. We have already touched on several of the issues that I was going to present today. Other speakers have already touched on them so I am going to move quickly here.

The important thing I want to make with this slide is that risk reduction is a multifaceted activity and cannot rely on some processing step all by itself. So in order to have an effective risk program not only do you screen donors. You have to have adequate donor deferral and tissue discard policies, single donor processing most likely, adequate cleaning and disinfection be batches, and we will come back to that later on, and certain processing standards to actually either remove or inactivate agents of infection.

Even though we have three different types of pathogens that can cause disease transmission in tissue we are going to focus today in my talk on viruses and standard non-demineralized grafts and viruses of concern are these.

Before we go right into inactivation and removal it's important to remember why a surgeon is doing a bone graft

and why we are providing that surgeon with allograft tissue. The primary purpose is to support bone formation across a defect. Not all processing has the same effect on bone graft performance. Essentially the bone graft can overwhelm the patient's ability to form bone at times and we have to make sure that we tip the balance in the favor of bone formation when we are processing.

Failure of a bone graft typically leads to revision surgery. So a failure of a bone graft is not without risk to the patient. It has direct morbidity and risk of disease transmission in the second procedure. So we have to be careful when we are thinking about processing for safety if that processing for safety is done in such a way it also limits the capacity of the graft to support bone formation.

Here are some of the types of treatments that can be used in bone processing or tissue processing which will

have at least an effect on bone formation. So, as you can see, a large number of things can impact on an allograft bone graft negatively. This is just an example of the way that you might process a certain type of tissue, demineralized tissue, with three different treatment programs or processing programs. This information was presented at the North America Spine Society and was conducted by another firm and they looked at the three different types of demineralized grafts from the perspective of osteoinduction and they found dramatic differences in the graft performance in the validated animal model. So I'm making a point that processing matters.

Well, when you start looking at risk reduction you need to start thinking about the tissues that you are going to process. We are speaking here specifically at Osteotech about musculoskeletal tissues, both soft tissues and hard tissues and with

hard tissues we are looking at cancellous and cortical tissues. Today's presentation is focusing on the hard tissue.

Now, we need to start thinking about the tissue that we are actually processing. So we can realize that bone tissue has two general phases, organic and inorganic. The inorganic phase is the largest phase of the bone. It's approximately 70 percent by mass so the bone is 70 percent mineral by mass. That means 30 percent is organic. So we have to begin to think about what diseases will be in the organic side. Since we are thinking today about blood-borne viruses we are going to especially focus on the blood supply.

Well, this is a nice picture of a bone. I know everyone is very familiar with it, but you need to put your hat on and say well, if you are processing bone what are you facing? You are facing a tissue which has both a long diatheses here with a large

intramedullary canal as well as cancellous bone up the metaphyseal area here.

And bone is heavily vascularized even though there is a large mineral component. Approximately 95 percent of the blood supply in bone is going to be in the intramedullary canal and about another five percent is going to be in the haversian and Volksmann canal systems. This is a picture of cortical bone and you can see even though it's very dense there's a lot of vascularity to this tissue so virus can in be a lot of different places in bone tissue.

Trabecular bone and cancellous bone, on the other hand, has a very different structure and is very open and this can be viewed as an extension of the intramedullary canal and that is how I categorized it before.

So now we know where the blood is and blood is being the primary vector here for these viruses of concern. We need to

start thinking about the maximum viral burden in bone before we start thinking about log reductions.

If we don't know how much is there, as was asked before about TSE, it means nothing to say I've reduced so many logs because that begins to become misleading and you think you are doing something good when maybe it is inadequate. So there are no published reports that quantitate viral burden in bone but you can extrapolate from blood because that is the vector.

We know that bone is divided into discrete compartments and we remind you again we are talking about primarily the medullary canal, the Volksmann, and haversian canal. The osteo-sites themselves are not directly connected to the blood system so that's not where we are going to find a lot of virus. But we are looking primarily at the Volksmann and haversians

system and the medullary canal.

We had to decide what was a dose of bone. What would a patient be exposed to? Looking at the records, we decided to be conservative. The virologists were telling us this constantly so we decided that 90 cc of nondemineralized tissue was probably a good dose to work with. It is probably more than most patients would see.

Occasionally a patient will have a whole bone replaced and then we are talking about hundreds of cc but for the most part the vast majority of patients are going to have less than 90 cc so we used 90 cc as a conservative marker.

We calculated the blood volumes by, to be honest with you, hiring a consultant who is a specialist in bone architecture, Dr. David Burdine at Indiana University Medical School, who calculated the blood volumes in the various compartments.

Then we were able to go with the virologist to calculate the theoretical maximum viral burdens so now we have arrived at what we have to remove.

So how do you go about this then? We'll show you some numbers in a minute. As was suggested by the FDA speaker right before lunch, we followed pretty much his protocol. We built a model facility in the lab which was qualified for this. You cannot introduce it into your factory, obviously.

We had a relevant panel of five viruses. We did not use the woodchuck virus. We used the duck hepatitis and bovine diarrhea viruses to get at the hepatitis issues along with HIV, CNV, and polio, which we used as an exceptionally resistant and stable virus. So we had a complete panel with the biophysical characteristics of the RNA and DNA.

As was suggested, we demonstrated

quantitative recovery so we could know how much we could claim. That was very important. You may not be able to recover any more bone but most likely you put more virus into the bone tissue than you could recover. You are limited to the amount that you could recover.

We go through a process step. We quantitate the viral log reduction and we calculate the difference between the maximum viral burden and the log reduction. We don't give any value to a step that reduces less than one log of virus and the viral clearance in total has to be at least in our opinion three logs greater than the maximal viral burden to provide a significant or acceptable level of safety.

We had first started working on demineralized bone. Demineralized bone is a type of bone which goes through a primary process first and then after it's finished, like most of the normal pieces of bone that

Dr. Hurwitz showed you, most of them, the larger mineralized pieces, it has been ground up, thoroughly demineralized, and in our case there's less than .5 percent residual calcium and the particle sizes are about 100 to 500 microns in size. So there has been a lot of processing. There is virtually no blood, cellular elements, or lipids left.

So the maximum viral burden we calculated for these three viruses, and these were the only ones we could get at that time information on from the blood values, was almost low three logs, 3.18 logs.

Then we did it for mineralized tissue and we looked at both log values for active and inactive marrow. Younger patients will have active marrow. They will have more virus in that marrow or at least can have more virus in that marrow so we went with the higher, more challenging

numbers. So we decided that this was going to be the basis in nondemineralized bone for the maximum viral load or theoretical maximum viral burden.

When we evaluated several steps in the demineralization process to take a look at what we could do there with that type of tissue we were able to achieve these log values for a demineralization step and, as was suggested before, we actually had two demineralization steps in this process but because they operate by the same mechanism you can only claim one. We then looked at ethanol, which also has two steps in there, but we only claimed one. These marks here indicate that no virus could be recovered after the treatment, but this was the maximum amount that we had demonstrated recovery of so we are limited to that number.

Interestingly enough, we found out that lyophilization had virtually no effect

on HIV or CMV and it was not done for the duck hepatitis virus, so that actually was a very minimalistic step. But the total log reduction values are at this line here. We take the maximum theoretical viral burdens and we subtract those and we come up to a safety assurance level or SAL. So these were the log reductions and this is the viral burden.

For mineralized tissue that had not gone through the demineralization step but was actually finished before that we used the surfactant and alcohol and removal as our three major steps and we were able to achieve these inactivation steps with alcohol. That was with surfactant. So total inactivation values were down here. Removal was also done and we then were able to get, and that is stated over here, got total clearance, clearance being the aggregate of removal and inactivation.

We compared to the theoretical

maximum viral burden, which we see here and we used a higher value, obviously, because we were being conservative. You see the safety assurance level.

So the take-away here is that current validation guidance documents and virology perspectives can be successfully adapted to the processing of bone tissue but not all tissue. I would not know how to do this for heart valves. I would not know how to do this for other forms of tissue. This does not apply to ligaments and tendons. That allograft performance can be maintained while producing a significant SAL using conservatively calculated theoretical maximal viral burdens. These are some of the guidance documents that we worked with. These are the old standbys. They were not designed with bone in mind but they can be adapted and here are some additionales.

I want to talk to you a little bit about TSE and then conclude. What we did

was look at prion inactivation by our existent or current cleaning procedures in an engineering study. I myself was not part of this study team, so I'm giving you the summary results of this.

They did spike samples using the hamster scrapie homogenate. They evaluated by the prion western blot assay and they looked at the solid surface cleaning procedures we used for tables and saws and things like that but mostly just on tables.

We used two different agents as disinfectants. One is Sporklens, which is a commercially available product that has peracetic acid, hydrogen peroxide-acetic acid, and has a pH down in the 1.5 to 2.0 at the level of use, and Expor, which contains chlorine dioxide and has a pH down at about 2.7. They did three runs, one with Sporklens, one with Expor, and were able to show significant value log reductions but the question is what's the burden. We don't

know. We don't know how safe this makes the surface after it has been cleaned yet. We need to come up with a maximum TSE or prion burden.

But we can adapt this validation process to this task and that's the important thing. Now, I also want to take a look at one other thing and that has to do with post-cleaning residuals of tissue on hard surfaces.

We were able to develop some methods for evaluating down to the limit of detectability of how much lipid, bone, and blood would be left on a table, a bandsaw surface, or a MedClean after it had just been physically cleaned. What we got were levels of lipids down here. What we're talking about is micrograms per square feet. As you can see, you can do a very effective job on blood, eventually. We had to develop some new assays to get down to that level. There weren't any commercially available.

But the important point here I want to make is that cleaning and the standard GNPs are just as important as the use of any more advanced or high-tech cleaning procedure or processing agent. So with that I think I've kept my promise. I think we are 12 minutes and we are done.

DR. BOLTON: Thank you. Questions or comments from the committee?

DR. DeARMOND: What was the cleaning? How did you clean again?

MR. RUSSO: Well, what we did is we have a written SOP that has been validated before for disinfection and inactivation of viruses. We took that same SOP which I can share with you at some point in time. It was really a simple SOP that is not so different than what should be done most places.

We used the Expor and the Sporklens and we were able to get the tissue burden, if I could call it that, the

residual tissue down to those levels. We were able to achieve those log reduction levels in the prions.

DR. GAMBETTI: You mentioned here that you Western blot. I guess it was used also to see how really the process of cleaning, how successful, so could you expand on that, on how you used the Western blot to prove that the tissue had been cleaned of prion?

MR. RUSSO: Well, these two sets of data were generated independently. So we had done a series of cleaning validations prior to that using different assay methods to detect the present of residual lipid calcium phosphate for bone and hemoglobin for blood.

Then separately or recently we did this engineering study on the log reduction. So we didn't use the western blot assay for the detection of the -- well, we may have in one of those. I'd have to get back to you

on that to make sure that I don't misspeak myself to let you know which actual test was used to determine the residual organic material. I will do that, okay?

DR. BELAY: I was just curious about how you actually spiked the different viruses into the bone. Were they injected in a vessel? And what kind of detection methods did you use for viral load?

MR. RUSSO: Well, I believe that actually two different methods were used. In the demineralized tissue, of course, we are dealing with something that's approximately one to 500 microns in size and that is going through these solutions. So essentially what is happening is that the tissue is spiked but then put into the solution and the whole bone, the non-demineralized bone, they create a cavity and then actually put in a stopper.

I might have to go back and review that protocol because it has been a while,

but it is actually a physical mechanism in which after a lot of validation to figure out how we could demonstrate recovery we actually drilled a hole and inserted a little piece of material containing the virus and then closed it back up.

So it's not the same thing as natively infected tissue which is going to be infected throughout the bone marrow cavity and throughout the haversian system but was the best that could be approximated using a three-dimensional object.

DR. BELAY: Did you use cell cultures to detect the viral load? What was the detection method?

MR. RUSSO: Pardon me? Jim, can you help? There were cell cultures that were used but I don't know precisely which. The experiments were done by Quality Biotech, which is now a division of ViroMed.

OsteoTech has an R&D Department but we're focused on bone formation and we

have to go outside for issues on safety and things like that.

DR. BOLTON: Other questions?

DR. PETTEWAY: You showed the potential to remove viruses using the system with several viruses so the potential may exist that you could remove prions. Have you thought about how you would do those experiments or if they are worth doing?

MR. RUSSO: No, to be quite honest with you. One of the things we did was come here today to learn about the state of the art so we could figure out if such a thing was possible. If you look at the data that I presented you'll notice that in one of the studies for the mineralized or the non-demineralized tissue we did not use HIV as the marker. We used murine leukemia virus.

That was because the laboratory became a little bit upset about us spiking bone and then using high pressure to force it through the channels of the bone in

producing a liquid spray in their laboratory. They felt that spraying HIV wasn't the best thing. At the moment, given what we don't know about TSE, we are little concerned about how to safely conduct such studies.

DR. BOLTON: A question from the audience?

DR. FARSHID: Farshid, FDA. I just would like to say that the estimate of viral clearance should always consider the worst-case scenario. The worst-case scenario would be the highest estimate of viral load in that given donor. That is how we look at it.

I think looking at the tissue separately and trying to determine the viral burden in the tissue will introduce a variable which is very difficult to control. Therefore in order to have a high degree of assurance that the system works you need to consider the highest level of the virus and

that probably for most of the virus will be the viral load in ---- period.

MR. RUSSO: We can talk about this later but we can possibly pick up the highest viral load that would exist outside of bone at the highest inspection period and use that. Then we can possibly compare the two and that would be an interesting thing to do. That is an interesting suggestion.

DR. BOLTON: I think we will move on to our next presentation. Our next presentation is by Dr. Randall Mills. He is Vice President of Operations for Regeneration Technologies and he also will be talking about process validation.

Dr. Mills.

DR. MILLS: So we don't cover too much of what has already been covered I'll try to skip as much of this that the other speakers have already touched on so we can go on to the next slide.

As the person responsible for

producing tissue at our facility, and we are an extremely large producer of human allograft, processing over 5,000 donors a year and about 200,000 allografts, I spend a lot of time worrying about the types of issues that are associated with allograft safety and making sure our recipients receive the safest allografts possible.

To that end we developed a process to actually sterilize tissue. There's a number of reasons why we did this but I want to point out there are really three typical reasons why tissue that would be used in allograft transplantation would not be sterile.

The first and probably the most significant out of these three is that these tissues are recovered cadaverically. So after a person dies there is an opportunity for bacteria that normally reside in the gut to cross over the gut lumen into the blood stream and contaminate the tissue. In

actuality this is happens quite frequently even though we recover tissues with inside the FDA, State of New York, and AATB standards it is not uncommon. As a matter of fact it is actually more common to see contaminants on tissue depending on the extent you culture them.

Now, some important issues about these contaminants where contamination occurs in this way is that these contaminants are typically pathogens and very often may be spore formers and you have heard about the clostridial transmissions that have occurred recently that the CDC is very interested in, too. Most likely occurred via clostridial spores.

The next type of contamination that occurs is during tissues recovery. Now, two points about this type of contamination. It's typically a very low bioburden and the second thing is it's typically a non-pathogenic bioburden, either

coagulate ---- staph propionic bacterium or the bacillus species.

Then the last type of contamination that could occur would be due to a screening failure. This is primarily for viruses and without a doubt out of these HCV represents the greatest risk to tissue banking right now, at least as best as we can estimate this. This is based primarily on the very high level of sero-prevalence we see for HCV among tissue donors being somewhere north of 1 percent confirmed RIBA positive HCV patient.

So with this in mind we developed a tissue sterilization process that would allow us to transition from the aseptically processed model to what we call the BioCleanse model. As occurs in the aseptic processing model, we use donor screening first. We follow that than by a sterilization process. We just thought that sterilization, although lofty, should be

accomplished. We are then able to conduct a sterilization review and then lastly we have a culturing scheme.

For conventional tissue, and this has been talked about, this is our viral screening panel. Before we process any tissue at RTI we do a significant amount of up-front screening to make sure that the tissue is safe and this is our viral testing panel.

For conventional agents on the back end we also do USP sterility culturing, 14-day destructive culture, aerobic and anaerobic, two-temperature, two-media culture. We think one used in conjunction with appropriate bacteria stasis and fungus stasis testing represents the most sensitive method for determining contamination. We also monitor the environment. We evaluate the strength of our tissues, residual moisture, and again, process run records.

With regards to TSEs we have

talked about this for some time with regard to donor screening. We do all of the typical TSE exclusionary criteria. We have also instituted the European donor exclusion criteria voluntarily. I can tell you as a point of interest we have not seeing this affect donation dramatically at all. We are quite comfortable with the decision.

We also do tissue exclusion. Now, we don't process dura mater but we also don't process vertebral bodies and obviously we don't process corneas. This is all because of the potential risk of contamination of cerebral-spinal fluid or brain tissue.

We have line clearance and tissue segregation and we have also, and what I'll talk the majority of the rest of this presentation about, processing measures aimed at removing different types of disease reservoirs.

So this is the process that we

developed to do this, BioCleanse. It's a low-temperature chemical sterilization process that is fully automated configured similarly to an autoclave. We load the tissue into this device, we seal it up, and it uses very rapid fluctuations between pressure and vacuum to fully penetrate and fuse the tissue with different cleaning solutions.

Also, during this process we use multiple fluid exchanges and that leads to massive serial dilution. So we end up with a very large reduction of organic material left in the tissue and then lastly we follow that up with rinse cycles to remove all the different chemicals that we use to sterilize the tissue.

A note about the process, it does not use any excessive heat, does not use any irradiation, and does not use ethylene oxide. This is an example. This is actually the BioCleanse system at our

facility in Alachua, Florida. What you are looking at, that's actually a two-story configuration that sits inside an 85,000 square foot manufacturing facility. It's, again, configured like a pass-through autoclave so what you are looking at is tissue would come in one side, be sealed into the chamber, be exposed to the process, and then be removed from the other side so we have unidirectional flow of tissue throughout this process. It's a very complex process, obviously, by this three-dimensional ---- it is also a very expensive process.

This is just an example of some of the support equipment that is required to run this process. Again, this is that second floor. This is where the different chemicals and solutions are prepared and delivered to the sterilization chambers below.

This is what a sterilization

chamber looks like. There are four in each bank. We have two banks of four. Each chamber is a totally a separate and independent unit in and unto itself.

This is an example of a technician loading tissue into the process. After he is done putting the tissue into the process he seals the chamber up and enters the donor ID. And then one of the nice things about this process is that it is completely automated. One of the reasons we were able to validate this process so successfully is because it is an automated process that is not technician-dependent. So the process is run and actually controlled and monitored from a remote location so we can avoid bringing contamination into our tissue-processing facility.

When we did the validation for this, unfortunately at the time we were doing the validation for this process, there was no road map so we really had to cast a

very wide net. So we thought long and hard about the types of parameters that we thought were necessary for tissue sterilization process.

The first we think is essential is to have complete matrix penetration and then followed by removal of potential disease reservoirs, particularly blood and bone marrow and lipids. Dr. Rohwer spoke earlier about it and he hit it dead on. You cannot sterilize what you cannot touch. So unless you get complete penetration and removal of these elements it is very, very difficult, if not impossible, to actually sterilize tissue.

Obviously we wanted to have a process that could eliminate bacterial and fungal contamination, including spores. That became important here recently with the recent reports of infection and a death. Enveloped and non-enveloped viruses have a process that we can remove the germicides

that we use and then lastly have tissue that is functional.

Now, as we were doing all of these we thought there were a couple of overriding principles that we had to keep in mind. One is we had to do all of these under worst case conditions depending on the type of study we are doing that factually defines worst-case conditions. We also wanted a validation that would account for both process variability as well as tissue variability so for all of these studies we had to look and consider whether it is a younger donor, a middle-aged donor, or older aged donor, male and female, and other types of tissue variabilities, the different types of tissues that we see.

We wanted the process to be, obviously, very repeatable and whenever possible we conducted all of our validation studies in full-scale production equipment using the technicians that actually run it

as opposed to scaled-down laboratory studies which for some agents obviously were important.

Now, what we ended up with at the end of this was a massive validation. We have over 10,000 pages of validation data on this BioCleanse process and I can tell you after having gone through a lengthy review of our validation studies with the FDA although it was a painful experience, and I'm not going to tell you it wasn't, it was a very constructive experience at the end. We think we came out on the other side of that process much better because of it.

I would touch on a couple of key studies we think are important. Obviously, with over 10,000 pages now I can't go into all of it but a couple of key studies that we think were pretty significant. The first is a tissue penetration study. In this study we added a tracing dye to our cleaning solutions. We actually complexed in this

study Fitzi (?) to a 65 kilo ---- protein. We loaded tissues into the chamber and we ran the cycle for only five minutes. Now, a typical full run of BioCleanse is eight hours long, so this is five minutes out of eight hours.

We removed the tissues and examined them histologically. As was alluded to in the previous presentation, this is what the haversian system or a vascular system of cortical bone looks like. So we evaluated tissue histologically and looked to see where dye had penetrated and in fact at a five-minute time point we were able to completely penetrate the deepest aspects of not just cancellous but cortical bone and in fact tendon sample size 59 times in a row.

In this study we were looking at the same thing but using endogenous substances as the surrogate marker. We evaluated histologically tissue for the

presence of blood and marrow elements. Now, obviously on the left is a proximal femur that has been sectioned open and inside is what you would typically find, blood and bone marrow. On the right is a BioCleansed femur that was processed whole and intact. There were no cuts, holes, or other manipulation done to the tissue before it was processed and it was sectioned afterwards.

Macroscopically it's very clear to see that the process does a very good job of removing blood and bone marrow from the medullary canal and from the cancellous bone. When we looked histologically we saw the same thing was also happening. Obviously on the left-hand side we can see haversian systems with blood elements. On the right side same haversian systems have been completely evacuated of blood and bone marrow.

From this study we actually have

started doing, and I have some preliminary results but not in slide form, a prion removal study using this concept. What we did was we spiked scrapies into the marrow cavities of these long bones, created a scaled-down model for this, obviously, and ran it through the process and then did a mass balance to see where the prion had gone off and obviously at the end completely homogenized the tissue and evaluated that by western blot.

Now, I will tell you with all the caveats this was a pilot study. This was to determine feasibility of a larger scale study but out of that we actually got very positive log reduction. It seems that log reduction of at least three logs is going to be very possible with this system.

This study actually combined two what we think are very important factors. One is being able to penetrate the tissue but the second is being able to penetrate

the tissue and actually penetrate it sufficient enough concentration and quality of germicide where you can achieve sterility inside the cortical bone.

So what we did in this model was we drilled small holes inside the densest part of cortical bone, again being part of this worst-case model. We took the densest, thickest part of cortical bone, drilled small holes, and we took Bacillus Stearothermophilus, which for this process is the most resistant biological indicator, and we had trapped it inside the bone.

We sealed it up with a self-tapping titanium screw and we ran this at one-quarter of the total processing time, about two hours of contact time in the process. At the end of that we removed the biological indicator. We cultured the biological indicator as well as the construct, using a test that would detect the bacilli stearothermophilus if it was

there.

We ran positive, negative, and recovery controls and actually had to spend a significant amount of time validating this model. The results at the end of that were 26 out of 26 times we ran this process we were able to sterilize without failure the biological indicator seated within the densest part of this cortical bone.

Now, that was a very good construct model that we prepared because it was using a very resistant organism in the most difficult part of tissue to reach. In this model we went with a little bit more of a relevant testing. We actually went out and recovered donors with premortem septicemia. They had multiple bacterial pathogens both gram-negative and gram-positive, aerobic and anaerobic organisms.

We took the bioburdens of those greater than 1900 CFU on Donor One per gram and greater than 1400 CFU per gram on Donor

Two. We processed these tissues within BioCleanse. Then we took the tissue out and we destructively cultured the tissue again in the two media, two temperature, culturing scheme recommended by FDA. Obviously we had all the necessary bacteria stasis and fungal stasis testing. What we ended up with was all of the tissues for this model ended up sterilized and completely free of contamination.

This is a broad range of all the different viruses and bacteria. Obviously here we were able to completely cure enveloped and non-enveloped viruses, RNA, DNA, small, large, resistant, and easy to kill. The different types of vegetative bacteria and fungi we established this process can kill are the typical types of things we either see contaminating the tissue at recovery or associated with orthopedic infection.

Then lastly we validated the

system against spores, both clostridium spores and bacillus stearothermophilus spores. None of this clearance took more than one quarter of the process time. So again we have a great amount of overkill and a tremendous amount of redundancy built into the process.

Really quick to go through strength testing obviously for our constructs this was alluded to earlier. If you sterilize the process but you damage the tissue during the process you really haven't done anything beneficial to the patient. So we needed to make sure that the tissue was functional so we tested it in a number of different applications.

This is axial compression. This is very good for spinal applications which the majority of our tissue is used in. Compared BioCleansed tissue to untreated tissue, untreated was not processed in any way, BioCleansed again for worst case, to

make this as difficult as possible, we actually ran it through the process four times so we would exaggerate the conditions the tissue was exposed to and noticed no difference.

This is by diametral compression. It is the most sensitive method for testing. Again, no difference.

Shear testing, this is good for torque. A lot of our tissues are actually machined in this shape that gets screwed into the patient. No difference again in shear testing.

Three-point bend testing for the most part is actually for bone. It is really a meaningless test but it is the one that is most often referenced in the literature so we did that and there was no difference there.

This is actual product testing. This is probably the most relevant thing we did because this not only picks up

differences for the process but also looks and sees if there's any downstream effect of either freeze-drying or freeze-drying followed by reconstitution and again no difference.

From the biocompatibility standpoint we needed to make sure that this tissue was functional when it was implanted. The first thing we did with regards to biocompatibility was we validated the process to completely remove all of the different germicides that we use.

So our starting point for these tests was essentially sterile, clean bone. We ran it through this biocompatibility problem and did not have any reactions going through it.

So looking back on what we were able to do, we were able to completely sterilize the tissue because we were able to completely penetrate the tissue and remove all of the blood and bone marrow. Our

process is not just bactericidal and spungicidal but it's also sporacidal.

We can kill enveloped and nonenveloped viruses. The tissue is functional and biocompatible but, most importantly, because this is a completely automated process it is a validated process. It is one that is not dependent on technician interaction for its success but in fact is reproducible.

So just to conclude there is a residual risk of disease transmission when only screening and testing are used but clearly the things that pose the greatest risks are HCV and bacteria. We believe that both tissue exclusion, not processing things like dura mater, vertebral bodies, and removal of diseased reservoirs reduce the risk of both conventional and emerging pathogens and our initial prion data seems to support that.

This process has been validated to

sterilize tissues without altering its biocompatibility or its biomechanical integrity. The process is completely automated which allows us to validate it. We have also implemented this process in anticipation of FDA's GTP requirements that are coming out which we think are going to be exceptionally important for the industry.

Then lastly we have had a tremendous amount of clinical success with this. We have had over 200,000 of these grafts implanted, we have had good acceptance by the surgical community, and we have not had a single infection since we have used this process.

So I'll take any questions. Thank you.

DR. BOLTON: Thank you.

Questions? Yes.

DR. LINDEN: If you are concerned about HCV why are you doing that only for HIV and not HCV?

DR. MILLS: That's a really good question. The answer, and we're working hard, actually, to do NAT for HCV. The problem is our samples are collected cadaverically. The problem we have had is sample stability because the blood sample gets drawn from a cadaver. If any of your are familiar with what that looks like it very frequently can be associated with a tremendous amount of hemolysis. That can be as late as 24 hours post-death.

Then the sample would need to get spun down and sent to our testing facility. Now, we could test it at our testing facility almost immediately but a lot of these tissues are being transported across the country so realistically there is at least a 48-hour period before we could actually get the samples up on test.

And what we have seen so far is we just have some sample stability issues. Now, we are trying to work on ways of

stabilizing the HCV nucleic acids but we are not there yet.

DR. BOLTON: Other questions?

DR. DOPPELT: You said a couple of times that in the BioCleanse process you are using chemical solutions and germicides. What chemicals and solutions are you using?

DR. MILLS: We use three fundamental types of solutions. We use alcohols, peroxides, and detergents. Now, how we mix that is a proprietary cocktail but we use those three in different forms.

DR. DOPPELT: Is that in any way different from what other people are using?

DR. MILLS: The cocktails are, absolutely.

DR. DOPPELT: But, I mean, they are all using detergents and --

DR. MILLS: I can tell you the order is important. Obviously the most fundamental thing that's important is you have to completely penetrate the tissue.

Dr. Rohwer said if you can't penetrate it, you can't kill it. So there's a big difference between taking a piece of tissue and soaking it in isopropyl alcohol versus taking a tissue and completely perfusing it with isopropyl alcohol.

DR. BOLTON: Ermias?

DR. BELAY: You would say you had already implemented the European deferral policy in your company?

DR. MILLS: Correct.

DR. BELAY: I was wondering if you could tell us a little bit more about the kind of policy that you implemented and your experience because there is a lot of data on the impact of that policy in cells and tissues and your experience potentially would be helpful for us.

DR. MILLS: The European exclusion, we have had that in place, I believe, for over six months now. I think we are obviously changing to the new

guidelines now, but I think the one we had was European travel over the last six months. We have had no impact, almost no impact. We have excluded some donors but there have been very, very few.

A point that should be considered is that we don't necessarily draw from donor pools that are around military bases and that is something worth considering. Just because we haven't had an impact doesn't mean the industry as a whole wouldn't have an impact.

DR. DOPPELT: I may have misunderstood. You said something about a log reduction of three for something. That was scrapie?

DR. MILLS: Yes.

DR. DOPPELT: What is your log reduction for the other viruses like HIV and HCV?

DR. MILLS: Well, starting out, the spores are greater than six log kill of

the most resistant spores at one-quarter cycle. Everything else we did we did with less than one-quarter cycle.

So, for example, with HIV we actually only tested two compounds against HIV. Both of them had greater than four log. We ended up with I think 8.8 log reduction for HIV just on two chemicals. All three compounds would have obviously inactivated the HIV.

In all cases all viruses were brought to non-detectable limits so it was really just a matter of what we could spike into the tissue, not on the tissue but actually into the tissue and then what we could meaningfully recover. For HCV it was something like 13 logs. They get absurd they get so high but the viruses actually are pretty easy.

DR. BOLTON: Maybe I misunderstood. Do you have a scaled-down version of your BioCleanse unit in your

system?

DR. MILLS: Yes.

DR. BOLTON: And that's what you conducted the prion studies in?

DR. MILLS: Yes. We actually have a couple of scaled-down versions depending on the types of studies we are performing.

DR. BOLTON: Other questions? Let me ask you this. Your full-scale version is for processing larger amounts of tissue in a maybe more efficient means. Is a scaled-down version something that could be used on a smaller scale by other tissue manufacturers?

DR. MILLS: The reason we went to the large, it's one of those machines, that enormous complex, actually processes a little chamber one at a time. It's one donor's worth of tissue through that at a time. So actually it's almost staggering to say that mammoth machine we put up there is pretty small scale already.

When we scaled them down to the laboratory standpoint they do get to the point where the reaction chamber could fit on a table and you would have a series of other chemical banks around you and the like.

The problem runs into this. If what you are trying to do is anticipate GTPs then you need equipment that's validatable. You need software that's validatable. There are just, like, a number of considerations like no threaded pipe, sanitary valves. The whole thing has to be sterilizable itself.

So you run into facility costs that I can tell you ran us about $25 million. It would be difficult to make, like, an autoclave version of this that you could just sell as a unit. It would be very difficult.

DR. BOLTON: Thank you. We will now move on and back to Bob Rohwer, who's going to tell us about the experience with

TSE agent clearance studies in experimental models.

Bob, I'm going to ask you if it is possible to go less than 30 minutes? Thank you.

DR. ROHWER: Over the last ten years we have done quite a large number of clearance studies on a commercial basis and some of them we did out of our own interest as well. That's what informs the presentation I am going to give you now.

These are some of the things we have looked at over the years. We have done a lot of these studies using blood and blood products both from bovine and human origin. A number of studies were done using the cone fractionation, but we have also looked at the Kissler-Nietschman fractionation System.

We have done quite a bit of work with various manufacturing methods for bovine collagen and then we have just recently have completed on bovine gelatin.

You will hear tomorrow from Whizzer Gregori about a series of experiments that were done characterizing the Asahi Planov filters with these agents.

So the types of manufacturing steps that we have looked at are given here, depth filtration, membrane filtrations, phase separations of various sorts, extractions, precipitations, column chromatography, thermal inactivations, irradiation, chemical inactivations, and others. What I'm going to focus on is rather than describe individual experiments which would go on and on and on I'm just going to give you the highlights of what we've learned from this experience and we have learned as we've gone along.

I think we always did design good experiments but we're designing even better ones these days with this experience behind us. The key elements in these types of studies are the scaled-down process itself,

the choice of agent and host that you're testing, the spike modality and how it's introduced into the material, and the processing itself and how you do that. We are just going to go through these things one at a time.

The scale down is usually left to the client. Quite frequently what we are brought is an existing virus validation procedure which they've used in the past for HIV, porcine parvo, et cetera, to look at conventional viruses and it is relatively easy to adapt that to the study of the TSE agents.

On the other hand we have learned some things as we've gone along. Some of these scale-downs are really scaled down and our preference has become, even though it would seem to be more convenient to work on the 100 mill or 50 mill scale, what often happens as you work through a process is your sampling starts removing volume and

material and by the time you get to the end you are working with very little material. You have very little room for flexibility in sampling an assay. If you want to take an extra pH sample it gets a difficult and, quite frankly, we prefer to work on a slightly bigger scale. The other thing is as you get to smaller scale surface effects begin to dominate the separations and we worry about that in the case of these very adherent agents.

Well, the agent is a real issue. We can choose from the various mouse strains of scrapie, the hamster scrapie strain, which is the one we prefer. There several strains of CJD out there out there that we can use and we have used some of them, the Fukuoka strain in particular. And we within the last couple of years brought the BSE variant CJD model into our lab using the model that was developed by Moira Bruce at the MPU.

The only one that is completely unambiguous is the BSE variant CJD model because it is fairly immutable in any animal system you put it in. If that is what you're looking at it is the relevant strain for that particular agent.

Otherwise our feeling is that the choice is pretty arbitrary. For example, it's not clear to me in particular whether the variation we've seen between these various models within strains is any greater than the variation between scrapie and CJD and because there is so much variation it's arbitrary and we use that as a justification for working with the hamster, which is our preferred model.

One you pick the strain you have to pick a host and there are lots of arguments out there. Various inbred mouse strains can be used with virtually any of mouse-adapted strains and the hamster strain, which is convenient and well

characterized, and then we have the transgenic mice models.

If the PRP molecule is the whole story then this is a model of choice, definitely, for doing these studies; however, there are some caveats attached to using these strains. That is that the most effective transgenic models are not real clean representations of the prion gene in the mouse.

The ones that work best are chimeras, mixtures between the host, either cow or a human, and the mouse gene. A lot of these vectors carry the doppel (?) gene in with them. The ones that have the short incubation times usually have many random and multiple insertions and the expression of the gene is actually aberrant compared to its normal context.

If we really wanted to get to something that was close to a true humanized mouse, for example, we would use one of the

replacement gene replacement models where it's a one to one replacement of the model gene with the mouse gene. The problem with these is that they are not necessarily as convenient to use as some of the others.

Let's go on since we are pressing for time. Another example of the things you have to consider here is that what we've noticed is that as a background it's important to remember that BSE, even though it seems to be a very stable strain in terms of its re-isolation from various animal models, nevertheless presents very differently in cattle and humans. You would never confuse variant CJD with BSE clinically.

On a laboratory scale this gives you something of a conundrum because we really feel that the hamster 263k scrapie model is clinically much more similar to BSE in cattle than the BSE strain itself is in the VM mouse.

On the other hand BSE in the VM mouse gives us a disease is clinically more similar to variant CJD in humans. So the point here is that even at the level of selecting a host strain to work with it's somewhat arbitrary.

Let's talk about spiking. The central problem with TSE spikes is that TSE infectivity is poly-dispersed. It has a wide spectrum of physical and chemical properties because it is typically in an aggregated state associated with other cellular components and these things affect the way in which it fractionates and partitions.

As a consequence to that it is quite a different situation than you would have compared to working with something like porcine parvo virus where you can make a very highly purified, highly uniform monodispersed agent which regardless of where you introduced it into a process

stream you would pretty much have what would be there anyway even if you had brought it all the way from the beginning of the process to that point.

In the case of these agents you may be selectively removing certain components of the distribution in earlier steps which then don't get tested in later steps or the converse of that is if you introduce the agent at a later step you are removing something that's already been removed by an earlier step.

Another problem with spikes is that the only source of high titer infectivity in these diseases is CNS tissue of the brain in particular. And brain drive spikes give you this tremendous advantage. In the hamster we have almost ten to the tenth (10^{10}) infectious doses per gram but when they're used to spike something like blood or a low titer tissue that has nothing to do with the central nervous system the

relevance of these spikes is questionable.

One way to get around that is to take some fraction of the brain, and a number of people have experimented with microsomes, liposomes, detergent lipid protein complexes. Aventis has been working with caveola domains, fibrils. The problem with these things is I'm not sure that they really represent the true *in vivo* situation, either. They behave better in terms of the fact that they are more homogenous when you put them in as a spike but they are not necessarily more relevant.

The one place where this is not a problem is if the source tissue actually is a brain, for example, if you were doing an experiment on pituitaries, or if your major concern is cross-contamination of the tissue you are collecting by CNS tissues, in which case a brain-derived spike is exactly the right thing.

The alternative is to use

endogenous infectivity. Here the relevance is not questioned but the problem is the clearance potential is very low because the titers are typically low for non-CNS tissues.

On the other hand in the case of blood we have demonstrated now that we can actually get very accurate measurements out of these low titer tissues and I will show you in a moment how this can work to your advantage in getting an accurate representation of what you're trying to treat. Another problem, however, is blood is a little bit unique in the sense that its physical state is the same in large animals and small animals whereas something like organs are quite another story. It's pretty hard to compare the heart of a hamster to the heart of a cow.

Heino Diringer ran into this when he did his dura mater validation tests using hamster dura. The hamster dura is so

fragile that it disintegrates under these conditions whereas the human dura would not.

Another issue is the introduction of the spike. There's been some mention of that in the last couple of talks but it really is a fundamental issue. Every tissue is going to have some intrinsic infectivity in it in proportion to the amount of blood that's in that tissue because we know that there is infectivity in blood and at least in clinically affected animals it's about ten infectious doses per mill in the rodents.

But there may be other sources of intrinsic infectivity and it's very difficult to mimic this intrinsic association of infectivity with solid tissues like dura mater, tendons, hides, bones simply because it's not clear how to introduce the infectivity into the tissue itself in a realistic way.

And if you fail to do it, on the

other hand, when you do this type of test you are measuring the way brain homogenate is inactivated in the presence of these other materials rather than how well the process actually can seek out and destroy the infectivity in those materials.

The converse of this is extrinsic infectivity, which comes from cross-contamination, and because the brain has such high levels of infectivity and the central nervous system has such high levels of infectivity cross-contamination is fairly likely during the collection of other non-CNS tissues. And in many cases it maybe the most significant source of infectivity, in which case again a brain drive spike is appropriate.

There are other issues we could deal with, sampling, sample preparation for titration, assay methods, cross-contamination issues, logistics, but I'm going to go on now and talk about

experimental design next. What we've learned and what I'm beginning to feel more and more strongly about is that wherever possible it's best to test endogenous infectivity first even if there's virtually nothing there. The reason for that is that you should at least do the test and carry it as far as you can until you run out of infectivity because sometimes it goes farther than you think and there's no question about its relevance.

We have the methods now for doing this. The end result is that if you take this approach it really supersedes any evidence that you develop from a spike that claims higher levels of infectivity if you can't develop the same level of infectivity with the intrinsic infectivity.

I'll give you an example. Here, this is the example I gave earlier. Let's go on. Here is where this fractionation diagram was that I was looking for in the

last talk. Again, this time I'm going to focus not on these two fractions, albumin and IVIG, but rather on the fraction four where after this extensive cone fractionation we still had two infections in this fraction. This is two infections out of the equivalent of 50 ml of blood inoculated.

These infections come, as you follow the cone fractionation, on this side they come very close to the end. The next thing beyond here is fraction five or albumin itself. So it has carried through all of these steps, one plus two plus, well, that is the soup, actually, all the way to fraction four and the fraction four pellet, which means that without any spiking at all, starting with ten infectious doses per ml and a significant volume of blood, we are able to carry it all the way here and demonstrate about two and a half logs of removal. That's really the only removal I

think we can reasonably claim in that case.

When you can't use endogenous infectivity we have come to realize that another very important aspect of TSE experimentation is this concept that we are calling conditioning of the spike. What I'm getting at here is that when you have a process that goes from step A, B, C, D, E, F, G, et cetera, to make sure that when you are testing step C that you are not just retesting and re-removing the same subfraction that you would have removed at step A it's important to run step A, possibly step B, and then take your value from step C.

In an ideal way the way you would run an experiment is you would spike here, collect and measure until you run out of infectivity, and then spike at B and do the same thing over again, spike at C and do the same thing over again, et cetera. Now, this would be an extraordinarily extravagant

thing to do. It would be lovely if we had the resources to do an experiment this way but we usually have to make compromises.

Nevertheless it is important to keep this concept in mind because, for example, in a scheme like this where in the very early steps we might have a solvent detergent treatment of cryo, for example, and a filtration step following it if we then come down here and have to re-spike it, step D, and we haven't gone through this solvent detergent step and we haven't done this filtration we may essentially remove the same material that we removed here again at spike D.

This secondary spike needs to be treated in some way to condition this spike for what's preceded it in this process diagram. Of course, the other corollary here is you want to carry the process as far as you can on a single spike before re-spiking.

The same thing would be true here. If this filtration is equivalent to this one then you really can't count the removal from this filtration because you probably already removed it there unless you establish, as you'll see in the next slide, an experiment and you do the experiment this way where you show that the removal by the filtration is completely stochastic.

In other words what we're doing here is this is the actually process and this has worked very well for us on a number of occasions. Again, there will be an example of this in these Asahi studies that will be presented tomorrow. But basically what we are saying here is if you spike at A and you get a certain level of removal and you're selecting a sub-fraction by so doing it if you then rerun that exact same step over again you wouldn't expect any removal at all.

On the other hand if you do that

removal and it's on the same level as you got here then you can say that the limitation of this step is a statistical one, it is not a selective one, and as a consequence it is valid to count this in multiple iterations through the process.

How am I doing here? Not too good. I'm almost done.

One of the last points I want to make here is another thing that we have learned, very importantly, is that you always want to track the product stream directly for removal and not get this information by some surrogate method. And I give you an example of a column chromatography here where, for example, if you were to challenge with ten to the eighth (10^8) infectious doses you might find when you assayed the flow-through that you had ten to the eighth (10^8) infectious doses recovered in the flow-through.

You might find the same thing in

the first wash. It might go down in the second wash or the third wash, but it is absolutely essential to look at the eluate because you could also have ten to the eighth (10^8) infectious doses here.

And to pass this just to another step without making this measurement is not valid and the reason for that is that the precision of the measurement is only about a half log and when you look at this you really can't distinguish these three numbers significantly so you can see these kinds of things and we have seen them.

A couple of comments about the evaluation of total clearance, in my opinion endogenous studies take precedence over any type of spiking study. The continuous processing takes precedence over stepwise values. In other words the data you develop from a continuous process takes precedence over stepwise values and you have to use great caution in interpreting cumulative

removal from similar stepwise steps.

When you start evaluating total clearance, I think it can be summed up this way. The exercise is worthwhile because high values are definitely better than low ones but I think it's a big mistake to make too much of this type of data and the actual values should not be interpreted too literally.

In the end the thing that gives us the greatest confidence in these studies is that as more and more of them are being performed using a greater number of models and modalities we can start to compare them and as the data accumulate for multiple agents, spikes, assays, scale downs, et cetera, the convergence of diverse approaches on the same result provides the greatest security for the ultimate outcome and interpretation of that outcome.

So there's great value in doing it more than one way in more than one

laboratory and with more than one model.

DR. BOLTON: Thank you, Bob. I'm sure we must have questions for Bob from the committee. No, you are going to stun me. Nobody has any questions?

DR. ROHWER: We're all getting tired.

DR. BOLTON: Well, I thank you. I think that you have made these points before to most of the people who have been on the committee in past years and you have made them well, I think, so I think it's all beginning to sink in.

Well, at this time we will move to the open public hearing and Dr. Freas will take over.

DR. FREAS: As part of FDA's advisory committee procedure we hold open public hearings for members of the public who are not on the agenda and would like to make a statement concerning matters pending before the committee.

Mr. Chairman, at this time we have received three requests to speak at today's open public hearing. They are from Margie Baker, Moira Kennedy, and David Korroch. Margie Baker, would you please come forward? You can either use the microphone there or come to the podium.

While you are approaching the microphone we are asking that you address any financial interest that you may have with any products that you may wish to comment upon. These presentations will be timed for six minutes. A yellow light will go on at the end of five minutes. Go ahead.

MS. BAKER: I don't know if Dorothy Scott is here but I would like to thank her because she advised me to attend this meeting and she and I both acknowledge that no one has ever caught CJD from beef insulin or from blood transfusion yet people who have used UK beef insulin are deferred from donating blood. I am a type 1 diabetic

survivor for 58 years since age 53 with basically no complications. My only complications were caused by three weeks on synthetic human insulins, the only insulin that is readily available in the US now. Like many thousands of our fellow citizens, I cannot medically tolerate the synthetic insulins. Pork insulin is only a little better, very difficult to find.

And I will tell you why these insulin guidelines must be changed. I have no financial interest in CP Pharmaceuticals from Wales, UK. My interest is caused by my need for their beef insulin, which I import, which should be readily available in the open US market because as I was here I broke my one bottom of isophane insulin and I am on the verge of being very sick because I can't get it.

I called for pork insulin at CV Pharmaceuticals and they don't think they can even get the pork so you might be saying

goodbye to me.

Many suffer or have been maimed or die because it is no longer sold in the United States. Please don't patronize us by saying import. Only 50 people in the United States know how to import. The rest aren't even aware that there is such a thing as bovine insulin, much less find out how to import it, that their health will improve if they use it.

If they do happen to read the FDA CDER beef insulin site they are scared away by the mad cow warning, which is very inaccurate, so they go to the local pharmacy and buy what is available as the people with diabetes have always done. Fortunately, I found CP's website, asked my doctor to sign a letter of necessity for my nonprescription drug for the FDA, paid the USDA for my import permit. It now costs $95. I typed my personal use letter for the FDA and then filled out and faxed the order.

That may sound very easy to you but it isn't. It's harder than paying taxes and the extra $145 permit and shipping is an expensive hoax. Beef insulin should again be available in our local pharmacies without prescription as it has been since 1921.

The tradeoff of a theoretical risk of mad cow disease for a real human need is a disadvantage to the diabetic and not a credit the FDA. The guidelines need to be changed. I understand that the FDA refuses to allow CP to market their 25-year proven beef insulin here, requiring tests as though it were a new drug and requiring CP to provide that its bovine insulin contains no BSE prions.

BSE in beef insulin have not even been proven by you, the scientists, but I can prove to you that many have died for lack of what I'm here to talk about. CP's insulin has already been proven safe over decades by tens of thousands in many

countries. This proven insulin has been approved for market by the UK Medicines Control Agency and regulatory bodies in 12 other countries.

The governments don't impose suffering on their needy diabetics, shielding them from a remote possibility of CJD. Is compassion not considered? As your committee ponders the issues before it today please review the risk-benefit equation as it relates to the diabetic and society.

Remember the polio vaccine? That had real risks but was allowed because of a desperate need. Remember, CJD is not contagious. Don't block the one manufacturer who is ready, willing, and able to supply our desperate need and involves no BSE risk. You will eliminate such suffering and expense.

Please change your guidelines on bovine insulin. CP has made application for it, I believe, in '98 or '99 and withdrew it

because of the FDA's total bogus reasoning. CP's pancreas crystals come from US cattle. Can BSE prions stick to stainless steel equipment for nine years? Beef pancreas crystals don't carry BSE so how can they stick to said equipment? Even if the cow had mad cow, well, anyhow.

Dr. Asher asked me, he said, "Mad cow prions might splatter on the pancreas at slaughter." Then are there still mad cow parts allowed to be sold in the United States? Think about it. The only insulins available in the United States are defective for many. If I am the one in a hundred million years of treatment that might get CJD from beef insulin then that risk is better that the agony of taking FDA-approved synthetic insulins.

The FDA is adamant about protecting diabetics from the theoretical risk of CJD if we take beef insulin. The USDA is concerned about our insulin-

contaminating animals. I hope you can see reality. We do not need protection from theory.

The FDA and USDA should change their regulations on the UK beef insulin. The possibility of transmission of BSE through beef insulin to humans has never been seen. It is an unproven theory. We ask that you remove the warnings of BSE threat from all beef insulin guides, including the Internet, and allow it to once again be marketed in the United States. No one has ever caught CJD from beef insulin but many have sickened and died from your guidelines of withholding on UK beef insulins.

Remember, as doctors you are required to do no harm and to save lives. Thank you, and a copy of my speech is out on the table and I've also put a copy around to all the committee members. Thank you.

DR. FREAS: Thank you, Margie.

Thank you for coming today and making this presentation and sharing your personal experience with us. Your hand-out will be posted on the FDA web site and we do appreciate your taking the time to come up here.

Our next speaker in the open public hearing is Moira Kennedy from San Francisco, California.

MS. KENNEDY: First of all, congratulations for pronouncing my name correctly. That's very rare. I actually come from Santa Rosa, not San Francisco, though.

I was very interested in the speeches this morning because I myself am an expert in transplant from a different point of view from the distinguished speakers. I myself had a transplant six years ago and I'm very glad that all of those very stringent methods and restrictions were not in place then or I would probably have died

waiting for my kidney.

I would like to thank the speakers, especially Dr. Hogan and several members of the committee, and I think you are one of them, who actually spoke for the patient and spoke for the needs of the patients rather than just for the scientific considerations about transmissible spongiform encephalopathies because our needs with the shortage of organs from my point of view that is the most important.

Like I say, I'm very thankful that I've got my kidney. I'd further like to add that it would be too easy to bring in restrictions that would cost more lives than actually save them by these methods that are supposed to make organs and tissues safer.

I came to address the advisory committee on a related issue which has already been referred to by several speakers. That again is bovine insulin. The FDA's web page actually devotes more

words to warning against bovine insulin because of BSE than it does to giving information about reporting which is what it is supposed to be doing.

We need bovine insulin available because the biosynthetics or recombinant DNA insulins are not the same as the insulin that your healthy pancreases secrete. They are chemically different even if they do have recombinant DNA.

Firstly, once a bio-synthetic human insulin is injected ---- molecules fold over. This is does not happen when insulin is naturally secreted. Secondly, all injected insulins, no matter what they are, follow a different path to the blood stream than a naturally secreted insulin.

Thirdly, as the previous speaker already mentioned, beef insulin has a greater ability to warn people of impending hypoglycemia. What has been established and in fact the biosynthetic insulin, Humulin,

was placed on the FDA's ten most wanted lists of most reported drugs some years back because so many people have died through hypoglycemic unawareness.

For those of you that don't know what that is a person who injects insulin can tell if they've got too much in their blood and they need to go and get some sugar or get something to eat. Mostly you get symptoms through your nervous system and you know to do that.

With the biosynthetic insulins this often doesn't happen so you might be speaking at a microphone and suddenly you keel over and black out. It's not really good if you are driving a car and that happens but that does happen.

This is why some of those people that Margie was talking about need to import at great expense from the United Kingdom bovine insulin because they know that they can't survive without it, especially if they