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I N D E X

Session 3

P R O C E E D I N G S

MS. MALARKEY: Good morning, everyone. Could we please take our seats?

We have a very ambitious agenda this morning, so it would be good for us to get started on time, if possible.

We would like to welcome everyone back, and welcome to those of you who weren't here yesterday.

I'd also like to again thank everyone for their participation in this workshop, particularly the speakers and the panelists. I thought yesterday's sessions were very interesting and informative. So I'm sure that the speakers and panelists today will do the same, myself not included.

But I would like to thank a few people that have made this possible, particularly those that are in our Office of Communication, Training and Manufacturer's Assistance at CBER, particularly Bernadette Kawaley, who has been out there signing people in and everything. They've really made all the logistics for this workshop possible.

Also I'd like to thank the NIH folks for

their work on getting all of the technical issues sort out for today and also for just making this possible again.

I'd like to reiterate what Matt Kuehnert said yesterday afternoon and thank AATB, and particularly Scott Brubaker for all of his efforts in assembling such a knowledgeable and distinguished group of speakers and panelists for these sessions.

Now this morning we'll do things a little bit differently. We've got a lot going on. I'm going to start things off, hopefully set the stage for the speakers this morning and I think also some carryover from yesterday.

Then there will be three speakers talking about disinfection and sterilization processes, challenges, concerns, et cetera. We'll have a break.

Then we'll hear something about process validation which, as I think Martell mentioned yesterday, is a huge topic because, of course, you have to have a validated process for any of this to be consistent and for safety to be assured. Then we'll have a panel.

So this is again quite ambitious. I look forward to all the speakers.

So what is sterile? This is actually not a simple question. One would think it would be, but it really is not. Hopefully, you'll understand what I mean when I'm done with this very brief presentation.

A few disclaimers. I'm going to use quite a few references that really are not specifically pertinent or applicable to HCT/Ps. However, I do believe that there are principles in these documents that can certainly be applied.

The one exception is, of course, the United States Pharmacopeia, USP, which we heard a lot about yesterday in terms of BNF testing. I think the importance of that was driven home by Arjun's talk when he talked about the 1 ml carryover and the inhibitory effect that had on detecting microorganisms.

I'd also like to apologize for my slide. It says FDA workshop. Clearly, that's not accurate. It is the FDA and CDC. So my apologies to our colleagues at CDC.

So what I'd like to cover briefly is

something about sterility, particularly some things in our regulations and some of the comments that we received to the proposed rule and what we said in our final preamble to our final rule on GTPs.

Sterility assurance levels, you heard about that yesterday. I won't spend a lot of time on that.

Sterilization and then aseptic processing versus what I'm calling aseptic processing. Hopefully, again that will be clear as I go through this discussion.

We received one comment. I think Leslie Kux alluded to this yesterday in her very good overview. We received a comment to the proposed UTP rule regarding sterility.

The comment was -- it was a concern that the FDA is now requiring that HCT/Ps be sterile and processes be validated to assure sterility and that that would, in fact, impair function. I think we heard a lot about that yesterday.

We did respond in the preamble to the final rule that, in fact, that was not the case. We were not yet requiring sterility. We understood that there were

issues with that. Impairing function was certainly one of them. However, we, of course, expect aseptic technique and control of activities to limit the introduction of disease agents. We will revisit this as technology progresses.

This last point is important because I think that's one of the reasons we're here today, is to encourage innovation and progress in this area where it can be achieved, understanding that in some areas it may not be possible today.

We have a regulation 1271.230 that is under our process and processing control. Written representation. Any written representation that your processing methods reduce the risk of transmission of communicable disease by an HCT/P, including, but not limited to, a representation of sterility or pathogen inactivation -- and here you see we've split the two out -- must be based on a fully verified or validated process.

So I think that's very clear. Any written representation, so this is not limited to labeling but anything that you put in writing.

On the labeling front, 1271.370(a), you must label each HCT/P made available for distribution clearly and accurately. I think that last word accurately speaks again to whatever's on the label has to be correct. It has to be really based on what is -- it is true.

So what is sterile? Well, here's the first reference. Again, USP was mentioned yesterday quite frequently. There is a general chapter, 1211, sterilization and sterility assurance of compendial articles that speaks to this question.

There is also the AAMI document that was discussed yesterday as well. I like this one because it's very, I think, to the point. It is sterilization of medical devices -- and it is for medical devices, not specifically for tissues -- requirements for product labeled sterile.

In these documents, if you pull from them, basically, the definition of sterile is free from viable microorganisms. I think we would all agree with that. I think we heard Webster calls them germs, but we can do with viable microorganisms.

But, of course, that brings up the question what is a viable microorganism. You'll notice I don't have a reference here because this is my definition for today, just what we're talking about here, which is HCT/Ps. These are biological produces derived from humans. So this is again my definition.

It's somewhat like the USP in that we're really talking about anaerobic and aerobic bacteria, molds, fungi, et cetera, basically, what you can test for using a validated or verified sterility test method.

By verified I mean if you follow USP with the exception of validating things like the bacteriostasis, fungistasis and the growth promotion testing, it's really an already validated assay. It's a compendial method. So you just verify that you can run it basically in your laboratory.

So that's the definition I'm using just today for this particular discussion.

What about viruses? Well, the AAMI document actually includes viruses in the definition of viable microorganisms. But there is a caveat that says that

it may not always be possible or it may not always be practical.

My colleagues from CDRH who were sitting behind me yesterday reminded me that in the medical device field, in fact, when there is a claim of sterile -- and I assume this means devices that are synthetic or manmade -- then that is, in fact, a claim that viruses are not present. But, of course, these materials would not be inherently expected to have a viral load to begin with. So I just want to make that correction or that clarification.

Now, of course, with viruses, we have donor testing and donor screening. Those are really a cornerstone of the safety. These, of course, have to be accomplished with FDA licensed or approved or cleared test kits.

The donor screening. We just came out, as Leslie mentioned, with the donor eligibility guidance that speaks to some of the risk factors for clinical evidence of what one would look at. So that is hugely important, obviously, for viruses.

Now there are also mechanisms to evaluate a

particular process for its capability or capacity to remove or inactivate viruses. Generally, this is demonstrated through small-scale laboratory studies. The scale down must be adequately validated or modeled after the large scale. It uses actual or surrogate viruses as opposed to testing of the final product.

Again, I don't know -- and I think our colleagues from CDC spoke of this yesterday. I don't think we have methods that are sensitive enough, for example, to do that, even in the plasma derivative area where many pools may be tested by PCR. But when you get to the final container product, the sensitivity's just not there.

Now, there is a document, an ICH, or International Conference on Harmonization document Q5A, viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. While clearly that is not specific to this field, it's also not specific to the plasma derivative field. Yet the document has very good information about how one could accomplish such studies and what viruses, for example, one would use as surrogates.

Now, this is an example taken from a package insert. This is an actual label of a plasma derivative. I just went on to our Web site and found the first most recently approved one. I won't say what it is.

But this is a common label. This is how the pathogen inactivation or reduction is expressed. Here at the top you have the list of viruses that were evaluated. Some of them, HIV and West Nile, are, in fact, the real thing. But then there are others that are used as surrogates often because the viruses really can’t be grown enough to really assess a log reduction factor.

So, for example, minute virus of mice is a parvo virus. It’s a DNA with no envelope. It’s one of the most difficult, obviously. B19 is a parvo virus that is of concern in the plasma derivative arena. So, basically, you see what the viruses are and what their properties are and then the manufacturing steps that have been looked at.

As you can see, each one has quite a good log reduction. Then there’s the cumulative effect. You

can see, as I said, with the parvo virus, it's the smallest reduction. But we still have close to 8 logs cumulative.

So I have actually seen this done, certainly in this industry. I think that that is certainly something -- I don't know about the label or if people would be interested in putting that in the label, but I think that that would be an accurate representation if these studies were, in fact, accomplished.

What about TSE-associated prions? Do we list those as a communicable disease agent? However, I think we all know that currently the technologies do not exist that we would like to be able to evaluate.

So we do have to a reg on dura mater. There were again comments to our proposed rule, GTPs. A number of the comments said we should just remove the provision for dura mater based on the TSE risk. In other words, we should not even be talking about it.

We disagreed, as it would eliminate any safeguards that are in place or that could be put into place at a future date when, in fact, there are validated processes that exist. So we basically left

it there. I think that's really all we saw about TSE-associated prions in the case of processing.

I do believe I have seen some studies on cleaning of utensils and facilities and equipment. But I think even that is difficult because of the stringent nature that is needed to, I guess, inactivate -- or whatever the proper terminology would be -- these prions.

I would say, of course, we do -- again, we have the donor screening questions around this risk. That is really what we have today. So we did keep the reg for dura mater. It says when there is a published validated process that reduces the risk of TSE, you must use this process for dura mater or an equivalent process that you have validated unless following this process adversely affects the clinical utility. So even then we put that in there because again, we understand the need for clinical utility.

Then, of course, when you used a published validated process -- and this is the case across the board -- you verify it. You don't necessarily validate it again, but you verify it.

Again, back to sterility assurance levels, I think we all know now, if we didn't before, that SAL refers to the probability of a single viable microorganism occurring on an item after sterilization. This is based on the kinetics of microbial inactivation. Again, these kinetics are bacterial based.

The SAL is normally expressed as 10 to the minus N. Historically, two particular SALs appear when we talk about sterilization. That is 10 to the minus 6 or 1 in a million probability and 10 to the minus 3, which is a 1 in a thousand probability of survival.

So what about sterilization? Well, terminal sterilization is just what it sounds. Of course, it is a validated process whereby product is in its primary packaging and sterilized. So nothing else is done. It is ready for market after that process. It may get secondary packaging, but it's in its package. Generally, this is done to an SAL again of 10 to the minus 6, but it's also based on the intended use of the product.

This is coming from the AAMI guideline.

Products intended to come into contact with breached skin or compromised tissue, invasive products that enter normally sterile tissue, and surgically implanted devices. So this would seem to encompass what we're talking about here.

However, AAMI recognizes that this may not always be feasible. It expresses some exceptions. I think this is particularly interesting. This is where things get complicated.

If a product is unable to withstand such treatment -- that is a 10 to the minus 6 treatment or either gamma radiation or steam sterilization, whatever the case may be, or other sterilization method -- if the product offers unique or superior benefits for patient diagnosis, treatment or care, if there's no alternative product that can withstand such treatment -- those might seem somewhat redundant, but I think they are somewhat distinct -- in these cases, validated sterilization processes to achieve either 10 to the minus 5, 10 to the minus 4 or 10 to the minus 3 could be used. The most rigorous, of course, that the product can withstand should be selected.

Moving to aseptic processing, now we are moving to the drug world because I think the -- I will call it definitive. But the normal interpretation -- or the convention I guess would be the proper word -- of what aseptic processing is, is all around our FDA guidance for industry on sterile drugs produced by aseptic processing.

There's also reference to this in AAMI because there are some devices that cannot be sterilized with heat or gamma radiation. So again, they must be aseptically processed.

But we say in this guidance that if you can terminally sterilize, you do it. This is only when you cannot terminally sterilize to any one of those levels that I just mentioned.

In an aseptic process, the drug product, container, enclosure, are first subjected to steam sterilization -- it's generally steam in the drug world -- separately as appropriate and then brought together.

So you're starting with all sterile components, if you will, including the project which is

generally filter sterilized using a .2 micron filter that has been validated again in a laboratory for its capacity to remove a certain amount of very small bacteria, brevundimonas diminuta. Then product is subject to a validated or verified sterility test method.

So I appreciate what Martell was saying yesterday. But I think when we get away from the 1 in a million concept and we get down to even 10 to the 3, you got a 1 in a thousand, then these tests are important. They are just another level of assurance.

The test beforehand is also important because you're relying on your sterile filter to render the product sterile prior to going through this processing. So I think there's a place in this industry, as well as clearly in others, for doing these tests. That's just my personal opinion on that.

In terms of validation of aseptic processing -- and again this is a drug thing, so it's not really application here. But the way it's done is one takes growth media, very happy media for bugs. You sterilize it. Then you put it through the process and

do all the manipulations with the people there and the equipment and everything. Then you basically incubate it.

The contamination rate -- what we'd like to see is basically zero. But there is an allowance for .1 percent, which really now is like 1 in 4356, I think, as opposed to -- anyway, I won't go there. But it's a 1 in a thousand is what it amounts to.

This is what is deemed acceptable. Of course, if you do get a positive unit, then we expect a full investigation as to why that might have occurred.

Generally, what we see is that if there's a media fill failure, it's a massive failure, I would say.

Now what about labeling a product as sterile? This goes back to AAMI. There's also discussion in the FDA guidance.

According to AAMI, the product may be labeled as sterile for the following: The terminal sterilization to an SAL of 1 in a million. When exceptions are met, terminal sterilization to the most rigorous SAL possible, so 10 to the minus 5, 10 to the

minus 4, 10 to the minus 3, may still be labeled as sterile. An example of a medical device with 10 to the minus 3 is, in fact, a xenograft, a heart valve.

Sterile products produced by a validated aseptic process and passing a validated sterility test. So in all these cases a product may be labeled as sterile. So, again, it's not an easy question.

What about aseptic processing? Well, I think for HCT/Ps, it's fair to say that the term is used, but it's unconventional in terms of what the drug world would say. Clearly, the HCT/Ps themselves are not sterile coming in, so you're not starting with all sterile materials an components.

There are, I know, some firms that do bioburden reduction. I learned yesterday that that's non-terminal sterilization, is the proper term. That occurs using sterilization methodology, gamma radiation generally prior to -- and this is probably not correct aseptic processing.

I think aseptic processing, which would be actually processing -- getting everything off of the tendon or bone or whatever might precede this -- but

the bottom line is before disinfection and those types of processes are done. I would assume that those are done using aseptic technique as far as possible.

So my understanding is in this situation, the convention appears to be that the product is labeled as aseptically processed whether it has that non-terminal sterilization step or not. If it's just subject to disinfection or it has the non-terminal step, then it is generally labeled as aseptic process. But that's my understanding, and perhaps I'll learn differently when we -- our other speakers come up.

So the questions we have for our three presenters on disinfection and sterilization processes are, what are the different disinfection and sterilization processes available?

What effects on the graft do different processes present that could be problematic? For example, decreased functionality.

Those are our questions. So, I guess, without further adieu, I'd like to introduce our first speaker who is Dr. Steven Arnoczky. Dr. Arnoczky is a doctor of veterinary medicine.

He is a professor of surgery in the College of Human Medicine and Veterinary Medicine at Michigan State University. He is also the director of a laboratory for comparative orthopedic research in the College of Veterinary Medicine at Michigan State University.

He received his Doctor of Veterinary Medicine from the Ohio State University. He is a board certified veterinary surgeon. He has numerous honors and many publications and book, and book chapters.

So it's my pleasure to introduce Dr. Arnoczky.

DR. ARNOCZKY: Thanks very much, Mary.

It's a pleasure to be here. There's some disclosures first of all. I'm a consultant for the MTF. I review grants for them for their allograft science competitions and also Regeneration Technologies. I reviewed the pre-clinical studies on the meniscus aspects of that. So I wanted to disclose that to you right now.

First of all, what is a veterinarian doing up here? I do a lot of basic science. I run the

comparative orthopedic research laboratory at Michigan State. Some of the things we do are involved with allografts. We did the first basic science work on meniscus allografts, looking at the biology of meniscus allografts. We also did the early work on patellar tendon allografts for ACL reconstruction.

So I'm coming from this whole aspect, from a little bit different perspective, being interested not only in the sterilization and safety but also the effect on the functionality of these particular tissues, something I don't think we've addressed too much. I think it's very, very important.

Also, I head up the task force at the American Orthopedic Society for Sports Medicine on allografts. We've put together a symposium that Scott was very, very helpful, and many of you in the audience were helpful with, as well in industry.

There are a lot of questions that the end users, especially in the area of sports medicine and arthroscopic surgery have. I'll try to represent to them, in terms of talking about their concerns, that you in the industry, in the regulatory group really, I

think, need to address and should be made aware of.

So first we're going to talk about what I was asked to speak on, the sterility of connective tissue allografts, a balance of risks and rewards.

We heard a wonderful lecture by Mary. I wish all the orthopedic surgeons and sports medicine docs could hear that because that's a big concern, and in terms of sterility, what does it mean and how it's applied.

Now, we heard about levels of tissue processing. Obviously, donor screening, laboratory testing are real cornerstones, as Mary alluded to. She alluded to aseptic tissue harvest and then the other secondary things that can go on in terms of the tissue sterilization procedures and terminal post-package sterilization.

But the first three are real key. We're going to talk a little bit about the aseptic tissue harvest and processing and then how that relates to why even people want to do secondary sterilization procedures.

If tissue harvest is aseptic, why do you need

to sterilize tissue? Well, again, Mary went through some wonderful definitions here. We're talking a condition of asepsis in which living pathogenic organisms are absent, a state of sterility.

But here really the aseptic tissue harvest, the way I look at it, we're talking about the process and not necessarily the tissue from the get-go. It's the process of surgeons being sterile or at least aseptic, I should say, not introducing any outside organisms.

So the reasons that people are worried about this obviously are the pathogens that have been talked about, bacteria and the new emerging viruses, and something that I have never heard talked about before until Mary talked about it in terms of allograft processing and that's the prions. That's a major concern that the orthopedic surgeons have or a concern, I should say, that they have.

So I think the major reasons for sterilizing connective tissue allografts on these secondary sterilization procedures is the idea that you could have contamination of intestinal bacteria in the blood.

People have identified these. These are maybe pushing the envelope a little bit because there's such a demand for connective tissues out there that perhaps 15 hours sometimes is pushed a little bit, maybe to 16 hours. You have a chance to get intestinal bacterial contamination of the harvested grafts.

Occult perimortem infections, here an example, three people died of rabies transmitted by organ transplants from an infected donor who had no symptoms prior to his death. So they're not always able to screen a hundred percent in terms of donor history and the serological testing for all the possible perimortem infections that could be happening.

Scott talked yesterday about Chagas disease, trypanosomiasis, new things that again have to be a concern when we have a global world here, people traveling back and forth. Again, may not be able to identify these at the time of death.

Contamination of tissue during recovery or processing. This is probably one of the biggest areas. The judicious use of these tissues that are gifted to us, the end users, necessitate that you try to utilize

as much as the tissue if at all possible. Again, you handle the tissue. The tissue by necessity has to be handled a lot.

People have shown that in terms of rank-ordered sources of microbial contamination in aseptic processing, personal contaminants and human error are the top two. They were the top two some 20 years ago. They're still the top two. So we have to be careful of that because, again, these are kind of labor-intense duties that are done in terms of processing these tissues.

Screening failure. Again, there is an infections window that not all these serological assays can detect. HIV and Hepatitis C, 7 days; Hepatitis B a 20-day window and so again, it's not a foolproof system. Donor screening is key, but it's not a foolproof system.

Again, the orthopedic surgeon, at least the sports medicine people are concerned about what happens in that window and should we have secondary sterilization procedures that maybe help override that chance.

Emerging disease. Who would have thought many years ago that West Nile virus would exist? Jakob-Creutzfeldt disease has been around for a while, but again, with the idea of prions and bovine spongiform encephalopathy; Mad Cow, being in the literature and in the public domain, people are more aware of these diseases and are concerned about them.

We've heard again -- you don't need it from me -- a very elegant presentation by Mary, but the question that comes in is, does this really affect prions. We really are talking prions here or just endospores and bacteria.

We know that prions are these self-replicating abnormally folded isoforms of cellular protein that are the quote, unquote, "infectious agent responsible for the spongiform encephalopathies." The TSE is transmissible.

They're more in my patients than in your patients in terms of animals, but again, Jakob-Creutzfeldt disease is certainly one area that is there and again is of concern to the end user.

Mary alluded to the fact that there are

materials out there or processes out there. But really, conventional sterilization methods from dry heat, ethylene oxide, formaldehyde, many of these have proven ineffective in inactivating these particular proteins.

The World Health Organization has looked at and is documented that there's basically a couple different recognized effective inactivation treatments, including exposure to sodium hydroxide. In some of the prion strains, steam sterilization seems to be effective.

Most of this is really based on the World Health Organization categories on the ability of these prions to be ineffective. It's based on basically bovine tissues and body fluids. Obviously, the brain, the spinal cord, dura mater are very highly infective or media of infectivity. When we get down to the connective tissues that I'm talking about, really no detectable infectivity.

So again, you kind of have an order of safety here, based at least on the World Health Organization, that they've not been able to document infectivity from

connective tissues, skin, musculoskeletal tissues and the like.

What is sterile? Mary did that very, very nicely. The key is that the orthopedic surgeons, the sports medicine docs, would like to see on the label what that is. If you get it from a specific tissue bank, they would like to know whether it's 10 to the 6, 10 to the third.

I think education is going to be important to let them know that heart valves, as was pointed out, the xenografts, are actually 10 to the third. I think that would give them some degree of comfort because right now they're believing that it needs to be 10 to the 6 no matter what, and everything needs to be sterile and removed, even viruses. That's just the mindset. That's something that maybe educational aspects will address.

One of the caveats that people bring up is, in general, most of these sterilization techniques are validated by these log reduction assays in spiked tissue samples; the idea that you dip and you dunk a tissues in a virus or a bacteria, and then you go ahead

and do these cultures. and you do your processing to see if you can eliminate the organism there.

I don’t know that people use systemically infected tissues. I wonder why. If they do, I would like to know about that because I think that’s important.

We look at a lot of things. We have some viral models that we use for connective tissue viral transplantation and transmission. It’s always a concern to me that I don’t see a lot of this. Maybe it’s done. But again, it’s not out there for everyone to understand. That would be something good to educate us on.

Then how can we sterilize tissues? We have all these as a preamble. How can we get out there and sterilize these tissues and maybe address some of these concerns?

There’s certainly numerous techniques out there. We can kill the bugs. That’s not a problem. I mean there’s ethylene oxide, vaporous hydrogen peroxide, radiation, chemical. All of these work very, very nicely. The problem is they have significant

limitations when it comes to sterilizing connective tissue because these have to be functional.

We'd like them also to be biologically incorporated in a normal setting. That's something that really I haven't heard talked about at all in the last two days.

In sterilizing connective tissue allografts, it's important, I think, to balance the rewards against the risks. The rewards are having a sterile tissue that's going to be safe. The risks are do we affect the tissue properties, not only the mechanical properties, but we'll talk about also the biologic properties, which I think are real key.

So the challenges are going to be the resistant forms of microorganisms and viruses which require longer exposure and higher concentrations of sterility, especially in connective tissues where you have a dense extracellular matrix. You need to achieve adequate penetration into this dense extracellular matrix, which is not necessarily an easy thing to do in all the tissues that we're going to talk about.

Most importantly, I think besides the killing

all the pathogens is preserving the biomechanical and biological character of the allograft. We always talk about in the FDA safety and efficacy. I think this is the efficacy aspect of it, that these tissues the surgeons can be relied upon or understand the fact that these techniques can be relied upon to maintain what they know is the normal biological incorporation and the biomechanical function of these particular tissues.

So what methods are available right now to sterilize connection tissues? Well, there's about four general categories. We're going to talk about each of these in terms of what is out there.

I'm going to talk about some proprietary names. That's not to say that we're advocating any of these. But I think for completeness sake, we want to be able to talk about this. In those instances, I've actually also added the Web site for you so you should go back and learn more about this by looking at the Web site, and talking to these people, and asking the questions that you need to have answered.

But ethylene oxide has been around for a long, long time. Its method of action is basically you

destroy or you alkalize the purines and pyrimidine moieties, and you really bugger up the cell resulting in DNA and RNA dysfunction. So you really go to the heart of the cell.

You also inactivate some of the cellular processes that are involved with enzymatic processes that allow for cell viability and cell maintenance. So as a secondary mechanism, you also can kill cells through this activity.

The positive for this, it's very effective sterilin against bacteria, spores, molds, yeasts and viruses. In fact, this is what we use to sterilize equipment that cannot be put in a steam sterilization, things that may have rubber on them or optical aspects of it that we don't want to screw up when we put in the autoclave.

The negatives, it's a very poor tissue penetrant. It's only good for surface sterilization. That's why it's good on these instrumentations that it's used for.

As was alluded to yesterday, there are some byproducts. These byproducts are nasty. Ethylene

glycol, ethylene chlorohydrin, they're mutagens and they're cytotoxic. These are only the top two.

Someone once told me there's about 40 of these different moieties that come out, potential compounds that are also mutagenic and cytotoxic. So we have to be careful about this.

That's always the tough thing about connective tissue. We can get it off by aerating an impermeable material, surface material, but we don't really have the algorithms, I think, normalized to what kind of aeration are needed for all the different connective tissues.

The negatives. It inhibits osteoinduction and reduces allograft remodeling. So here we're talking about biology. It affects the biology. It may not change the mechanical properties, but it does affect that ability for that tissue to incorporate into the host. That's a very, very important concept.

There's also been reports of patellar tendon grafts causing an immune -- basically, we think this is a foreign body reaction, resulting in dissolution of the graft. It's called the applesauce reaction. What

you see here looks like applesauce basically once was a patellar tendon inside the intra-articular space of the knee used to reconstruct the anterior cruciate ligament.

So it’s an idea -- probably a reactivity to the residual materials left in that patellar tendon causing not only the synovitis but the dissolution of that particular graft. So this is not really a good system for connective tissues.

Gamma radiation. We know the mechanisms here. We dictate cobalt 60 to nickel 60. We produce some photons. The photons then go right again to disrupt the nucleic acids leading to dysfunction of the whole cell and the messages that the cell needs to maintain viability.

It also generates free radicals from liquid water. One of the things we want to remember is that connective tissues that we’re talking about loaded -- the extracellular matrix, with collagen, mostly Type 1 collagen for bone, tendon, ligament and menisci -- these free radicals damage the collagen molecules. So they’re doing something bad for the

mechanical properties of that.

We have to also remember that if we freeze or freeze dry the tissues, this changes the moiety a little bit. We have to require higher doses to achieve the same sterilizing effect as the dehydrated and non-frozen tissues. So manipulating these tissues also affects the dose that is required to get the action that we need.

The positives are its outstanding penetration. We can know that these rays penetrate throughout the entire tissue. So it ensures that the sterilizing effect is ubiquitous throughout the structure of the tissue. It can get into nooks and crannies and zap those viruses and bacteria, which is very, very important.

So it’s an effective terminal sterilant. You can wrap something in the package. You can sterilize it using gamma radiation because we know it’ll go through that packaging.

Low doses, which most people are using -- I tend to use the mega rad aspect of it. It’s just a decimal point over. This would be 20 kiloGrays -- are

able to kill most classes of microorganisms.

People have talked about what these doses are. You need two and a half to get up to bacterial spores. We'll find out you need a lot more to get to some of the viruses that are inside the connective tissue matrix.

The negatives, HIV inactivation in connective tissue, require higher doses. Patellar tendon grafts, 4 mega rads; bone, 3 and a half mega rads or 40 kiloGrays or 35 kiloGrays; that's pretty high.

It's because -- someone mentioned yesterday it's difficult to imagine bacteria being in the extracellular matrix of a tendon. It's not difficult to realize that viral particles are being shed and they are in the extracellular matrix of these connective tissues, especially retroviruses.

We know that doses greater than 3 mega rads and 2 mega rads can adversely affect the mechanical properties of bone and patellar tendon grafts respectively. We said yesterday that 2 mega rads people liked. Well, 2 mega rads does change it. It does change it.

So we have to look as a dose-dependent change, but it's still changes the mechanical properties. So we have to be careful about using these levels of radiation in this kind of tissue.

There's been some elegant studies done, peer-review studies done, showing that, again, it's a dose-dependent effect. It changes the mechanical properties, not only the strength but also the viscoelastic properties that we're going to talk about later on, that I think are going to be the important thing that we should be looking at in terms of evaluating these processes.

Gamma radiation is so good. Can the tissue-altering effects of gamma radiation on tissue be minimized to take advantage of its excellent penetrating ability? Can we do something with this?

It's quite innovative that people realize that what you can do is if you can remove the free radicals, you can take away some of those things that are deleterious to the collagen and maybe make it a little bit safer from a functional point of view. They can do this by freezing at ultra-low temperatures,

dehydrating the tissue and adding free radical scavengers.

This is the mechanism done by the Clearant Corporation. They're using it on bone, tendon and bone-patellar-bone processes. The bone-patellar tendon-bone is what you use basically for anterior cruciate or cruciate ligament reconstruction. That was pointed out very nicely yesterday.

The tissues are incubated in the solution containing radioprotectorants. They're dehydrated or freeze dried in the case of bone. They're irradiated at a very low temperature, minus 65 degrees at about 50 mega rads or 50 kiloGrays.

So high doses but at the frozen temperature and in a dehydrated state to minimize the deleterious effects on this. It allows this terminal post-packaging sterilization because, again, these things are very good to penetrate it.

The nice thing about it is there is -- the process appears to have no effects on the mechanical properties of tendon and bone, both cortical and cancellous. There are peer-reviewed articles in the

literature that document this. It's very, very important. I think I'll harp on this a little bit, that we need to see more of these peer-reviewed scientific articles to help us understand what's going on and see what has been done.

Indeed, preliminary studies also demonstrate some efficacy in the process of inactivating prions. So again, there is some hope there that this could also be important in inactivating these prions.

The next level we talked about -- we talked about radiation. We talked about ethylene oxide. Chemical sterilization, again, we have to have the same criteria for all the processes.

The chemical sterilizing solutions must not alter the properties of the tissue. They must penetrate the tissues completely, which is the key. But also importantly, they must be removed completely.

It's easy to get stuff in connective tissues. Sometimes it's very difficult to get some of these things out. That's the real key and a challenge that has to be overcome for these chemical sterilization methods.

In some tissues, it's a little bit easier than others, at least I believe. Things like bone, the microstructure of bone, we can see that we have a Haversian system in a cortical bone. These are tubes, if you will, that allow passage of vessels and the Volkmann's canals that go horizontally. So there's a nice system of conduits there. Under pressure, it's been shown that you can really get to all axes of the bone.

When Mother Nature designed this, she designed it in such a way that no bone cell is more than 300 microns from a blood vessel. They don't really have a capillary system in our bones, but the design is such that it's pretty darn close.

So if you can, under pressure or however you want to do it, you can go ahead and reach all these areas. Here it shows very nicely using a classic technique of disulfide blue added to profusion solution allows you to get to all the levels of the bone. That's a real key.

Bone is good. The other tissues, they have a bit more dense cellular matrix. So with things like

tendon, here we have just in our laboratory a static -- most of these are not done statically. They're done actively.

But in our lab, just to show you, give you a ballpark idea here, dye penetration to a tendon after 24 hours of static incubation, most of the tendon, no penetration of the dye. Dye penetration in the meniscus after 72 hours -- that central portion of your meniscus doesn't get anything. Even as we get older, it's very difficult for the nutrition, even by walking on our meniscus, to get to that central area.

That's why in most of us, once you reach 50, 55 years of age, we start to have degeneration here because diffusion into that area is compromised. It must be also compromised, I would imagine, trying to get some of the factors in there from a chemical sterilization point of view.

The techniques are out there. One from LifeNet and we have Lloyd Wolfinbarger in the audience that I'm sure can address specific points about this. But it's a whole six-step process.

All these are not in isolation. There's not

an island here. You have to do all these things. All the companies are involved with this. The bioburden control, bioburden assessment, then you get into the cleaning aspect of it.

There are different techniques. Again, we're not going to spend a lot of time on this because, again, some of these are proprietary solutions that are basically put through a series of steps that allow this solution to get in contact, hopefully, with all the areas of the extracellular matrix.

Then they're terminally sterilized at a very low level like I would imagine just to take care of any potential contamination that would occur from handling these tissues and doing all the different procedures that are there. Again, the in-house testing suggests it has no effect on mechanical properties of the tissue, this particular chemical technique.

BioCleanse from Regeneration Technologies, again, a chemical cocktail, a chemical profusion technique that is there under extreme pressures to drive that into that connective tissue, that extracellular matrix that is so dense. Rinse cycles

with water for injection, very sterile water going through the whole system.

This is also terminally sterilized at about 2.5 mega rads, not the bone-tendon-bone, because remember we said 2 mega rads of radiation is bad for tendons. So they don't do that with their bone-tendon-bone. Again, in-house studies at Regeneration suggest that the process has no effect on mechanical properties of the tissue.

Tutoplast from Tutogen, a system of processing the tissues using, here in this case, hydrogen peroxide, solvent dehydration and terminal sterilization with a gamma radiation, a maximum of about 20 kiloGrays. Again, they're using bone, bone-patellar tendon-bone. In-house testing suggests that this technique does not have any effect on the mechanical properties.

They also have a treatment for prions. The tissues here are treated with one normal solution of sodium hydroxide for one hour. The solutions they use at this, however, are limited to fascia, pericardium and dermis.

We have to remember that this treatment of sodium hydroxide also effects collagen in a time-dependent manner. So the longer that you have to have it around to permeate these particular dense tissues are really going to be deleterious. So it really cannot be used on bone, tendons or ligaments without a significant reduction in the biomaterial properties. So that's a real key here for that.

Finally, NovaSterilis, being used right now in bone-tendon-skin in terms of the experimental use of it right now. They use supercritical CO2 on the dense gaseous space. This gas has excellent penetrating ability.

What happens is that there's a switchover in terms of the formation of carbonic acid. Carbonic acid basically acidifies the environment, thereby killing the microorganisms through this transient acidification. Again, their in-house testing suggests that no apparent effects on the biomechanical properties or biological incorporation of the treated tissues.

Now, there are other methods out there

certainly. We can glean from this some of the tissues that have been used, the xenograft tissues specifically. Parasitic acid, ethanol has been used in the small intestine submucosa from swine that has been used for some of the patches, this ligament augmentation device that is used for rotator cuff and other soft tissue aspects of it. This is very effective against bacteria, spores, viruses and fungi. But it's also very, very, if you will, traumatic to the tissue, some of these soft tissues.

It's been used a long, long time in Europe. It's been recently applied to bone-patellar tendon with no apparent effects on the mechanical properties. There's a couple peer review articles out there that kind of document what they've done to look at this.

Now, in conclusion, kind of maybe talking a little bit now about my perspective – my thoughts also of some of the end users in the sports medicine community.

Most of these techniques have only been applied and validated in bone or bone-tendon constructs as has been alluded to. The mechanical effects of the

procedures have mostly been limited to time zero testing data.

So what you do is you take a process. You maybe take patellar tendon from an individual donor. One you treat, and one you don't. Then you compare what those processes are at zero time, the mechanical properties and perhaps even the biological biochemical constituents of that particular graft.

The problem is is that there have been no -- to at least my knowledge – no standard testing protocols have been established to evaluate both the mechanical and biochemical or biological factors that are involved with these grafts after the sterilization procedures.

Many of the outcomes have been ultimate tensile strength. How strong is this material? That's pretty easy to measure. You can do that pretty easily. But I don't think this is valid. I think we talked about yesterday having a patellar tendon graft that's 2200 Newtons.

Grafts do not fail by large part by rupture. That's very, very rare. Most surgeons will tell you

that the ACL allografts fail by stretching out and the patient has a residual joint instability and then that is deemed a failure. So I think what we really need to be looking at is the viscoelastic properties.

Someone mentioned yesterday, what about the activities of daily living. These are the ones that affect the viscoelastic properties. A graft that stretches out over the course of time can be very, very strong, but if it stretches out over the course of time, this is not what you want from a functional graft.

Testing conditions, again, a varied amount of testing conditions. We've asked in conjunction with the American Orthopedic Society for Sports Medicine to have these companies tell us what they've done and send us their data so we could look at it independently. We've had one taker in the last six weeks. So I think there's a variation there.

We should come to some agreement, I think, in terms of what the environment is. Is it tested at room temperature at 37 degrees in a water bath and not a water bath? All of which have an effect on changing

the mechanical properties.

The strain rate, this is the viscoelastic tissue. How fast you pulled it apart affects what the material look like. Now, obviously, if you design your studies to do the same thing, different control, that's going to be quite similar. But it doesn't allow us to compare them across different processes. So I think that's going to be real key.

Statistical analysis, remember what you're trying to do in essence is to show no difference. So that's pretty easy to do with just a few samples. But that gives you a chance for a Type 2 statistical error. You just don't have enough there.

So what we should be asking for is what is the power of your statistical analysis. Is it that .8 and the statistical significance being equal to or less than .05 as we do in the scientific community? I think that's going to be important.

Also, variables. Age is shown not to be a real factor in terms of ultimate tensile strength. It may be a factor in some of the viscoelastic properties. As we all get older, we tend to get stiffer. That's

just a rule. We lose water. We should be looking at what the effects those are. Maybe we should be having some biomechanical limits on the tissues that have been used.

Also, as was pointed out yesterday, perhaps gender could be an issue, especially as we get onto people in the 50s that are donating tissue that might have some decreased bone mass.

Now, also the effect of sterilization on the biological incorporation of connective tissue has not been rigorously documented. We don't have a lot of pre-clinical studies out there. Some people have, but it's not uniform. We don't have a uniform method to look at this.

What happens when you put it in an animal model? Does it, in fact, remodel in the same time frame in the same way as a fresh or frozen allograft would? I think we need to be looking at that.

Most biological data, in fact, has been gleaned from the clinical use of the material. At our meeting this past summer, one of the tissue banks said we have not heard any negative comments regarding the

thousands of processed allografts that have been implanted. This is very good. I think this is important information to have.

But as I tell my residents and graduate students, absence of evidence is not evidence of absence. We need to look at this perhaps a little closer to find out exactly what's happening to that. Just because we haven't seen any problems doesn't mean problems may not exist if the tissue has some changes in its mechanical or biological properties.

In peer-review publications regarding the safety and efficacy of the various secondary sterilization procedures are in general lacking. In the scientific community, we need to publish to see if our data is valid so other people can test it against their own laboratory techniques and verify it or maybe even challenge us. So I think we need to see more of this. That in general has been lacking.

I'll finish up with other connective tissues. We just talked about bone, bone-patellar tendon-bone. But what about tissues such as menisci and articular cartilage, which were alluded to yesterday?

Menisci can harbor and transmit infectious retrovirus. Jean Nemzek did her graduate work in my laboratory and showed that the meniscus, which is a very minimally cellularized tissue, dense extracellular matrix, has virus in terms of a retrovirus in our systemically infected feline leukemia virus model which is kind of a -- it's a decent model for HIV in terms of the ability of the retrovirus to reproduce and how it behaves. It doesn't produce the same symptoms. That's why we -- or the same disease processes. That's why we use it in the lab. It's safer.

It shows that in the meniscus -- in fact, most of the connective tissues we'll talk about can transmit these viruses because they're in the extracellular matrix.

Sterilization techniques, which adversely affect the collagen architecture of tendons and bone, will also affect the meniscus because it is made up of primarily Type 1 collagen in terms of its extracellular matrix. So it's difficult, again, to do this. It's also very dense. As we said, the dense extracellular nature may inhibit the access of sterilants into the

areas of the meniscus.

There has been one set of data out there that's been published from BioCleanse who've applied this to the meniscus. Lloyd has told me that the Allowash has also been applied to the meniscus and perhaps he can answer questions if you have specific ones at the discussion or the panel area here.

But again, there is some peer-reviewed literature out there that shows that there's no significant biomechanical differences and some wonderful pre-clinical data that exists that shows that there's a normal biological incorporation. These are the type of studies that we need to be seeing.

Finally, articular cartilage. Articular cartilage is a bit different because we cannot process this because the clinical success of articular cartilage allografts has been shown to be related to the transplantation of viable chondrocytes.

From the early work of Henry Menkin and Bill Tomford, in terms of trying to cryopreserve this, to now, the techniques now where we can preserve about 75, 80 percent of the viable chondrocytes for up to about

28 days using different technologies is key because one, it allows for some serological testing to be done and bacteriological testing to be done, but it also is important because viable cells are required to get good results. Doug Jackson in a classic study showed us that the whole amount of success is related to the number of chondrocytes you transplant.

In last month's *Journal of Bone and Joint Surgery*, our lab published a paper that showed that contrary to previous reports, articular cartilage can harbor and transmit infectious retrovirus.

People were assaying articular cartilage from HIV-infected people by looking for proviral DNA. Proviral DNA does not get into the cartilage cells. The cartilage cells themselves are not infected.

What's happening and what you're seeing here is the P27 antigen all throughout the extracellular matrix. It stops at the chondral. It doesn't go inside the cell. What happens is if you transplant this, there's enough of the component in there, the genetic material, that cells can then uptake these parts of the virus and start to replicate this virus.

So I think we have to be careful because, again, we don't have secondary sterilization techniques that can be used on articular cartilage because we want to keep those cells viable. So it underscores the importance and significance of donor screening when considering the use of articular cartilage allografts.

Finally, in summary, there are a variety of these techniques out there that exist for bone and tendon. While classic techniques have been shown to adversely affect the mechanical and biological properties of many of these tissues, when used at the levels needed for viral inactivation, newer sterilization techniques claim to obviate that concern.

Most tests, however, have been done in time-zero specimens and have not been standardized in terms of methodology and outcomes variables. Peer-review publications, the impact of these techniques on host incorporation or remodeling are significantly lacking.

Again, we cannot necessarily apply all these techniques to the meniscus although people are now looking at that. Hopefully, we'll have more data as

the time comes.

The questions still exist. Mary did a wonderful job of alluding to that and perhaps changing the minds of some of these surgeons. But questions still exist within the surgical community regarding the precise sterility assurance level achieved from these sterilization techniques.

This will be something that they would love to see on the label, as well as the efficacy of these techniques in activating or destroying viruses and prions. That is a concern of the sports medicine community.

So the questions I would have is what infectious agents have been used to validate these procedures. I know what some of these are. Mary talked about what has been done for viruses. I think these are things that we need to educate the end consumers because these questions still come up.

Have these agents been spiked on the tissue or has systemically-infected tissue also been tested? I think that’s an important point to make.

What outcome metrics can best characterize

clinically relevant effects of these sterilization procedures on the biomaterial characteristics and the biological incorporation of the connective tissues being processed?

If we could come to some agreement -- we have a lot of standardization for what is safe in terms of sterility. We should maybe focus also on what are some of the outcome metrics that we also all can agree on that are clinically relevant, that may be geared up to look at not only the safety but from the efficacy point of view of these processing.

So I'll leave you with the idea that what more can be done to ensure that when it comes to the transmission of infectious diseases, allografts can be considered completely safe and effective.

I'm a long suffering Cleveland Indians fan. So I had to put this baseball analogy in here. After 53-some years, they're finally getting back into the playoffs. We're going to keep our fingers crossed tonight. For those people in Boston, I'm sorry about that. But we'll cheer for the Indians tonight.

Hopefully, that will give you some idea of

what goes on in connective tissue and what we think, at least from a basic science point of view, and some of the end users in sports medicine feel are clinical concerns that we'd like to see addressed.

Thank you very much.

MS. MALARKEY: I just need to take a second to replace a battery here.

Our next speaker is David Fronk, who's vice president of Regulatory Affairs and Quality Assurance at CryoLife, Incorporated.

David joined CryoLife in 1992 and was appointed to the position of vice president of Regulatory Affairs and Quality Assurance in 2005.

Previous to this position, he served as vice president of clinical research for six years. He has over 20 years of experience within the medical products industry.

Prior to joining CryoLife, he held research and product development positions at Baxter Healthcare Corporation and Zimmer, Incorporated.

He received both his BS in mechanical engineering and his MS in biomedical engineering from

the Ohio State University.

David.

MR. FRONK: Thank you, Mary.

I would also like to thank the organizers, the FDA, the CDC, for putting this symposium together.

I'd also like to give some special thanks to some colleagues of mine at LifeNet: Perry Lang, Lloyd Wolfinbarger, Linda Weiss and Lisa Williams, who were instrumental in helping me put this presentation together.

Let's take a step back before we get started here in looking at disinfection and sterilization of cardiovascular tissue. It's going to take a little different position than what Dr. Arnoczky talked about with regards to musculoskeletal tissue, but I think it's important to take that historic perspective.

Just to give you a little bit of a snapshot, the use of allograft tissues has been going on for about 50 or so years. You can see the first implant is some arterial vascular tissue back in the late 1940s. The first use of cardiac allografts in 1956, this particular allograft was put into the descending aorta.

Back in the early ‘60s is when the first use of valves for orthotopic or implantation backing of the valves took place.

Most of the rest of the presentation, I’m going to be focusing in on cardiac tissue, though it has applicabilities across vascular tissue as well. Most of the literature and most of the research that has been done has really focused on cardiovascular tissue.

I want to go back in time. The philosopher Santayana has a wonderful quote that those who cannot remember the past are condemned to repeat it. Now, the corollary of that is from my wife whose comment is Dave, you idiot, I’ve already told you that.

So I think we need to go back and take a look at what has taken place in the past and kind of contrast that to what we’re going with going forward. We have to always keep that retrospective eye in place.

Now, the beginnings had a very humble start. The use of this tissue was recovered from a morgue, a very unclean environment and very uncontrolled processes and environment. The tissue was placed in a

balanced salt solution. It was stored in refrigeration for up to 16 days. But importantly, no antibiotics were used.

Now, surprisingly, the clinical outcome was phenomenal for this first patient. It was done in a teenage girl 13 years of age. As a follow-up that was reported about 7 to 10 years out, she was doing remarkably well.

But because of the environment and the conditions in which these grafts were prepared, and maybe because there was no antibiotics used, people were concerned about the sterility of this product or this tissue as it was going in. So a fair amount of early work looked at sterilization methods.

I'm not going to go into the absolute definitions of the sterility that they had with regards to this, but they were in essence trying to make this tissue or render it sterile.

As you can see from the list here, a variety of different entities were used, chemical entities like beta propiolactone, gas ethylene oxide. E beam energy was used at 1 and a half to 3 mega rads of radiation.

You heard from Dr. Arnoczky some of the concerns with regards to musculoskeletal tissue. I think there's even greater concerns with cardiovascular tissue, particularly if you have an opportunity to look at a heart valve leaflet, what its infrastructure is like. The impact of radiation can be quite detrimental.

Other chemical entities, formaldehyde, chlorhexidine were also investigated. Most of the published literature talks about the outcomes with beta propiolactone and E beam energy. That's where I'm going to spend the next few minutes, giving you some sense of what type of outcomes took place with those sterilization methodologies.

There was some pre-clinical work done. I can't necessarily say if this was done prior to the implantation of these grafts or if this was done after the first clinical uses were done.

But if you take a look at this, using some coarse mechanical testing of ultimate tensile strength of the aortic wall, not necessarily the aortic leaflet, you do see some significant differences with beta

propiolactone use as compared to E beam energy when it comes to material strength.

From the data that was presented here -- and this was work from Columbia University -- they really showed no time zero difference with E beam energy with respect to ultimate tensile strength of the aortic wall.

From a morphologic standpoint, they did also see differences with beta propiolactone compared to E beam. They did see some swelling within the leaflet and some thickening with the chemical sterilization. That was absent with the use of ionizing radiation.

Well, how did they perform clinically? This is a series of data published from the Mayo Clinic. This gives you a sense of how these grafts performed with respect to a statistic of freedom from re-operation. As you can see, at 10 years out, only 40 percent of the patients did not have to go re-operation for this valve operation. Those results are clearly unacceptable.

The interesting thing -- and it’s probably worth pointing out a little bit here -- is if you take

a look early on, within the first year or two, the actual clinical outcomes are arguably acceptable. 90, 95 percent freedom from re-operation -- I'll show some more data a little bit later on -- is not too bad. So from the early data or if you're only doing clinical evaluations after a year or two, you might get a sense of false hope with regards to some technology.

What did the researchers ultimately conclude with regard to these forms of sterilization? There was a high incidence of graft related death, a high incidence of graft failure. Macroscopically, they did notice that there was cusp rupture within these patients. Microscopically, they did see some damage to the extracellular matrix.

So all in all, this use of sterilizing methodologies fell out of favor in the surgical community. As a matter of fact, if you would talk with surgeons today that happened to be practicing cardiovascular surgery in the '70s, they will give you a very visceral response to the use of some form of sterilization technology for heart valve replacement.

When the time was taking place when these

outcomes were initially coming about, additional methods of preservation of these cardiovascular allografts were also being investigated. The technology and how it was termed, “antibiotic,” quote, unquote, “sterilization” became very commonplace.

The use of sterilization is in quotes here. When you look at the literature and they talk about their outcomes, they would talk about that 92 percent of their valves were actually deemed sterile after it underwent this process. So I think clearly that would not fall into something that we would want to call sterile or sterilization.

When you look at it, antibiotic sterilization or antibiotic treatment, it’s really treatment with a tissue with a variety of antimicrobial agents. As you can see, various methods were used in terms of treating this tissue. The range of incubation and antibiotics ranged from 6 to 24 hours as well as the temperature in which the antibiotics were incubated in ranged from refrigeration to body temperature.

There’s a confounding factor in all of the data from this early time period with regards to how

the tissues were stored after their disinfection in antibiotics. Some of the grafts were stored at 4 degrees C for a period of up to 6 weeks. Other tissues were cryopreserved.

Let's take a look for a second at what these outcomes are. If in your mind's eye, you can remember what the freedom from re-operation data looked like for the beta propiolactone and E beam sterilized grafts, contrast that with what we're seeing here for the 4 degrees C and cryopreserved antibiotic disinfected grafts. We see a significant difference.

At 10 years, you're looking at an 80 percent freedom from re-operation. That's the data over here. Then if we take a look at freedom from structural valve deterioration, again at 10 years, we're seeing very good clinical outcomes.

Just for a point of reference, freedom from structural valve deterioration really is a hemodynamic function variable. It takes into consideration if the grafts are exhibiting some form of regurgitation or allowing backflow of blood back into the heart or stenotic events, the valve is becoming obstructive.

I think there's one additional point that is worth bringing out with regards to this data. You do see some difference in freedom from structural valve deterioration between the 4 degrees C and the cryopreserved grafts at 10 years, no difference from freedom from re-operation.

But when this data was carried out a little bit further and you take a look at the outcomes at 16 years, you see a definitive widening of the outcomes between those two storage methodologies. I think that's an underlying issue. It's not the premise of this meeting or this presentation, but I do think we need to be mindful of the storage aspects of grafts as well, and what impact they might have on clinical outcomes.

Where are we today? I think when you take a look at the data from the past, we as a processing industry view the method that provides the best outcomes to patients, best outcomes to the surgeons, are antibiotic or antimicrobial disinfection methods. That is uniformly employed throughout the four processors that do process cardiovascular tissue. In

addition to that, we also uniformly use a method of storage of cryopreservation.

What is our objective with processing cardiovascular tissue in light of the fact that sterilization technologies, at least historically, have proven not to be very successful?

You heard from the surgeons yesterday of the expectation that the tissue is free from contamination when they get it. I don't think that's fair for what we do here with cardiovascular tissue. I think there is a presumption of free from contamination implies sterile. If it is free from contamination, then there should not be organisms present. We've heard that from Mary's presentation this morning.

I think a more appropriate goal that we should be shooting for with regards to disinfected tissue is an assurance that the tissue is reasonably free from contamination. So what do I mean by "reasonably free from contamination"?

We want to put in safeguards as best as possible to say that when we release that tissue, to the best of our abilities, that that tissue doesn't

have organisms on it, but it is not sterile, therefore there is the risk that it very well may.

So how do we go about meeting this objective? There's a variety of different things that we do as processors to try to minimize that risk. It's a whole issue of mitigating the risk and trying to remain with a graft that will provide good clinical outcomes. I'm going to just spend the next few minutes talking about these five different areas.

Selection of the antimicrobials. Across the industry we use a very common set of antibiotics. There is an occasional -- there is a processor that does use antifungal agents as well. They're broad based antibiotics that affect both gram negative and gram positive organisms. Again, the use of antifungals obviously would be addressing any fungal contamination that might be present within the tissue.

Well, how -- that's great. We've picked these antimicrobial agents. What does that mean? It's clearly important if we're going to disinfect this tissue to ensure that it actually does what we want it to do, and that is to kill the organisms that might be

present.

This is data from two different processors, their kill curves that they have generated for their antimicrobial or antibiotic disinfection solutions. Interestingly, the different processors also have different incubation temperatures in which they decontaminate the tissue, ranging anywhere from refrigeration to 25 degrees C as opposed to a 37 degree or body temperature in evaluation.

I think it’s critical that we do these kill curve analyses. There’s a standard USP panel of organisms that are traditionally used. We as processors typically will look at organisms that are also within our environment or common organisms that are in our environment and also throw those organisms against the decontamination solution.

There’s also some work in looking at some pathogenic organisms that might be of particular difficulty in comparing and seeing how well your disinfection regimen works with regard to clearing these organisms. I’m going to have a little bit more to say about that a little bit later on. But again,

it’s important to know what your process can do and what it can’t.

We’ve heard a lot about this yesterday with regards to microbial detection testing. When you look at the overall process flow, it is absolutely imperative to do some form of testing, particularly if we’re not going to be terminally sterilizing these grafts. We need to know what is on our tissue prior to our decontamination step as well as post-decontamination step. There were beautiful presentations yesterday talking about that. I don’t want to spend any more time with that, but it is critical for us to know what we have going into our system, what happens afterwards. I have to acknowledge that really from a post-antimicrobial detection standpoint, we’re really interested in mostly presence or absence. There is -- as Martell was talking about -- a very good scientific reason to understand what is there and how much of it is there.

I do think that is important. But clearly, from a clinical perspective, we want to ensure that there is at least absence of organisms or if there is

presence, that those tissues are subsequently discarded.

There were many discussions yesterday with regards to how these microbial detection tests are done. I don't want to spend much time on it other than the fact that almost all of those that were discussed yesterday are employed whether that is swabs, fluid extraction or rinsate work or a maceration or destructive testing methodology.

What's the next step that we used to help minimize this risk? There are plenty of standards in place. I'm not going to go and spend a tremendous amount of time. Scott did a very nice job yesterday. We do have AATB standards to comply with. Again, these standards help to mitigate the risk, particularly with this disinfected tissue.

As you can see here, the requirement from the AATB is that we do have a list of organisms that we will discard tissue when those organisms are detected. Fungi, clostridial species, streptococcus pyogenes, Group A strep are three such organisms. Each of us processors also have an independent list that we work

with that that will also necessitate discard of the tissue.

AATB standards to require pre-sterilization or pre-disinfection culture results to be done. Again, a series of organisms that when detected require discard of the tissue and then post-processing, or final, or pre-packaging results are also required.

Now that we've done all of that and we have our tissue put into a bag, we've done our testing and we find that it is free from contamination as of detectable contamination, what do we do with regards to labeling? There are AATB requirements for labeling. But what additional should be put on the tissue? Should we label it non-sterile? Should we label it aseptically processed? That's with quotes from Mary's presentation.

I think it is important in distinguishing what Mary discussed in terms off what is traditionally or conventionally reviewed as aseptically processed compared to what we do within this industry.

Do we need to talk about the antimicrobial treatments that we use? I think these are all

important factors. I think the various processors do to some degree put information like this.

I'm going to throw one additional statement that maybe we need to be putting in our labeling. That is some form of warning statement. Do we need to talk about that this tissue is only disinfected, therefore there is the potential that contamination might be present?

Clearly, we know from our viral studies that we do, and the fact that we do testing and screening but we do not have viral clearance steps, that there is the potential presence of virus within this tissue. Again, should we be warning the surgeon who then has the obligation to warn the patient that that is the case with regards to this? From a products liability standpoint, I will give a plug based on our attorneys, and the answer to that question is absolutely yes.

What other additional points should we give consideration to with regards to disinfecting of this cardiovascular tissue? Again, it all centers around organisms and our disinfection steps and methods that we use.

Should we be looking at accept/reject criteria based on our AMS or antimicrobial or antibiotic cocktails based on organisms in which we know the cocktail has limited effectiveness? We don't have perfect detection methods. This is all sampling. Everything we do has some level of success, has some level of error. They're all additive throughout the process.

So if we don't have perfect detection methods, should we be eliminating tissue from the processing which we know bugs were detected in which we have limited antimicrobial effectiveness on?

Likewise, if we are doing re-numeration of our microbial detections, we do counts. If we get excessively high bioburden on our tissue, whether it's an in-process or an incoming bioburden, should we be eliminating this tissue from the field just because of gross levels of contamination? All food for thought, I don't think there's any consensus within the industry how we do this. But they do allow us to give further discussions to try to assure that that tissue is as safe as possible acknowledging that we cannot

terminally sterilize it.

Well, that's where we are today. But what does it mean for us going forward? You heard from Dr. Arnoczky's presentation, there are other methods of sterilization out there from musculoskeletal tissue. I know the various processors and various researchers are looking at its applicability to cardiovascular tissue.

So what should we be doing? Should we decide how to modify how we process our tissue, modify how we disinfect it, or take that leap of faith in how we go about sterilizing the tissue?

I think we've got a variety of different assays that we can look at should we choose to undergo some changes to our processing methods, some laboratory pre-clinical testing that can be done, some animal testing. I think ultimately we do need to look at the human model. Clinical testing, as we saw from some of the early data, is very important and is going to be the ultimate prediction.

Biomechanically, what can be done? Very common biomechanics tests, I have not included here. It is to Dr. Arnoczky's point that we do need to look

at the viscoelastic effects of the tissue. Valves are opening and closing once a second, so they are obviously undergoing some form of cyclic loading. So it is important to look at that as well.

Clearly, what I've listed here is a lot of ultimate failure properties. I think they are good coarse measurements if you've done anything significantly wrong with or detrimental to the tissue.

There are other means of hydrodynamic performance; putting the tissue in some form of cardiac simulation model to see how well the valve opens and closes, if it has levels of regurgitation and the like, and ultimately, you can consider doing accelerated wear testing. These are standard panels of testing that our brethren in the mechanical and bioprosthetic heart valve device companies do when they assess their valves.

What other methods are used from a pre-clinical assessment? Collagenase digestion is an assay that can be done to look into what impact the process might have had on the collagen ultrastructure. Collagenases are used to digest the links that bind the

collagen together.

If you subject tissue to these collagenases and then assess how much collagen is present or where the weight of the tissue free, in post-treatment you can get some sense of how well that tissue has been maintained to its native level or control value with the process. The more you cross-link the tissue, the more you toughen it up as you do with a glutaryl treatment or the like, the greater the amount of collagen material that will be left afterwards.

Likewise, if you do something that is significantly damaging to the collagen structure, you're going to see a significant reduction in the volume or the weight of the tissue post-treatment. Again, it's a good coarse assay to use. It's really beneficial for weeding anything gross that might be affecting the properties or the structure integrity of the tissue.

A corollary testing to that is thermal denaturation, is looking at a heat impact on the collagen structure. Again, the higher the temperature that is used to digest the tissue or disassociate the

tissue or denature the tissue, the higher level the collagen cross-links are. The lower the temperature, the more likely that you've done something detrimental and weakened the tissue's ultrastructure.

So again, some nice assays that can be used to give some coarse assessment of how the processing method has affected the structure of your tissue.

Clearly, you have animal testing applicability. The standard models of replacement heart valves is a weanling sheep model. That is predominantly used to assess calcification. It has been proposed that primate models may be a better model to use to look at an inflammatory response or an immune reaction to the tissue. That's all up in the air.

I do think it gives you some level of data to assess your tissue. It does require control testing. These are very expensive. Obviously, the next step would be how well these to work clinically.

To summarize, I just kind of want to give you my general take of where we're at with regards to this. I think the data clearly shows that the sterilization methods that were used historically had poor clinical

outcomes. So it's something that we want to have our eyes wide open should we investigate sterilization methodologies going forward.

Clearly, over the last three or so decades, disinfection methods are the method of choice and have provided very good clinical outcomes. I think what we saw from the data from the E beam and the beta propiolactone use, that early clinical results may not be predictive out of late clinical outcomes. I think some of the pre-clinical testing is also -- may or may not be predictive of the clinical outcomes. So we need to be mindful of that.

Really to emphasize the point, here's the data again looking at cryopreserved disinfected or antibiotic disinfected tissue with regards to freedom from re-operation compared to those other sterilization methods. Again, when you look at the one- and two-year data, you don't see a whole lot of difference. It could give you a false sense of hope.

So should we undergo things like this, I think we have to be very mindful of this data and consider that longer-term follow-up may be requisite in

order to assess these types of technologies.

Lastly, I want to leave you with a little bit. We're talking a lot here about disinfection sterilization of these tissues. In the heart valve world, the outcome of a graft or a replacement valve device that has organisms present on it or colonizes organisms is endocarditis. It is well understood that even sterile tissue, mechanical valves that would probably go an SAL of 10 to the minus 6 or a bioprosthetic valve that would have an SAL of a 10 to the minus 3 has some level of early onset prosthetic valve endocarditis.

There is no presumption that these grafts were contaminated when they were put in. This is just an inevitability with doing valve replacement surgery. You can get a sense of what the percentage incidence of early prosthetic valve endocarditis for both these types of valves.

Looking at it a little differently, this is some data from New Zealand in comparing the relative risk with regards to incidence or prosthetic valve endocarditis, comparing mechanical or bioprosthetic

valves to allograft heart valves and, clearly, you see in that early post-operative period, despite the facts that these grafts are sterile, they do have a higher incidence of developing prosthetic valve endocarditis.

Clearly, the allograft tissue has been show to be relatively free from acquiring prosthetic valve endocarditis. So I think it's important when we look at the -- trying to come up with a way to have tissue that absolutely has no contaminants on it to begin with, that we're still going to have issues associated with prosthetic valve endocarditis. It's not going to eliminate that potential adverse consequence.

I think in final summation, we do need to look at risk/benefit. Dr. Arnoczky talked about that with regards to musculoskeletal tissue. I think we clearly need to look at that from cardiovascular tissue, maybe even more so with regards to this. This is life-saving surgical procedures. The last thing a patient wants is to have to undergo re-operation or have some significant adverse mechanical effect of their valve replacement.

So with that, I thank you very much for the

opportunity to present.

MS. MALARKEY: Our next speaker is Joel Osborne. Joel is currently the vice president of quality assurance and regulatory affairs at the Musculoskeletal Transplant Foundation. He's responsible for maintaining worldwide regulatory compliance in accordance with applicable governmental regulations and industry standards.

Prior to joining MTF, Mr. Osborne held several positions with the American Red Cross, including manager of quality assurance, American Red Cross tissue services at HQ, director of tissue services and assistant laboratory director for the American Red Cross blood service located in Springfield, Missouri.

Mr. Osborne has worked in the field of tissue banking and blood banking for more than 25 years. He received a BA degree in medical technology and is a certified tissue bank specialist through the AATB. He's a member of the AATB and the American Society for Quality, the American Society for Testing and Materials, and the Regulatory Affairs Professionals

Society.

So, welcome, Joel.

MR. OSBORNE: You may ask yourself why a guy from the Musculoskeletal Transplant Foundation is speaking on skin. Several years ago, we had the opportunity to acquire the American Red Cross tissue services. As part of that, we acquired a facility out in Costa Mesa that was processing heart for valves as well as skin for burn.

It was something that prior to the acquisition of that facility -- the Red Cross made a voluntary decision to shut down operations approximately 6 months before we acquired. The reason for that was because of an FDA warning letter that was regarding validation work, which they had done that they received a warning letter on. As such, they decided to shut down that facility. When we acquired that facility, I was charged with the opportunity for revalidating that system. It was a big challenge. It took us about a year and a half. We opened the operation.

We called the FDA district office, told them

that we were ready to start processing and distribution. We had one of the investigators come out. He reviewed all of our data, which I was very proud of. We spent the next couple of weeks discussing why isn't skin sterile. It was something that it was an educational experience for the investigator, but it was something that was a misunderstanding when it comes to this industry.

Now, the next three slides are not on our handout, so don't be looking for them.

This is a quote that is about as old as I am. It comes from a *Lancet* article in 1972. I'm just kidding about my age. I'm a little bit older than that. But it is, I think, very important to read this quote. It goes over these next three slides.

Although sterility is in theory an absolute term, in practice it may only be regarded at best relative and at worst misleading. It's a philosophical concept that can never be unequivocal in a real world.

Experience has shown that it's virtually impossible, even if it's honest, to change the definition of a term that has been in use for many

years. We may need a new term to indicate -- listen to this – “the state of having been sufficiently freed from microorganisms to be deemed safe in some special purpose by some competent body.” I termed the phrase virtually sterile. It’s something that I do think we need to really define as part of what we’re doing.

The abandonment of the term sterility and the acceptance of some other term would remove confusion and enable an important manner of providing microbiologically safe medical products to be more rationally and realistically considered. Now remember, this was written in 1972. This was not written today.

Well, the purpose of what I’m about to present today is really a -- what I tried to do is I tried to poll some of the processors out there of skin. It is something that in the skin banking world -- there is kind of a unity that -- most skin bankers are willing to share information on processes. There’s not a lot of proprietary information out there about skin processing.

This is what I call my lasagna slide. Just for terminology, you have the epidermis layer, the

dermis layer, which is in the middle, and the hypodermis, or the fat layer, which is a kind word for fat.

What we're going to be talking about today is split thickness skin, which is just that. It's the epidermis layer. It's part of the dermis layer. Then full thickness skin, which is the epidermis and the full dermis layer.

Now, something about tissue recovery. In this case, this is a dermatome recovery. It's done in a aseptic fashion. The skin is prepped very similar to a surgical procedure. Then you have a dermatome, which is a big razor blade. Those of you who have never seen this before, beauty is only skin deep.

This is an example of unprocessed split thickness allograft. You can see how thin it is. When you hold it up to the light, you can actually almost see through it. On the other hand, you have full thickness. It is something that when this is obtained, it can be obtained using a dermatome. It can also be obtained using what's known as freehand dissection, which is just a surgical scalpel procedure. It's

something that can be recovered on both living and deceased donors. Now, I'll get into the living donor issue in just a second. But as you can see, we can recover much larger sections of skin.

Living donors is something that is very new to the skin recovery. It is something that patients undergoing removal of excess skin after significant weight loss can actually donate that skin. That happens to be something you have a very dedicated donor there. It's something that they're very amazed to find out that they can donate something that would otherwise be unusable. It's something that with our program, at least, we're trying to look at these donors as a living donor. They meet all the FDA requirements. They undergo the medical history, all the testing, everything that's necessary.

I won't spend much time on the slides going forward. These few slides deal with the use of skin that was really covered by Dr. Kagan very eloquently yesterday.

I will say that there is a difference between the dermis skin and the split thickness skin in the

fact that the full thickness skin is used for dermis. This is a relatively new procedure. Dr. Kagan mentioned this yesterday. It's something that is implanted.

The differences that you have between the dermis skin and the split thickness skin for burn are pretty significant because split thickness skin for burn is used as a temporary covering whereas dermis is used as an implantable.

Now I'm not going to cover all this. This was covered by different speakers. I will say that the AATB standards has pretty significant standards dealing with skin. What they don't have are standards dealing specifically with dermis. That's something that we need to take a look at.

This is a graphic of the differences between split thickness skin that is used for burn and dermis skin. Usually with recovery, again, split thickness skin can be recovered with a dermatome, whereas the dermis is actually recovered using either freehand dissection or a dermatome.

The differences in processing, the split

thickness skin is usually processed using antibiotics. You're trying to maintain the cell viability and the matrix whereas with dermal skin, it goes through a chemical disinfection.

Preservation. Dr. Kagan talked about the use of fresh skin for burns as well as cryopreserved skin. In the case of dermis tissue, the skin can be lyophilized or refrigerated.

The properties here again are very important. With skin for burn, we're trying to actually maintain the cell matrix, whereas with the dermis skin, we're actually trying to decellularize the matrix and preserve the matrix. Again, the application for split thickness skin is a topical dressing, whereas the dermis is actually implanted into the surgical site. There is a difference, as I mentioned with the disinfection procedures. A lot of speakers have talked about antibiotics versus chemical disinfection. I will say that it's something that is with split thickness skin; again, we're trying to maintain the cell viability, so we use antibiotics there. With the dermal skin, here again, we're trying to maintain the

actual matrix of the skin, not the cells, so that we generally use a very harsh process.

Labeling, both for split thickness skin and dermis are generally not labeled as sterile. The processing of split thickness skin is just a picture of usually we're using a certified clean room with laminar flow hoods. Generally, with split thickness skin, we're trying to keep the skin to maintain the cells at a low temp, 2 to 8 degrees. The processing of skin, again, we're trying to maintain that environment so we don't cross-contaminate the skin.

We do have pre-processing skin cultures. I'll talk about that a little bit later. But it is something during the processing, we've got various rinses and soaks that the tissue goes through. It's usually balanced salt solutions, isotonic solutions. The grafts are actually transferred to the antibiotic solution. All this is done at 2 to 8 degrees centigrade.

This is just another example of some of the processing that is done. In this case, it's a manual method. Most skin banks have not gone to automated

methods for the processing of skin.

Packaging is very simple. It is something that -- all skin is packaged within the hood. The tissue is not labeled sterile. We usually package in half or 1 square foot sections. Generally, skin, as far as MTF is concerned, we validate it to their expiration date on the packaging.

This is just an example of the final packaging that is used.

When the skin is generally control rate frozen, we're using a 1 degree centigrade per minute type of cryopreservation. It's something that we do not -- and I don't think any other skin processor out there actually has data on cell viability. It is something that here again may need to be looked at in the future.

The skin is stored at minus 70 degrees. We transport to hospitals on dry ice. We're trying to maintain the cell viability. That's why we cryopreserve it. The literature's pretty clear, as Dr. Kagan mentioned, for viable skin. We're trying to maintain that skin viability, so when it's used, you have a way

of actually preparing the bed for future grafting.

Again, we're using cold temperatures. With fresh skin, which Dr. Kagan mentioned yesterday, there's usually an expiration date of anywhere between 10 and 14 days after process.

The processing of dermis, it's very similar to the processing of split thickness skin. Again, the recovery is done in an aseptic fashion. When it's recovered, it's brought back. We do a preliminary process on that. The skin is frozen. We actually wait for the skin to be cleared by the medical director until we actually start processing it.

The skin is soaked in hypertonic detergent solutions followed by a series of rinses. There is a chemical disinfection that we use as part of the process. That's peracetic acid. We then package the dermis in a sterile foil tie-back package.

The processing of dermis is, as I said, a little bit more automated than the processing of split thickness skin. In the case of the dermis, we're actually disinfecting in a closed system. It's placed into a stainless steel vessel.

At MTF, we have a semi-automated process. This is a Wisconsin milker machine that we've developed. It is something that, as I said, the systems are closed. The series of disinfection solutions as well as the rinses are done in this closed system.

Packaging is done in a laminar flow hood. There's representative samples taken. At this point, they're packaged. We do not cryopreserve this skin. This skin is either freeze dried or it can be packaged for room temp storage.

The microbiological testing, there's been a lot of discussion about the difference in samplings that are done. In our case, we're sampling recovered skin as well as the transport solution from the recovery. Post-processing, we do a final rinse sample as well as a sampling of the representative sample of the processed skin during packaging.

Validation, the next speaker will be talking about. But these are lists of the various studies that we've performed. Obviously, bioburden equipment validation, package validation, the test method

validation, this is important because a lot of the speakers have talked about BNF studies. With skin, because of the solutions that we're using, the skins really permeate into the tissues so that you have to be very, very careful as far as the testing to make sure that you have no interference with the chemicals that you're using, the process or the antibiotics.

Residual studies are also important because -- and biocompatibility studies, because, again, those solutions permeate into the tissues.

We do stability studies on the tissues at packaging. We do physical testing -- and this is really on the dermis -- for suture pullout, primarily. We do a hundred percent destructive testing of tissues from -- usually it's up to somewhere in the neighborhood of 20 batches of tissue that we actually take through and destroy. We destructively test to validate the system.

Then we're also doing environmental testing studies and clean validations when we do validated process.

Now, with microbiological reduction with

skin, as you can imagine, skin is very dirty going into the process. It is something where we have -- when we develop a process, we're trying to show what the process capabilities we have. Normally, this is a spiking study. It's something that we try to spike to at least 10 to the 6 CFUs of challenge organisms. This is something that's pretty standard now within the industry. The challenge organisms used in our studies are representative of different types of groups of organisms that are considered pathogenic or highly virulent.

Inoculated tissue sections are exposed to all of the disinfection solutions. This is done at a one-half normal exposure time. This is to demonstrate a worst-case scenario. Two tissue sections are tested for each organism. You use some sample for pre-disinfection and then one sample post-disinfection.

This is just an example, the half cycle study that we did. This was on one of our dermis processes. You can see it's a fairly robust process with significant log reduction.

One thing I do want to mention is that using

mixtures of organisms, there is a kind of a symbiotic type of situation you run into if you're just testing one organism at a time. So it's very important with any type of reduction studies that you're using mixtures of organisms.

For those of you who don't know the clinical use of dermis tissue, this is a very good example of how dermis can be used. This was a nationally televised story that you might remember. It was back in September of 2006 or August 2006.

They were 4-year-old conjoined twins. They were joined at the torso. The procedure was done at the Primary Children's Medical Center in Utah. It was a 26-hour surgery to separate and reconstruct and over 50 nurses and surgeons participated.

In this case, MTF actually supplied allograft dermis for this procedure. There were 14 pieces of dermis that were actually implanted to cover the organs and replace all the soft tissue.

These are the two girls several weeks after the surgery. So this is a very good example of how the use of these types of tissues can actually impact

somebody's life.

The next two slides are slides that I -- are not a part of your handout. But it is something that I've talked with a few folks, and I think from this meeting, at least from an AATB perspective, what we'd like to do is develop a focus group. This should be made up of industry experts and stakeholders.

What I think needs to be done is that we need to review the standards related to validation of aseptic processing and terminal sterilization, specifically with a lot of the information that was presented today. I think we need to revise those standards dealing with critical issues about aseptic processing and terminal sterilization.

We need to develop guidance within AATB that is specific for tissue types, processes, as well as pre- and post-process sample procedures. We also need to develop guidance related to labeling. This is an initiative that I'm the vice chair of the standards committee. I will try to move this initiative through.

The takeaways from this workshop, at least for me, one size of standards and regulation or

guidance doesn't fit all tissues. You must have a clear distinction between tissues that cannot be terminally sterilized and tissues that can be terminally sterilized. The sampling methods will differ between those tissues that are aseptically processed and only those tissues that can be terminally sterilized.

So I think we need to focus on that because that is an area of confusion not only for our industry but for the users of tissue out there.

Thank you.

MS. MALARKEY: Well, according to my schedule, it's break time, so I guess we will go ahead and take a break. We're supposed to reconvene at 11:15, but we're a little early. So I think if I say let's get back by 11:05, we should start on time at 11:15. So enjoy your break.

(A recess was taken.)

MS. MALARKEY: All right. Well, maybe we could go ahead and get started. As I predicted, it's just about 11:15, so we remain on schedule.

Before I introduce the next speaker, a couple

of items. You may have noticed that Martell presented some slides that weren't in his package yesterday. Those slides are available up at the counter on your way out.

They will also be e-mailed. That is, his whole presentation will be e-mailed to everyone as will Joyce's because it's not available here today. So everyone will get that by e-mail.

So our next speaker is Joyce Hansen. She is currently the president of J. M. Hansen & Associates, which is a consulting firm formed to enable manufacturing companies to have more control of their use of contract sterilization and/or provide guidance to support optimized use of internal sterilization and laboratory services.

Ms. Hansen has more than 28 years of industrial sterilization experience. She most recently held the position of vice president of Sterility Assurance for Baxter Healthcare and Sterilization Core Competency Champion.

She's held numerous other positions in the industry. She's currently the convener of ISO TC198

Working Group 2 on radiation sterilization, past co-chair of AAMI radiation sterilization working group, and co-chair of the AAMI sterility assurance level working group.

She has many other honors, including she's the winner of the 2001 International Meeting on Radiation Processing Award for outstanding contributions to radiation processing.

So, Joyce.

MS. HANSEN: Thank you, Mary.

Good morning, everyone. Just trying to make sure I know where the slides are, how to move this here.

All right. What I was asked to talk about today has to do with disinfection and sterilization of tissues. There were a couple of questions that I was given to answer with my presentation. I'm going to answer a couple of these altogether.

The first are how are disinfection and sterilization processes validated. The second is what are the challenges and concerns in process validation. The third is what are the expectations and what is

industry's experience. Now, all three of those questions are going to actually be addressed together as I go through the process here. Then the last is how does process validation differ as it determines sterility and viral inactivation.

What I thought I'd do is set the stage really with regards to what kind of documents might be out there discussing disinfection. There is an AAMI document, ST58, that was published in 2005 that deals with chemical sterilization and high-level disinfection in healthcare facilities.

Now, this is meant for manufacturers of products that are actually supplying products to the healthcare facility. This is not specifically addressing how products will be addressed in the hospital environment.

One of the first things that I should point out in this is under the scope session there's a general comment section. One of the statements in there that I thought I would bring to your attention is that processes that use liquid chemical sterilants in high-level disinfectants and gaseous sterilization

processes are validated by different methods. Therefore, they do not provide the same level of sterility assurance.

Another thing that I wanted to point out from this document is actually in Annex A, which deals with microbial lethality, materials compatibility and toxicity. In that, under the microbial lethality section, it actually says that the FDA recommends that processing by chemical sterilization or high-level disinfection be limited to critical devices that are heat sensitive and incompatible with other sterilization processes.

There's actually a reference in that document to the FDA guidance on the content and format of pre-market modifications, submissions for liquid, chemical sterilants, and high-level disinfectants that was published in January of 2000.

So there is recognition in the standards that we have in the industry today when we're dealing with primarily medical devices, that we do recognize that it is recommended to go for terminal sterilization. You would only not use terminal sterilization if they are

not compatible with terminal sterilization.

When I thought about talking about the validation for disinfection and sterilization, really, I thought about what are the differences between the two methodologies to help understand why these might be different from a validation standpoint.

One of the first things that you recognize when you think about disinfection and sterilization is the difference in the packaging technique. You heard several individuals talk about how disinfection was used when packaging occurred versus terminal sterilization.

When you think about disinfection, typically, products are packaged following exposure to the disinfection process versus terminal sterilization, where products are packaged prior to the sterilization process.

When we think about sterilization, we think about it as terminal sterilization because the products are in their final finished form, final packaging that is then exposed to the sterilization process and sterility over the product shelf life is maintained by

the integrity of the package itself versus disinfection, whereby you have the process occur and then there's an aseptic handling and then the packaging. So this is the primary difference and the primary difference with regards to how you handle product throughout the process.

The second difference that we think is critical when thinking about different validation techniques has to be with the microbial lethality that is occurring with the processes.

The first is that with disinfection, it may not exhibit what we call log linearity kinetics. The shape of the survivor curve may vary depending on the formulation of the disinfectant, the chemical nature and the stability of the disinfectant over time and over its use versus terminal sterilization, where we are looking at sterilization methods that have proven to be log linear in activation. This information can then be used to extrapolate, to demonstrate the achievement of a sterility assurance level.

Typically, when we think about this and we think about application of the validation method, we

look at disinfection typically demonstrating something called spore log reductions or log reductions of the organisms that you might intentionally put on the product and look to see how many of those organisms are killed in the disinfection process, whereas with sterilization because we have demonstrated log linear inactivation, we do use that linear inactivation to demonstrate a sterility assurance level.

Now, Mary talked about what sterility assurance levels we typically demonstrate earlier. So I'm not going to go into that at all.

However, what I wanted to talk about was the difference between a spore log reduction and a sterility assurance level. There's a lot of confusion sometimes when I talk to people as to what does this mean. All right?

When I think about inoculating a product with a number of organisms, you can inoculate a product with a variety of a number of organisms anywhere from let's say 10 to the 3, 10 to the 6 organisms. Then you expose the product to the process. You look to see what number of log reductions you saw.

So let's say you started with a population of 1 times 10 to the 6 or 1,000,000 organisms. You saw something greater than 6 spore log reductions as we saw in Joel's presentation earlier. What he's demonstrating is that during that process, we've inoculated a certain number of organisms and we see a number of log reductions. Typically, you can only demonstrate when you have zero positives or zero growth at the end of that that you have achieved equal to or greater than that number of spore log reductions.

A typical experiment for spore log reduction might be to inoculate the item with a known number of spores or organisms, depending on the challenge process, expose that inoculated item to a defined process, and determine then the number of surviving spores or organisms after exposure and calculate the SLR. It's pretty straightforward.

An example of that might be where if you were to inoculate a product item with 2.5 times 10 to the 6 spores, expose to the full disinfectant process and conduct a quantitative test to determine the number of surviving organisms. If the number of organisms

surviving equal 2.5 times 10 to the 3, then all we saw was a spore log reduction of 3. This testing is typically conducted following full exposure to a defined process.

When I started putting this presentation together and trying to contrast between spore log reductions and SALs, and where we are with disinfection processes versus terminal sterilization, it's very difficult to talk and say definitively there's one way for one process and one way for another process because there are multiple ways to address validation of your sterilization and disinfection process.

What I'm actually starting to see -- and I think Martell and I have talked about this -- is that you actually start to see people move into calculating a sterility assurance level with disinfection processes. So what I just talked about, which was a spore log reduction, this is really the traditional. This is what's been done in the past.

What we're starting to see from an industry standpoint is that even with disinfection processes, we're starting to see people look at spore log

reduction going to sterility assurance levels.

When you think about a sterility assurance level test or experiment, you might think about conducting experiments to define the relationship of the amount of exposure to the sterilant or disinfectant to the inactivation of the microbial challenge population.

For terminal sterilization processes such as moist heat, ethylene oxide and radiation, microbial inactivation has been characterized by a log linear relationship for many years. Therefore, in other words, we have defined an increment of exposure to the sterilization process to deliver 90 percent reduction of the organisms to be considered what we would call a D value and that we can use to predict the sterility assurance levels.

When you think about how that is done, how the testing is done, sterility level assurance level, typical experiments that were done originally on sterility assurance levels were to inoculate an item with a known number of spores.

Again, that’s typical of what you saw with

the SLRs or potentially you could determine the bioburden. That was something that was suggested earlier, which is to look at the known population on the product itself. You then expose the items to several increments of the defined process, not the full sterilization or full disinfection process but several increments along the way.

With that, to then determine the number of surviving organisms following exposure to each increment either in something that we call quantitative or fraction negative testing. Quantitative meaning that we go back and count the number of organisms that are remaining following each exposure, or we do something called fraction negative testing, where we're looking for placing the product that has been inoculated and exposed to equal increments of the sterilization process.

We then conduct a test of sterility to determine how many positives or negatives we had. With that, we can conduct a calculation that would be like a most probable number calculation to determine the resistance of those organisms to the sterilization

process.

One of the first things we're attempting to do is to demonstrate the log linear relationship and to calculate the D value itself. Okay? So the important thing is the demonstration of the log linear relationship, especially when we're talking about disinfection.

The second thing is then to understand what that resistance is and to calculate or determine the sterilization process to provide the desired SAL, whether it's 10 to the minus 3, 10 to the minus 4, 10 to the minus 5, or 10 to the minus 6, and conduct a validation of the sterilization process.

Again, one of the things I wanted to point out with regards to sterilization processes and what I'm also starting to see with disinfection is that this information is typically being done during what we call cycle development.

It's way before validation. It's really to gain an understanding of the sterilization or disinfection process and its kinetics with regards to how well or how poorly it is capable of killing the

organisms that exist on the product.

When we think about log linear and sterility assurance level and we think about the organisms that we might put on the product, let’s say we start with a 10 to the 6 level of organisms, we’re looking to understand what the quantitative or qualitative response might be.

If you start with 10 to the 6 organisms and you’re testing for quantitative, you’re going to be testing in the region whereby we’re looking for somewhere less than 10 to the 6 to down to, let’s say, 10 to the zero.

So in this region here of the curve is where we can quantitatively count the number of organisms. The region when we’re talking about fraction negative, most probable number calculations of resistances, is we’re talking in the region where we have, let’s say, 10 to the 1 to down to 10 to the minus 2 or 10 to the minus 3 actually depending on the sample size that you use.

I would really just give you a comment. When you think about the quantitative part of the curve

versus the fraction negative part of the curve, to get a better understanding, you actually have to have a bit of both. The reason for that is because what happens in this part of the curve may be different than what happens in this other fraction negative part of the curve.

So I would challenge anyone who's doing disinfection and looking to move into sterility assurance level assessment to really look at both of those parts of the curve and make sure that they have a consistent log linear inactivation.

From that information, then you can actually determine to go from 10 to the minus 2, 10 to the minus 1, and with your log linear understanding to then predict the sterilization process that would be needed to go down to a 10 to the minus 6.

Another difference between disinfection and sterilization really has to do with the accessibility of the microorganisms to the sterilant. With disinfection, we may not penetrate barriers presented to the disinfectant itself.

So I know earlier there was some discussion

about the fact that some of the disinfectant will permeate through the entire tissues and fabric of the materials that we're trying to disinfect. It may or may not occur, depending on how long you leave the tissue in contact with the disinfectant.

So when we think about disinfection, this is one of the issues or concerns that we have with regards to disinfection of tissues, whereas sterilization can penetrate barriers such as biofilms, tissue and blood, and go right through the entire sample to deliver a sterility assurance level throughout the entire product.

Another difference between the two methods really comes into play when we're talking about routine processing and how you monitor the effectiveness on a day-to-day standpoint. When we think about process monitors, for disinfection because we don't want to inoculate or put organisms into the disinfectant process, we typically see that process monitoring may not be present with the device that's being processed.

So this presents an issue with regards to making sure that over time you have a good disinfection

process and that it’s consistent time to time. This may mean that we need to think about putting process monitors at the end of the day of using the chemical for disinfection. Right now, I don’t think that’s actually been a thought that’s really been addressed with a large number of the disinfection processes. I challenge that maybe that’s something we need to think about.

When we think about sterilization, we are always able to put a chemical or biological indicator present of some kind, during the sterilization process, to demonstrate the full achievement of the sterilization process. So this is a difference that we need to keep in mind when we think about validation in routine monitoring once we’ve completed our validation.

Now, we’ve heard a lot today about the impact of the disinfectant versus the materials themselves. When you think about the choice of a disinfection versus sterilization process for the tissue products, materials compatibility is the first thing we should be thinking about.

When we think about medical devices, this is

also the first thing we think about. So no matter what type of product we're talking about, materials compatibility immediately following sterilization or disinfection and long-term following sterilization to disinfection, we should consider not just the materials compatibility initially but over the shelf life of the product. So one of the things we have to be concerned about is, again, not just compatibility but functionality of the device.

When we think about another choice, it is whether or not we need an SAL versus a contamination rate. When Mary talked earlier, she talked about aseptic processing. With aseptic processing, we can only guarantee rate to be less than or equal to, let's say, 1 in a thousand.

I bring that up because when we're thinking about disinfection, we're combining disinfection of a product and then handling of a product to aseptically process it into a package. Again, we have a contamination rate potential here.

One of the things that I've seen has been probably a lack of an area that needs to be addressed

in the future, is really how to demonstrate that aseptic processing part.

How to validate that has been demonstrated for the pharmaceutical industry for many years. We've adapted that for the pharmaceutical industry to give us a contamination rate. But when I've worked in the industry with individuals, I haven't necessarily always seen validation of the aseptic processing part to give us a contamination rate.

So that's an area that I would just bring up just to say that do we need a sterility assurance level. Do we need a contamination rate? Do we need to know what that aseptic processing provides us, or are we just accepting the fact that we have delivered a disinfection process and we have good handling technique?

Obviously, the choice of disinfection versus sterilization also comes into play with regards to personal preference primarily with the physicians that are using the devices. Primarily with the negative impact or view of radiation sterilization, there's a lot of pushback to say no, we don't want terminal

sterilization.

It's this whole definition between what is the goal of sterilization versus aseptic processing. What do we gain from this for a physician's standpoint? Sterility, the acceptability for placing this in the patient versus what we think with regards to where we should go for processing our products for the future. So I think that is something we need to keep in mind.

When we think about product application -- and this applies to both disinfection and sterilization. I think about product application because when you think about this, you should always say not only am I looking at the sterilization or disinfection process, but now I'm going to put a specific product into that process.

Validation of individual products types should be conducted whether it's disinfection or sterilization because there are different inactivation characteristics that might occur.

We also talk about something called product families. Validation of product families may be adopted based upon tissues used or similarity of

treatment of tissue prior to disinfection and/or sterilization.

So there's a couple of different ways we can think about product families to help reduce the amount of validation that needs to occur. I particularly like the combination of using the tissues themselves and the similarity of treatment of tissue prior to disinfection or sterilization as a way to think about the bioburden that might be on the product that represents the challenge to the sterilization or disinfection process. So that's typically how I would look at product families is those two things in combination.

When you think about disinfection validation, what does that encompass? I broke this into manual and automated processes.

Manually, what are we looking at? We're controlling the preparation of the disinfectant, the concentration, the water quality if you are mixing the disinfectant up and the containers used to make sure that we don't have a negative impact with regards to the materials of the containers themselves.

We have to think about controlled time and

conditions of exposure to the disinfectant. We have to think about controlled time for the use of the disinfectant. Possibly there is an expiry date with regards to the length of time that you could utilize that disinfectant.

We have to recognize that longer exposure time may not achieve more spore log reductions. This has to do with the fact that there may or may not be a log linear relationship with regards to the disinfectant and the organisms on the product itself.

We need to validate each step of the sterilization process versus the entire sterilization process. One of the reasons why I bring that up is a lot of times I find if there are multiple steps in the disinfection process, you'll find that a lot of people will just go for looking at the entire process itself.

I would recommend that you actually look at each of the subcomponents of the total disinfection process to make sure that you understand at what point you are seeing an effective kill. That may give you a clue as to what's happening with the non-log linear inactivation.

So when you think about this, you also have to think about what documentation is already out there. There may be some R&D already out there that's published in the literature that you can access and use to support how you're viewing the total process versus each of the subprocessing steps.

The manual process also should use microbiological tests for qualitative or quantitative results. Typically, again, we're using a series of microorganisms to challenge the process. Typically, AOAC series of microorganisms, and those are inoculated on the tissues.

Tests are conducted to determine total kill which is the qualitative or determine the number of remaining organisms which is a quantitative, which I mentioned earlier. Both qualitative and quantitative allow for the calculation of a spore log reduction. Then the product is aseptically packaged following the process itself.

When I think about the automated processes that I see for disinfection, however, I have been typically starting to see -- and I know Martell and I

have talked about this for some time because we've started to see more movement into the automated processes and more typical quality validation, such as an IQOQPQ, starting to get more permeated into that environment.

When you think about an IQOQPQ, we're first talking about IQ and OQ as the equipment testing itself. IQ really is the insulation qualification saying that my equipment is installed and operates as intended. My calibration and my monitoring and controlling equipment is acceptable.

Then when we think about the operational qualification, we're looking at what we call an empty chamber, in other words, with no products. It doesn't mean it doesn't have anything else in it. It just means you're going to conduct the process without products in it.

We're going to look at the distribution of the disinfectant and/or temperature across the chamber. We're going to look at conducting a minimum of three runs to demonstrate consistency and reproducibility. This is an area where I don't see we're always looking

for consistency and reproducibility. In the questions that were asked of me upfront, I was asked to point out some concerns. I would say this is one of those areas I have a concern.

A lot of people do a lot of different microorganisms, a lot of different types of organisms. But I don't see them repeated two or three times. In any good validation, you should see a minimum of three replications to demonstrate that you see consistency over time.

Then obviously, one of the other things when we're talking about the chamber itself is that we're looking for identification of minimum and maximum locations within the chamber.

A lot of times you might think about disinfection and making sure that there's a good means of making sure that there's an even distribution of the disinfectant across the chamber itself, making sure that you're not seeing concentrations occur that give you stratifications over time, and when you put your product in there, that this then keeps that in mind with regards to the minimum and maximum locations

within the chamber and its potential effect on the sterilization process.

When we think about PQ, now we're talking about tests with the product in the equipment. We're looking again at minimum and maximum product configurations. So now we're looking at the different kinds of products that you might put into that disinfectant and how that might impact the capability of the disinfection process.

We're looking at the fill of the chamber as well; how many of these can you put in the chamber and still see effective disinfection for the product itself?

Tests of microbiological monitoring system. Again, in many cases we choose an inoculated product to demonstrate spore log reductions. If a biological indicator is chosen for disinfection, there needs to be demonstration that the monitor is more difficult to sterilize than the product bioburden itself.

So keeping in mind there should be some link to the kinds of organisms you typically see in your product, in your tissues. That might be a wide variety

of organisms. But there needs to be some link to demonstrate that the biological indicator chosen to monitor, and in essence validate your disinfection process, is appropriate for that disinfectant.

Then we need to challenge the process to demonstrate the desired spore log reduction is achieved and product is aseptically packaged following a process. Again, what I am starting to see with the PQ part of this is that it has been, traditionally for a very long time, where you just see the spore log reductions for the full sterilization process.

We're now starting to see people move into the sterility assurance level approach where we're looking for the linearity of the resistance of those organisms to the disinfection process. So instead of just doing product exposed to the full sterilization process, you're starting to see like Joel mentioned. He mentioned a half cycle processing, or half exposure he called it, for a worst-case processing.

I'm actually starting to see more people do multiple fractions of their disinfection process, where they might see an eighth of the process, a quarter of

the process, half of the process and look to see over time what's happening for, again, definition of potential log linear relationships.

Now, the challenges and concerns. The SLR achieved with the disinfection may be different depending on the types of tissues being processed. We've seen that over and over again. A more rigorous disinfecting process may be used with bone versus tendon because the bone from a material compatibility standpoint will withstand that.

Manual disinfection. Concern that I have is that delivery of the disinfectant to the tissue may not be consistent time to time. In fact, this has been proven to be seen with some testing that I've seen recently.

Again, none of that published, but the thing that I bring to play here is just to keep in mind when you think about manual processing, there needs to be really, really strict controls on the preparation of the disinfectant, how many product samples you can put in that disinfectant, how long it's left in there, and the expiry of the disinfectant itself needs to be well

controlled.

The last challenge or concern is that disinfection requires a product is packaged following treatment, which requires additional handling for aseptic processing. This processing may introduce contamination. I have not been seeing validation of that aseptic processing step to look at how frequently might you be recontaminating the product from the time that you move from the disinfectant to the time that you get it into the package.

Detailed disinfection validation methods have not been defined for the tissue industry, i.e., consensus standards are not available. I was really happy to hear in Joel's presentation where he was proposing that there needs to be a little bit more development of standards in this area. I think there needs to be requirements as to what should we achieve with the disinfection validation and how might that be linked to labeling for the products themselves.

There's no specified requirement at this point in time as to what should be achieved with the disinfectant, e.g., a spore log reduction. There is no

defined requirement at this point.

One of the things I want to remind you is that 3 spore log reductions does not give you a 10 to the minus 3 SAL. I do get this a lot, where people say well, I have 3 spore log reductions. Therefore, I have a 10 to the minus 3 SAL.

Go back to those slides I prepared earlier and look at what a 3 spore log reduction means. It goes from a 10 to the 6 calculation to a 10 to the 3. That's 3 spore log reductions, okay? That does not demonstrate a 10 to the minus 3 SAL.

If you have a population of organisms that starts at 10 to the 6 and you want a 10 to the minus 3, that's 9 spore log reductions. Okay? So that's one of the things that I think is not fully understood in a large variety of industries, to be honest with you, not just the tissue industry but a large variety of the industry.

I know Vicky Hutchens is here today. We worked quite extensively on the sterility assurance level document which is SE67. We still feel that we are training people into understanding the differences

between spore log reductions and sterility assurance levels in the medical device industry as well. So again, just to make sure that we clarify that. That's an area of concern that is that people do equate those two things.

When we think about sterilization validation, again, we see IQOQPQ. IQ and OQ are, in essence, the same as what we saw as the disinfection validation for the automated processes. The differences might be that we have a little bit more understanding of the characterization of the differences across the sterilizer versus the disinfectant process itself.

Again, when we think about IQ, we're thinking about making sure the equipment operates as intended and is calibrated.

When we think about OQ, we're thinking about an empty chamber. Again, no products in the sterilizer and what do you achieve with the distribution of the conditions across the sterilizer.

We're thinking about moist heat or ethylene oxide. We're thinking about temperature distribution, humidity distribution. When we're thinking about

radiation, we're looking at radiation dose distribution.

Again, when we talk about sterilization validation, we do have a minimum of three runs to demonstrate consistency and reproducibility. So this is very well controlled and very well defined for the terminal sterilization processes today.

Again, identification within the sterilizer of the locations of minimum and maximum conditions. With that information from the OQ process, you then move into the PQ process. You have to worry or be concerned about the minimum and maximum fill of the sterilizer in relationship to where you identified the minimum and maximum locations for temperature, relative humidity, dose. You have to keep those in mind.

When you think about the fill of a sterilizer, again, we have to be worried about making sure that we have some constraints for the volume fill, or as small a volume fill, because there is an impact, whether it is a minimum or a maximum fill of the sterilizer.

Now, when we talk about the choice of

microbiological monitoring systems, specifically with moist heat and ethylene oxide, we typically go to the use of a biological monitor. When that biological monitor is chosen, we do need to demonstrate that the monitor is more difficult to sterilize than the bioburden in the most difficult to sterilize location within the product actually and within the sterilization load.

So when we think about distribution of temperature, relative humidity, ethylene oxide concentration, if it's an EO sterilization process, we have to be fully challenging to the worst case within the product and within the sterilizer itself, which is why we then equate it to the minimum and maximum conditions with the sterilizer. I'd like to see more of that applied to the automated processes within the disinfection validation. So we're more looking at the minimums and maximums there as well.

When it comes to radiation sterilization, typically, we don't have a biological indicator that's used to monitor the process. We do typically use a chemical indicator, but we do have a microbiological

validation step to demonstrate the achievement of the sterility assurance level.

When we think about PQ for sterilization validation, we challenge the process to demonstrate the desired sterility assurance level. And in the case of majority of tissues, I would think a 10 to the minus 6 would be desired is achieved with the sterilization process.

Sterilization validation concerns. Challenges that we might see with terminal sterilization is the materials compatibility and/or functionality of the product over time. One of the things I'm really concerned about is a lot of times we think about historical data to help us understand the materials compatibility. It's the functionality of the product over time.

I would challenge a couple of things that I saw earlier today primarily because this is an area that we start to see an issue or a concern with regards to materials compatibility. I think a lot of the older testing that was done, whether it was radiation, electron beam, or gamma sterilization, we do have some

concerns with the stuff that was conducted back in the ‘50s and ‘60s because the dosimetry systems that were used to monitor the doses delivered may not have been accurate for that time. They have actually understated or overstated the doses delivered to the product. So when I think about looking at older data from materials compatibility and functionality of the product over time, I would challenge you maybe to look at some newer data, to look at some more well controlled delivery of the sterilization processes, and to then have a better understanding of what might happen with that tissue.

Obviously, another challenge or concern has to do with the physical appearance of the product. It does with a lot of the terminal sterilization appear to be not acceptable because of the color changes. The issue may be that we need to -- if we want to go to terminal sterilization to prevent or to reduce the contamination issues, then maybe we need to have an education as to whether or not that physical appearance is good or bad.

Sterilization is a relatively new process for the tissue industry. There has been some attempt

recently to assist in the development of some standards that would give how to conduct validation for the tissue industry.

Right now, there are four documents that are out there that specifically deal with the validation of terminal sterilization processes. The first of which deals with alternative sterilization processes, maybe not the primary three, which are moist heat, EO and radiation. You'll get these document numbers when you receive a copy of my presentation today.

The other thing that's not on this slide is something that I think was mentioned yesterday, which is the fact that I think AAMI has been producing or is in the process of developing a application of the radiation sterilization process to tissue products. I think I'd like to propose that I think we should see more of that with regards to what is the next step.

So, Joel, I agree with your thought with regard to the fact that we need to come up with consensus standards on how to validate some of these sterilization processes for tissues specifically because there are specific concerns or applications

issues that are different from some of the medical devices that these documents were written for.

Those validation differ to determine sterility versus viral inactivation. Viruses are typically addressed through donor screening processes. We do not address viruses through the sterilization validation process. In fact, we typically exclude them primarily because the majority of the documents that you just saw up there are for medical devices, hard plastics, typically not tissues that we're talking about. So right now, the documents do not typically address the reduction of viruses.

Validation for sterility does not address viral inactivation, but viral inactivation validation is currently not required. So the question that I would pose back is, if there is a requirement for viral inactivation validation, then that would be another area that I think we should start proposing some defined validation criteria for.

Primarily with validation of medical devices, we choose not to address viruses because of the fact that they will either not be there existent in the

first place or if there is any viruses there, they will typically not survive any length of time once they have been placed or they have gotten onto the product prior to sterilization. So typically, we don't address viruses at all for the sterilization validation processes.

With that, I'd like to thank you for the opportunity to talk to you about disinfection and validation and sterilization validation techniques.

MS. MALARKEY: Thank you, Joyce. That was very informative as well. I hear you about the spore log reduction versus SAL. I think that is a common issue or problem, misconception.

Well, could the speakers from this morning please join me up here?

Then I'm also going to add to our panel with three additional individuals.

David Shriver, if you could please come up as well. David has been the director of Regulatory and Clinical Affairs at LifeCell Corporation since 2004, has over 30 years of drug discovery development and business development experience with major

pharmaceutical companies, FDA and PhRMA or the Pharmaceutical Research and Manufacturers of America.

He obtained his BS degree from Purdue and his MS and PhD from the University of Iowa, David.

Dr. Lloyd Wolfinbarger, he's the chief scientific officer at LifeNet Health and professor at the Department of Biological Ssciences at Old Dominion University; a managing partner of Bioscience Consultants, Incorporated; consultant to tissue banks and major biotechnology and pharmaceutical firms.

Finally, Carrie Hartfill, there you are. Carrie is with Regeneration Technologies, Inc., or RTI, which we've heard about many of these companies this morning. She was named VP of quality assurance and regulatory affairs. I'm sorry. She has been with RTI -- she started RTI as executive director of QA and RA in 2001 and was named VP of QA and RA in January of 2003 and chief scientific officer in March 2007. She's worked for 18 years in technology development as a freelance consultant before coming to the tissue business, I guess. She has a BS in health science from Birmingham University Medical School in England as well

as a masters in management from the University of Wolverhampton in England.

So, welcome all panelists and speakers and thank you all this morning for the wonderful presentations.

These are some questions that we're going to put out there, but I hope there will be many questions from the audience as there was yesterday because I think that's really where we got into the nitty-gritty.

I also noted that some of our speakers had some suggestions or questions of their own to put out to the industry and perhaps the regulators as well. So hopefully, we can address what we can in the next hour.

The questions that we will pose to the panel: How can FDA leverage with industry to address all these challenges and concerns we've heard about?

What information is needed to move forward with possible FDA guidance? I've heard that perhaps AATB will be looking at quite a few potential guidance documents. Scott is nodding.

What are acceptable expectations with regards to sterility? This is really something that's become

very clear this morning. That it is not an easy business and what exactly does it mean and how do we deal with it. Virtual sterility is I think one of the suggestions on the table.

Do these expectations vary by tissue type? I think we can all very definitively say yes, at least I certainly got that message.

What is actually the reality? What new methods of sterilization are being developed? We heard about some of them this morning from Dr. Arnoczky, but what else is in the pipeline? If there are things, are people willing to talk about them, I guess would be the other thing.

So with that, I will see if we have any specific questions.

Oh, yes, this is Dr. Dennis Guilfoyle with our FDA Office of Regulatory Affairs. He's a microbiologist.

DR. GUILFOYLE: In regards to tissue products that are disinfected with a chemical reagent, when you perform interrupted cycle assays where rather than run your whole process, you take interrupted times before

the completion, and you take those samples and you analyze them for the level of remaining spores after you've inoculated the 10 to the 6, could you address the concern of being sure to neutralize the residual chemicals that are still in that product? Because in some cases companies are mailing these out to private labs and the residual disinfectant is still working on the spores as they're being sent to the lab two days later; and the levels, even for interrupted process, is showing sterility where, in fact, it's just the non-neutralization of those original samples -- you know what I'm saying?

MS. HANSEN: Yeah. Thank you. You had a very good point.

One of the things that we're actually doing to prevent that from occurring is that when you think about doing incremental exposures to the disinfection process and when you think about what the end goal is to remove all of those residues to make sure that you don't have any residues remaining in the product that might harm the patient, obviously -- when you do those fractional kills, you actually should terminate the

sterilization or disinfection step but then continue to go through all of the other removal processes before you then send out the samples to the laboratory.

The other thing has to do with making sure that when you have those samples removed from the process, that you should have some control mechanism to make sure that they are frozen or refrigerated to make sure that there's no residual kill that's going on in that length of time.

So those would be the two things that we've -- that I know I've implemented. I know Martell and I have talked about how to stop that residual kill.

Again, it's that we make it go through the entire disinfection process, which includes the removal step. But definitely we've seen some big differences when people have not done that because they do see early kill of all of the organisms. It has to do with the residual kill.

Terminal sterilization saw this as well. When we were dealing, let's say, specifically with ethylene oxide sterilization, we saw that for many years people would actually expose their biological

indicators. The ethylene oxide was remaining in the materials. It continued to have residual kill. We saw that there was actually non-linear inactivation because of that.

So I think when you start looking for the linear inactivation part and you start putting in play the control measures to prevent some of these additional things from happening, I think you can start to see some linear inactivation.

Good question, though, because that's one of the things we've dealt with in a couple of locations.

MS. MALARKEY: Joel, I think you had several questions. You want to --

MR. OSBORNE: Yeah. I understand I'm a QA guy, not an R&D guy. I think these are R&D questions for -- some of this is. So I'll try to answer from a QA regulatory aspect, but don't hold me to it.

Skin and dermis, are there any test methods to measure the integrity of the final product after sterilization, e.g., radiation? Can you use collagen measurement quantitation to address this?

Second question. Are there any specific

considerations or set of specifications for skin dermis used near the CNS areas?

The first part of this question regarding final product after sterilization, I think with the dermis, I'm not familiar with anybody that is providing dermis that is irradiated. It is something that the reason why we don't irradiate dermis is because it does affect the matrix.

It is something that we do as part of our validation. We do look at various both biochemical and biomechanical tests of the dermis as part of the validation. We don't routinely look at each piece, though. We don't monitor that, per se, during processing.

Then are there any specific or set specifications for the dermis used for CNS areas? I guess the concern there is that with anything that is considered -- that would be possibly neurotoxic.

As I mentioned in my presentation, we do a number of tests to look at the residuals that are left in the dermis, peracetic acid being a major concern there. We do not have currently any warnings about the

dermis next to CNS, however. So it is something that we feel that right now the residual levels that we have are not toxic in any way.

The second question for full thickness dermis recovered using a freehand technique may be too thick for dermal allograft application. If thickness is reduced during processing or post-recovery, would its properties be similar to split thickness skin?

Well, interestingly enough, we provide various thicknesses of the dermis. We can actually control the thickness of the dermis that we are providing. So we provide a thin, medium and a thick dermis for various types of application. So it is something that we look at as part of control and processing.

The second part of this is we are trying right now -- a question was asked of me earlier about the epidermal layer. What we're trying to do to actually not get rid of tissue unnecessarily, is we're trying to develop processes that would include taking a split thickness allograft and then getting the dermal tissue from that full thickness recovery so that we

aren't -- right now we're just actually dissolving the epidermal layer as part of the process.

We're hopefully going to be able to provide both a split thickness and a dermis graft from one section of full thickness skin.

Does split thickness skin retain basement membrane possibly needed for barrier properties?

This is more or less a clinical question. I'm not sure what is meant by basement membrane. Here again, my lack of really knowledge on the, I guess, more technical side of the use of dermis clinically.

I know that from a barrier perspective, it's very important that the dermis be able to retain its ability to stretch and contract. Certainly, from a membrane perspective, you don't want a situation where the dermis stretches and then isn't able to contract.

So we do look at that as part of animal studies that we're conducting. It is something that bulging certainly of the graft is a big concern here. It's something that from our standpoint we try to take a look at through animal testing.

MS. MALARKEY: Does anyone have anything to

add?

MR. SHRIVER: Yeah, I'm from LifeCell. We also process dermis and cryopreserved skin.

The question related to the orientation versus the basement membrane, our experience is that there is no difference in performance regarding the orientation of the basement membrane; that if it's used, it will function very well. Now, that's not true with cryopreserved skin because you have the epidermis on top and you do want to put the proper orientation with cryopreserved skin.

The other question was what now?

MR. OSBORNE: The other question was, are there test methods to measure the integrity of the final product after sterilization and are there any specific considerations or set of specifications for skin near the CNS area?

MR. SHRIVER: We don't have any specific. We're very similar. We don't address that issue. We haven't had any issues related to that. The tissues that we use and the process that we use has been tested in standard pre-clinical biocompatibility. We have no

signal that there's any toxicity related to the processed dermis.

MR. MALARKEY: David, did you have --

MR. FRONK: Yes, there's one question with regards to heart valves. I'm going to pass this one down. I'm going to read it and let Lloyd, since he seemed kind of lonely over there, give him a chance to speak.

The question is related to heart valves. Are there any initiatives for standardization by setting specifications in ranges for things like cell viability, tissue strength, microbial limits, et cetera? In other terms, can you set criteria for pass/fail? What is the consensus thoughts?

So, Lloyd, I'll let you go ahead and give a whirl at that. Then I can chime in as well.

DR. WOLFINBARGER: I'm sorry. I'm hearing several echoes here. Can --

MR. FRONK: I'll go ahead and give my take on it with regards to setting specifications and standardizations for some of these various criteria.

At least from CryoLife's perspective, no, we

don't. We don't test the tissues individually to find out if they have certain criterias or certain strengths.

You have to keep in mind that we get tissues in from newborns to donors of age 55. We have causes of death that range from motor vehicle accidents to myocardial infraction. You've got warm ischemic times ranging from hours to 24 hours depending on refrigeration. So there's a lot of factors that play into – a part with regards to a lot of those variables.

I think the other aspect of it is it is very difficult to choose to test those pieces of tissue. They are destructive in nature and therefore would render the clinical utility of them moot.

Lloyd?

DR. WOLFINBARGER: My turn? Okay. How to answer some of these, I'll take the -- there's a question here for cell viability.

I think for cellular viability in a cryopreserved heart valve -- I remember in the mid '80s when we were setting up a cryopreserved heart valve program at LifeNet, there was two opinions. One was do

you want a viable cell population in a heart valve. The other was do you not want cell viability in a heart valve.

The physicians won. So essentially all programs that were distributing cryopreserved heart valves back in the mid ‘80s strove to retain a viable cell population. That viability of cells within those heart valves depends on where you assessed it and how you assessed it. It was usually some kind of an assay to measure cell function as a function of viability. As a general rule of thumb, that was a 35 percent of the viability of a native heart valve. That seemed to be the consensus of time back then.

You have to also appreciate then that cryopreserved heart valves went through a period where they were actually a device. They’re back now to a human tissue per the FDA. So in some respects, people kind of got locked into not doing a lot of changes because they didn’t want to really lose their grandfather clause within the ability to distribute cryopreserved heart valves.

I think that we learned very quickly that a

transplanted heart valve became acellular. The cells became apoptotic and usually died within the first 3 months and never recellularized over the life of the valve. So the interesting thing there is that an essentially dead tissue continued to function for upwards of 20 years, calcification being its major cause.

I think if you ask both LifeNet and CryoLife where they're going now, I believe we're going where we should have gone 20 years ago. That is, we're all looking at decellularizing a heart valve now, taking the cell population out and changing the matrix such that it will recellularize and repopulate when it's put into the patient, which means that over time that heart valve will become mutagenous and grow with the -- well, grow with an infant. I don't think I'm growing anymore. I hope I'm not. In some ways, I'm growing.

Tissue strength on heart valves has probably been the most rigorously tested. Groups have pressurized from the outflow side to see if the leaflet structures would maintain and manage the pressure. Conduit tissue has been stretched, pulled, bitten,

chewed on and whatever you can think to do with it.

As far as microbial -- and I guess I would say to you there that cryopreserved heart valves retain the same biomechanical properties as the native tissue and that that strength does not seem to change over the lifetime of the valve in the patient.

Microbial limits, I think I can speak for both CryoLife and LifeNet. We don't distribute any cryopreserved cardiovascular tissue graft that we cannot demonstrate is culture negative. That culture negativity is through the use of rinsates and representative tissue samples.

The question more or less comes down to how do you test for that microbial contamination. I think we in the industry have known for at least 20 years that one had to do bacteriostasis and fungistasis testing always to ensure that the antibiotics that are used in the disinfection of these tissues don't preclude you from detecting the microorganisms that might be there.

The last thing I think here is in other terms, can you set criteria for pass/fail? Goodness,

pass/fail. I think pass/fail for at least LifeNet is on a functional test. Every valve is tested for functionality. That means that the leaflets are intact. They coapt. There's no regurgitation. We test -- look at the tissues for abnormalities. Those don't get past. Anything that we can't disinfect is discarded.

MS. MALARKEY: Thank you. Carrie, did you want to address a couple of questions?

MS. HARTFILL: Perhaps I'll leave some of the stingers till last.

One question is what are the validated fingerprint methods or techniques used or recommended to evaluate prions in terminally sterilized bone allograft?

That's almost a 10,000-dollar question. The challenge in validating screening and testing methodologies relative to prions is that there are very few that are unequivocal -- very few test methodologies that are not equivocal in some manner or another.

In other words, there are really no validated methodologies that are accepted consistently across

regulatory authorities, across government authorities in general terms, not even for testing cows with BSE frankly, and leave alone testing for the presence of prions in a matrix, where the ability to detect prions to begin with, a level of infectivity has not been proven.

You saw the World Health Organization categorization described earlier today. The challenge is how do you detect something -- how do you prove something's not there? You're trying to prove a negative. If you can't identify it as being present, then how do you demonstrate removal? It's a huge problem.

Several tissue banks I know have taken a run at prion removal exercises. But beyond the theoretical, I think we have -- I know that the Clearant process has been tested. Beyond the theoretical, it's very difficult to actually prove the negative with no validated testing out there.

The next one is actually addressed to both the agency and the panel. It asks that one of the presenters briefly mentioned ICH viral clearance work.

What is the agency -- I'll defer to Mary for that -- or the panel's opinion of the EMEA definition of robust viral inactivation. EMEA is the European agency that screens for safety and efficacy of biologics.

Is it applicable to tissues and so what's my comment on that? In a past life, I worked in an advisory capacity to EMEA, actually, in terms of viral clearance, many years ago as EMEA was being formed. So my perspective is somewhat historical. It was very much related in those days to my experience with plasma fractionation. But it's absolutely relevant to viral inactivation or viral reduction.

There is an analogy. There is FDA guidance out there relative to viral inactivation techniques applicable to materials of biologic origin. They are very similar to the EMEA approach.

It's actually the approach that has accompanied RTI when we were looking at our tissue sterilization viral inactivation processes.

You want to go to somebody else?

MS. MALARKEY: I actually had a question for Dr. Arnoczky.

You had a lot of -- in your summary in areas of concern -- and this came up several times during your presentation -- you mentioned the lack of good peer-reviewed studies in a variety of settings. What do you -- how can we make this happen? I mean how can we encourage these studies to take place?

Do you have any thoughts on that, or does anyone else on the panel have any thoughts on that? Because it does seem that many of these areas, there is a lack of good data.

DR. ARNOCZKY: Yeah, I think -- I'm on the editorial board of several of the major orthopedic journals. I think one of the problems we run into is that it's difficult to get a peer-reviewed publication out there when the methodology is basically a black box because you're unwilling or unable to talk about what the proprietary aspects of it is. So you have a graft. You put it in a black box. It comes out of the black box. Then you test it.

I think one of the ways of getting around it, and something we're trying to do with the American Orthopedic Society for Sports Medicine, is having an

independent individual test processed specimens across the board and not really worrying about what the methodology is, but saying that this is a product from, let's say, LifeNet. This is a product from RTI. This is the product from tissue bank X, Y or Z and look at it independently.

I think that's one way of doing it. But I think even more incumbent upon us is to come up with a series of mechanical and biological tests that we agree are going to be clinically relevant. That's the real key here.

I think a 5 percent alteration in the mechanics or the biology may not be clinically relevant. Although we can measure it, that may not be a good assessment. We have to kind of come up together with what we think is going to be important, asking the surgeons, asking the basic scientists, and asking the people from industry and the regulatory group what we think is going to be clinically significant.

A 25 change in the temporal incorporation is going to be significant. But unless we do this together, I think that's what's also going to be the

problem. Everybody is doing their own thing, which I think they all think are right. Maybe they are. But unless we really have something that's standardized, it's very, very difficult to compare apples and oranges.

So tissues banks A, B and C, we really can't tell because they're probably doing well controlled studies. But because they're using different strain rates or different boundary conditions, there's no way to compare across the board.

So I think first of all, we have to kind of set the playing field equal, kind of set the rules by what we want to look at. Then I think maybe the peer-reviewed publications will come a little bit easier.

MR. OSBORNE: One of the things that is really interesting about this business, unlike medical devices where you have a raw material that's made out of stainless steel or you have a drug which is made out of a drug component, a chemical that can be well defined, the problem is human tissues are like snowflakes. We're all different.

It is very difficult to come up with clinical studies because you not only have processes but you have differences between donors. I think that it is something that the sports medicine surgeon out there is screaming for, give me data.

But sometimes it's very difficult to conduct these clinical studies in an environment that is equal because you have a non-standard raw material. You have a baseline that can be established; sure, biomechanical testing. But it is something that, again, you have to understand that this is a non-standard raw material.

DR. ARNOCZKY: Well, yeah. But there's a good study that actually was funded by the NIH for the MOON Group, which was headed up by Kurt Spindler out of Vanderbilt, that actually looked at this in a prospective way to show that at least in deep frozen allograft tissue, there was really no difference in the clinical outcomes between deep frozen and autogenous tissue.

So there's a way to do it, but we really have to look at individual specimens across there. I understand what you're saying, but I think there is a

way at least to give us some idea, even if it's just a pre-clinical.

I mean I would be happy to see pre-clinical data. We don't see that out there where you take an animal tendon and process it whatever way you want to do it and use the counterpart to look at a paired test.

That's being done in some areas, but it's not universal. So I'm just looking for a start that we can hang our hat on something because that's what the surgeons are asking for and that's what the basic scientists would like to know as well.

DR. WOLFINBARGER: Maybe if I could chime in on that. I think one of the things that has plagued the -- and I'll just call it the allograft industry -- for quite some time is that we can do a great deal of pre-clinical testing on all varieties of human tissue grafts, but essentially all you're doing there is you're demonstrating that that graft is not going to fail at time zero.

When a physician puts it into the patient, the heart valve is not going to burst or regurgitate. An anterior cruciate ligament tendon is not going to

fail in the patient. That's your pre-clinical data.

You can assure that physician that that graft's going to work at zero time. What I think we've -- and I guess I'll even implicate myself as a primary person. I've pushed for years for consensus clinical outcomes studies preferably that would be headed up by some of the larger societies, whereby for all allografts that are sent out there are -- the FDA obliges, thank goodness -- for all human tissue grafts to be tracked right to the patient.

It would be really advantageous if we could have and set up registries where physicians could report into a central database how their patients failed with a particular graft.

We've been pretty successful, for example, in setting up what's called the Ross Registry. This is a registry where allograft human heart valves are used in what's called the Ross procedure, which is replacement of an aortic valve with the patient's pulmonary valve.

The surgeons are reporting their data into that. They're following their patients. Irrespective of where the allograft heart valve came from, it's

going into the central registry. So that is something that I think is paramount for clinical outcome information.

We approached the AOSSM -- I think it was two years ago -- to help set up a registry of looking at allografts in sports medicine applications. As far as I know, we're still working on that. But I don't know if anything's come from it.

The biggest impediment for the allograft industry right now is, quite honestly, allografts work extremely well in a patient, so the patients just simply disappear on us. So it's hard to get long-term information back from them, since after a certain while, you get a human heart valve and you're not on any medication. So you go on merrily about your life. No one ever hears from you again until one of these days you die and there's an autopsy and somebody says they've got something strange here.

So you don't get the information back. So I think for the allograft industry, that's really one of the biggest problems. That is getting the clinical information back from the patients.

MS. MALARKEY: I'll try to answer. I have a few questions here. So I'll try to knock a couple of them out.

One of my slides indicated that products may be labeled sterile if they were produced by a validated aseptic processing process and pass a validated sterility test. My understanding was that you could only label products sterile if they were terminally sterilized.

Would you please give an example of this and expand upon this?

Actually, in the drug world again, I think -- as Joyce mentioned this before, in the drug world, of course, many of our biological drug products, particularly, you simply cannot terminally sterilize. They have to be filter sterilized, as I mentioned, and then subject to a validated aseptic process.

It is not a SAL. It is an acceptable rate of contamination of generally 1 in a .001 percent. With those provisions, you may label a product as sterile. Some examples of that are the plasma derivatives, in fact, albumin or immunoglobulin, and many of our

vaccines.

So this is a well accepted, though different, way of achieving that sterile claim, if you will. So I hope that clears up that particular question.

In the absence of a guidance document, what kind of data is the FDA looking for for HCT/P products to be able make a safety claim regarding viruses? In essence, free from risk from viral transmission.

Well, I don't know that that would be the kind of claim that we would be going for here. I think that's again a very absolute statement. But I think what I was showing was what is done in the other industries in terms of labeling where you actually are looking at log reduction.

I know that those studies have been done in this industry. I was just noting that these labels have been around for some time in the plasma derivative as well as the biotech industry, where all you can really do is say that your process has been evaluated for a log reduction factor and these are the factors.

So you're never saying absolute, but it's a measure of safety in the plasma derivative and other

industries.

The other one real quick, are viral inactivation studies required? I don't believe our regs do at this time require them.

I think people do them. I think if they do, that's the question, how to express that to the public. I think that's open for comment.

MR. OSBORNE: Well, one of the things that AATB standards does have as far as the labeling is concerned, we all have to label tissues by saying that they are able to transmit the viruses.

It is something that -- I mean regardless of what you're doing, I think, that is something that we looked at as being very important, at least on the surgeon perspective, to say that although we carefully screen and test, it is still possible to transmit viruses.

MS. HARTFILL: I have a quick comment. That is, while under 361 HCT/P regulation, tissues are not required to -- there are no specific requirements for any performance or criterion to be met other than true and accurate labeling reflecting what was done to the

tissue and what was the outcome.

When those of us that have been involved in producing a human tissue based product that has been designated as a medical device and therefore under CDRH jurisdiction, my experience is that we have very clearly been asked and required to provide viral inactivation studies as part of the 510K clearance process for those products.

MS. MALARKEY: I would highlight while there's no -- I would agree and even highlight that even though the 600s and the 211s which would be applied to the biological drug products, that could also be -- cells could obviously fall into that range.

There's not an explicit requirement for viral inactivation, but it is an expectation. There's often, entering the IND phase, it is an issue with safety right from Phase 1 on, has been my experience in the biotech and plasma derivative areas.

DR. WOLFINBARGER: Mary, if I can add a little bit.

From my perspective, as far as viral disease transmission by allograft tissues, I've always operated

more under determining what is the probability of viral disease transmission by that tissue.

Tissue banks screen donors. We know what the sensitivity of what our assay tests are. We know what the windows are for when we might miss that. So we know the probability of missing an infected donor.

But we know pretty much what the incidence of viral diseases are in the general population and, hence, in the population of potential donors. We know what our viral log kills can be based upon our processing or our viral clearance studies. We know how many virus particles we can inactivate with terminal sterilization at a given radiation dose.

So theoretically, we can look at all of those factors and we can perform -- admittedly, it’s a theoretical calculation. But we can calculate what the probability is of a specific viral disease transmission through a specific allograft might be.

Of course, that probability is going to change a little bit with the volume of the allograft. If you get a large allograft -- for example, if you get a proximal femur for a hip replacement, your

probability of getting viral disease transmission from bone from that donor is greater than if you're going to get a small graft for spinal fusion.

But I think the tendency to rely upon a viral log inactivation or viral clearance studies, just by the process, it is utilizing only about a third or maybe a half of the information that's available to the allograft tissue banking industry.

MS. MALARKEY: Thank you.

Joyce, I think you had a couple of additional questions.

MS. HANSEN: Yes. I have two other questions that came in.

The first is to show consistency and reproducibility. Should the three runs that are recommended for validation be conducted consecutively? The answer is yes.

In fact, even though we do talk about a minimum of three runs, many times you may actually require to do more than three runs. If you have a process that is variable and you go through an FMEA to look at what are the variabilities and how might they

change over time, even three runs conducted consecutively may not be enough.

We talked three as a minimum. But I would suggest that maybe there's even a potential need for doing more than three runs.

Again, from a validation standpoint, we're looking for consistency and reproducibility. At least a minimum of three gives you a good understanding. If you conduct them consecutively, then you demonstrate that you can reproducibly do this.

The second question that I got had to do with in general with disinfection. Is the rate of bioreduction linear or non-linear? They actually drew a curve with regards to disinfectant versus irradiation.

Irradiation is a linear inactivation. However, with disinfectants we actually can see linear or non-linear curves. It depends on the disinfectant itself, the length of exposure time, how well the expiry of the disinfectant is controlled.

In fact, you can see biphasic or triphasic inactivations, meaning that there may be several

portions of an inactivation curve that might actually have different D values, as I mentioned earlier, where we look at the time to reduce the population of organisms.

So sometimes with disinfection, I'm seeing either biphasic or triphasic inactivation, just again depending on the disinfectant practice that's being used.

But that is one of the big concerns with regards to comparison, with regards of disinfection versus terminal sterilization, is we have truly defined terminal sterilization as log linear, whereas disinfection there is the potential for it to not be log linear and definitely to have potential different phases in the curve that predict the inactivation rates.

MR. FRONK: There is one question that was listed for cardiovascular tissue, but I think it might apply to all the other type of tissue.

The question was for tissues after disinfection or sterilization, do you do biocompatibility tests such an endotoxin, hemoloysis,

irritation, complement activation, cytotoxicity, inflammatory responses?

So for us in the industry, they're going to be standardized to a 10993 panel of testing. For our conventionally or standard processed cardiovascular tissue, we have not. As you saw from the presentation, there is a long history of clinical use. I think that grandfathering, if you will, of the strong clinical performance, at least obviated my predecessor's at CryoLife's thoughts on doing such tests.

Now, I will acknowledge that in some new technologies that we have developed that might be more classically defined as a device, we have done these types of tests. We have found biocompatible, if you will, passing results from all of these battery of tests.

MS. MALARKEY: Does anyone else have questions down there?

MR. OSBORNE: Well, yeah, we've -- biocompatibility, I think it really depends upon the tissue, too, because with skin, the uptake of chemical is tremendous. You can end up with toxic

situations whereby you get chemicals leaching out. So that anytime you have a chemical disinfection process, I think it's very important.

The other thing is antibiotics -- I'd really like to ask the panel about this because I know that antibiotics aren't typical disinfectants. They're antibiotics.

It is something that from -- I'm often confused as to -- we, for example, treat our soft tissues with antibiotics. We don't use chemical disinfectants.

Are antibiotics -- as part of this discussion we're having, should they be considered disinfectants or should they be considered antibiotics?

MR. SHRIVER: One of the things that I think that is coming up as sort of a general theme is transmitting risk information. We need to transmit that as processors to our regulatory agencies so that they can understand what we've done and they can do an independent risk versus benefit assessment.

The issue with antibiotics are that where they're used to mitigate risk, and they make a better

quality product, and they do reduce the risk of whatever pathogens could be in the product that is sensitive to that antibiotic, there are a number of things that go on.

The question is how do we communicate that information to the surgeon who is going to be using the product so that he can make an independent assessment for that patient?

If you'll recall in the tissue regulations, there are caveats that do allow surgeons to use material that has not been fully screened or tested if they are aware that the patient is -- the surgeon and the patient are willing to take that risk.

So that it's -- we need to always come back to that risk assessment and how do we express that in the most appropriate way for our consumers.

MS. HARTFILL: I have a couple of questions.

This one's addressed to AATB representative, so I'll give it my best shot, Scott. Then if I don't do well, you can come and help.

The question is the AATB guideline for microbial testing specifies the type of organisms you

should test for but it does not say how to perform the test. Any suggestions to what type of procedures can be followed?

I think the reference is to the AATB standard that specifies the types of organisms that should be addressed. Those organisms do differ relative to different types of tissue currently. I would expect that would continue to be the case.

What AATB does not do currently is specify the nature of the sampling and/or extraction methodology, the actual culturing methodology or the method of assessment of the results, so acceptance criteria.

So if the question is AATB planning to work more on doing that, I think you heard earlier this morning from Joel that most definitely those guidances are under active discussion. We expect to develop them I think is fair to say, to say the least.

If nothing else, as well as enjoying these two days of workshop, the last two weeks of e-mail exchange amongst many of the people in the panel here and others in the industry has been really quite

exciting.

So more to follow. Is that fair to say, Scott?

Then I have one last question. It's addressed to RTI.

First of all, it's a two-part question. First of all, does your process sterilize to 10 to the minus 6 SAL?

Let's deal with my assumption that this is a reference to the process that we apply to sports medicine related soft tissues, tendons, menisci and to bone products. Bone constructs we call them, and we call that process BioCleanse.

The answer to that is yes, it does achieve a sterility assurance level of 10 to the minus 6. We also have a separate process that we apply to our demineralized bone based products that also achieves 10 to the minus 6.

The second part of the question is if so, why isn't the standard of care 10 to the minus 6 instead of aseptic processing?

I'm not sure that I'm the best qualified

person to answer that. But what I will say is that surgeons are becoming increasing more educated, that there's more education to come in terms of what does 10 to the minus 6 even mean. I think we've heard a lot of that discussion over the last couple of days.

RTI also fairly recognizes that while we are adamant that wherever we can achieve the greatest sterility assurance level, we will. We do elect to include viruses as part of our assessment.

We also recognize that there are some tissues for which the robust and sometimes disruptive nature of those processes cannot be applied and keep the tissue integrity remaining.

MS. HANSEN: Mary, if I might just go back to the question that was asked slightly earlier with regards to the antibiotic and whether or not that should be considered part of the disinfection process.

From a sterilization, scientific pure standpoint, I would not consider the antibiotic to be part of the disinfection process at all. Again, it is a means of reducing the potential for organisms to be there. But the disinfection process should be an

active mechanism for elimination of those organisms.

So I would not consider the antibiotic step to be part of the disinfection step. Again, it might be a way to reduce the initial population or just suppress any growth.

In fact, when I typically do validation for disinfection processes, I would prefer to not even have the antibiotic included in the disinfection step and actually have that as an added value of safety. Just a personal opinion. We can definitely discuss it.

DR. WOLFINBARGER: Okay. Let's discuss.

I'm sorry. I consider the antibiotic step in any processing an active disinfection.

MS. MALARKEY: Maybe the term is not quite correct.

DR. WOLFINBARGER: Yeah. Disinfection, for me, is anything that reduces the microbial bioburden by either removing it or killing it. So why wouldn't you consider an antibiotic a disinfection step?

MS. HANSEN: Again, it has to do with the term, okay? It has to do with what are you looking for and how are you demonstrating the inactivation or the

killing process and the starting versus ending process.

DR. WOLFINBARGER: See, I did -- years and years ago, we did -- within the Allowash process, we look at viral and bacterial log kill by each of the reagents used at the various steps in the total Allowash process.

In a sense, each of the reagents -- and that includes antibiotics within that process -- gave us the unique bacterial log kill mechanisms. We basically felt it okay to add all of those solution log kills. When we added on to the top of that the fact that the very early steps in the Allowash process clean -- by that I mean removes bone marrow in which bioparticles or bacteria would reside -- since we were able to show we removed 99.9 percent of that bone marrow, I always argued that I got a 3 log reduction by cleaning and anywhere from a 6 to 20 log kill by the reagents.

MS. HANSEN: Again, I think this is actually a good point for debate because when you think about what you're getting with the different parts, there's always different parts with regards to the treatment of the samples, right?

When you think about what's my initial population on the sample, you are looking for how to minimize that population. That's really what you're looking at with an antibiotic. You're minimizing it. You're minimizing it so that you're not having a proliferation over time.

So I look at it as a means of keeping it from having proliferation as opposed to an active reduction process because you're not going to have an active reduction process whereby you can say I've got an antibiotic and I've reduced my population by 3 log. I've got another step in the process and I've reduced it another 3 logs. I have another step in the process and reduced by another 3 logs. Therefore, if I add them together, I get a 9 log reduction.

That's not appropriate to look at that from a validation standpoint.

MS. MALARKEY: Joyce, I have a question back to you then.

We have heard that there are some products where that is the process. I mean that is what the extent of the process is for various reasons.

So how would you look at, then -- because there needs to be some expression of what is happening on -- David showed some curves of the effectiveness of the antimicrobial treatment. So I'm just curious.

MS. HANSEN: Again, part of it has to do with the validation mechanism and how you can add or not add sterility assurance levels or log reductions, okay?

It really has to do with how -- what you can prove from a disinfection standpoint.

AUDIENCE MEMBER: Can I just add a question to that? For the antibiotics, wouldn't it, in part, depend upon whether it's bacteriostatic or bactericidal?

DR. WOLFINBARGER: Absolutely.

AUDIENCE MEMBER: So it's not bactericidal. It's just bacteriostatic. It would just help keep the population down. But you couldn't really -- I mean you're not really killing them. You're just sort of keeping them from multiplying.

DR. WOLFINBARGER: Right, but -- see, I guess I don't mean to be a contrarian here. But when I look at processing protocols for the removal and/or killing

of viruses or bacteria or whatever in the tissue, if you think about bacteria, I don't really see how any process can ever give you a sterility level of 10 to the minus 6 because to do that you'd have to have at least a 12 log clearance kill.

So I've never thought of the Allowash process as giving any sort of sterility assurance level value but rather as a bioburden reduction through either cleaning and disinfection and/or disinfection.

Then for the sterility assurance level, I utilized the terminal sterilization where I basically can calculate what that sterility assurance level is for those tissues, based upon the fact that I'm going into terminal sterilization essentially with a culture negative population of tissues, meaning that, to me, I've always thought of culture negative tissues as kind of a probability as 1 in a thousand.

It's probably not really accurate, but culture negative means I can't culture anything off of the tissues. If you go in to that -- if you go in to terminal sterilization with culture negative tissues, you're able to get a sterility assurance level of 10 to

the minus 6 at a very low gamma radiation dose.

So you don't really cause harm to your tissues. But it's dependent on you having culture negative tissue to go in to that determination of an SAL.

MR. OSBORNE: Well, the reason I posed that question is not because -- I don't think there's a clear answer here. I believe this is probably an area whereby you have different ways of treating tissue. You have antibiotics. You have chemical disinfection. You have terminal sterilizations. So that I believe that here are -- we really need a definition as far as from the AATB perspective. I'm willing to tackle that challenge. But certainly those are some of the challenges that we face.

I know that's -- the antibiotics are really kind of a loaded question.

DR. WOLFINBARGER: Well, I know for much of my life in the tissue banking industry, LifeNet has always distributed what I always called culture negative tissue meaning that we couldn't culture any microorganisms off of representative grafts. But I was

never comfortable labeling those as sterile. So for years in the early days, tissues went out as culture negative.

It was only with the ability to implement terminal sterilization, where the graft's in the package that -- where we could achieve a sterility assurance level through a validated -- we use the -- I hate this -- ANSI, AAMI, ISO 111 37, and had to modify it to put in a media for anaerobes because that method was originally set for devices which -- and there's never -- I shouldn't say never -- there's never an anaerobe on a mechanical heart valve.

So we implemented additional culture requirements in there. But it was only after the implementation of the terminal sterilization that we ever put sterile on the packaging.

MS. HANSEN: I just want to go back to a comment that Joel made.

I do think this is an area that we need to fully define. In fact, in the medical device industry, we've talked about whether or not antibiotics give you reduced population, how should that be viewed, how

should it be viewed as a manufacturing step to reduce the proliferation of organisms from a static standpoint as opposed to a -cidal standpoint, and what does that mean to the manufacturing process, and what does it gain for you.

We've never actually gone in the medical device industry to defining that any farther because there's always been some discussion about what does it truly mean, how should you validate it, how should you validate a process that has an antibiotic. In fact, the medical device industry has typically taken out the antibiotic because of the potential for the impact on the samples to be negative or to demonstrate the negative growth of organisms; so therefore prevent us from being able to look at a log linear inactivate rate.

Now I think I personally would love to see this as be part of the thought process for how to move forward because can you add an antibiotic step that reduces, let's say, 3 logs or 2 logs or reduces the proliferation, and then can you then add an additional terminal sterilization? And from that starting

population following the antibiotic manufacturing step, can you then add it to 10 of the minus 3 or 10 to the minus 6?

I think that's something that needs to be fully explored.

MS. MALARKEY: We only have a few more minutes. So we have somebody at the microphone in the audience here.

AUDIENCE MEMBER: Thank you.

Joyce, I was listening to this dialogue, and it just hit me why it is this difference in opinion in that antibiotics cannot reduce or kill spores.

During the disinfection monitoring process, you're monitoring the reduction of the spore population where disinfectants can, in fact, kill spores to a lower level, which can be measured, whereas antibiotics primarily attack viral organisms in non-spore state. That's where they're most useful in reducing the population of a tissue when they're viable vegetative organisms present. But it cannot be used in terms of measuring the reduction of a spore population with disinfectants.

So both have their purpose, but I don't think one's exclusive of the other. It's just the non-ability of antibiotics to reduce spore populations.

MS. HANSEN: Agreed.

MS. MALARKEY: I think clearly we have something else to add to the list of -- Scott, did you -- anyway, well, there's just two minutes left.

So I would ask the audience if there are any more questions that they have. It looks like Martell is -- wants to say something.

MR. WINTERS: We've touched a little bit on number four with you guys, some of the new sterilization methodologies, but I just want to throw out there so you're aware from a radiation standpoint what else might be coming down.

There are a handful of us on the radiation working group of AAMI and ISO who are spending a lot of time and effort on developing new methodologies, which will allow for use of these much lower sterilization doses that can be applied for tissue and still allow the sterile label claim.

There are a couple of different approaches

we're taking to that. If we can get those details out, I'll see if I can't get someone to talk about that maybe in the spring meeting, to provide some details on our approaches there. But just be aware that that is the case.

MS. MALARKEY: Thank you for that.

Anyone else?

Well, it's been quite a day and a half, I would say. Thank you again to everyone for their participation, and particularly our esteemed panelists and speakers from the three sessions. Thank you, audience, for your participation.

I don't know if Celia would like to say any words of parting. But I would just say thank you very much.

DR. WITTEN: As you said, Mary, it's been a very excellent day for presentations and really great questions. I think it's given us a lot to think about, and, hopefully, also the tissue industry as well.

So I'll just add to her thanks, for the speakers, for our organizers, for the help that we got also from OCTMA in helping us put things together, and

to NIH for hosting this. Thank you.

(Whereupon the meeting was ended.)