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Processing of Orthopedic, Cardiovascular and Skin Allografts Workshop

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P R O C E E D I N G S

DR. WITTEN: First, I'd just like to introduce to myself and to welcome everybody who's come to this workshop today on the processing of orthopedic, cardiovascular and skin allografts.

I'm Dr. Celia Witten. I'm the office director at FDA of the Office of Cell Tissue and Gene Therapy, which is the office in the Center for Biologics Evaluation and Research that has the overall responsibility for administration of the program for tissue regulation in concert with other colleagues across the agency.

This workshop is co-sponsored by the Center for Biologics, the Center for Devices and Radiologic Health and ORA and FDA, as well as our sister agency, the Center for Disease Control.

I'd like to thank the organizing committee which spans, as I said, FDA as well as CDC. The members are listed in your program.

I'd like to thank the speakers who have come today and tomorrow to share their knowledge and experience over the next two days, which will help the

FDA and the industry move forward in enhancing tissue safety.

This topic is certainly of great importance. There’s upwards of one and a half million tissue transplants performed annually. These products overall are very safe, but it is critical for FDA and industry to continually re-examine how to enhance safety of these products, and that’s what we’re here for today.

There are several key components of safety, and we’re not going to talk about all of them. But one important consideration is processing methods. That is what the workshop today is focused on.

So this workshop is to share scientific information and gain knowledge of current practices concerning processing methods for orthopedic, cardiovascular and skin allografts.

We appreciate the opportunity to learn from the expertise in this room. The information we gather today and tomorrow will assist us in determining whether there’s a need for additional guidance in this area and point us in some direction for guidance development.

The workshop is going to be divided into three sessions. First, however, before those sessions start, there'll be an overview of the regulations provided by FDA.

The key areas for the three sessions will cover surgeon clinical practices, including expectations for the graft, experiences with processing effect on function and practice regarding graft management and assessment and management of adverse events.

The next session will focus on pre- and post-processing cultures from microorganisms, current methods and limitations, reliability and validation and standards in this area as well as scientific challenges and concerns regarding current culturing methods and potential future scientific methods development.

The last session will focus on disinfection and sterilization of tissues, definitions of terms and challenges and concerns in process validation.

Each of the sessions will have a couple of speakers followed by a panel discussion. This is an ambitious agenda. It's an important topic for us and

for public health. We have a distinguished panel of experts in the field. I'm looking forward to a productive two days.

Now I'm going to turn it over to Dr. Ruth Solomon to give a little housekeeping details and also introduce our first speaker.

DR. SOLOMON: Thank you, Celia.

I'm Dr. Ruth Solomon. I'm the division director of the Division of Human Tissues in the Office of Cell Tissue and Gene Therapies.

I would also like to welcome you to this workshop this morning. I would like to give you just some housekeeping information. First of all, you may have noticed the rest rooms are right outside, women to the right, men to the left.

There will be breaks at 10:30 this morning and 3:00 p.m. this afternoon. Lunch is approximately at 12:15. It's lunch on your own. There's a cafeteria one flight down and a café also, and another smaller cafeteria one flight up.

Also, we will be taping the sessions today and we will be making a transcript. So I'd appreciate

if when you do talk, you would introduce yourself first.

As Dr. Witten said, there are going to be three sessions. Each one will be followed by a panel discussion. We'd like these panel discussions to be interactive. Therefore, you may find in your folder -- or they're available at the desk outside during the break -- these index cards on which you can write any questions for the speakers.

Then Dr. Melissa Greenwald and Dr. Laura St. Martin -- they're the women in uniform here -- will be coming up and down the aisles to pick up the cards.

It's my pleasure to introduce Leslie Kux. She is the deputy director of the Office of Compliance and Biologic Quality. Thank you.

MS. KUX: Can everybody hear me?

Yes, thank you, Melissa.

Good morning. Like Dr. Solomon and Dr. Witten, I'm also very pleased to welcome everybody here today to our processing workshop.

We are really looking forward to learning

more about the needs and expectation of the surgeons who use human tissue, or the terminology that I will probably be using is HCT/Ps, as that's what we call them at FDA, and also learning about some of the processing challenges here.

My job is to give you guys an overview of the FDA regulations to set the stage and then to focus in a little more detail on the regulations that apply to HCT processing and establish the regulatory requirements for processing operations. I'm not planning on taking very long because, like the rest of you, I want to get to the panel.

So the purpose of our regulations is to prevent the introduction, transmission or spread of communicable disease. There are really two means to this end. The regulations focus on two means.

The first is preventing the use of tissues from ineligible donors and then also preventing improper handling or processing that might contaminate tissues. It's very simple, a straightforward goal. It's focused on communicable disease.

So what is a communicable disease? Well, it

is a disease that is transmitted by viruses, bacteria, fungi, parasites and TSE agents. The potential sources are obviously donors. But in addition to donors, something that's important from a processing standpoint, it includes the environment, including the processing environment, as well as equipment and utensils and other materials used in processing and materials used for shipping and storage and packaging.

So we're not -- the regulations and the processing regulations in particular, manufacturing regulations, focus not just on donors but also on other possible sources of contamination and other means of spreading contamination.

The regulations consist of several parts. The first is registration of manufacturers. This is obviously an information gathering tool for FDA, so we know basic information about who's manufacturing human tissue and what activities the firm engage in.

In our regulations, manufacturing is very broadly defined to include recovery, processing, storage, labeling, distribution, screening, testing, a whole range of things. Processing is just one part of

that.

Obviously, a key component of the regulations is donor eligibility. It’s a -- we have a two-pronged approach in the regulation. There’s donor screening to screen donors for risk factors for clinical evidence of relevant communicable disease agents and diseases. Then as well donor testing must be done for certain relevant communicable diseases.

As part of that testing, the tests must be licensed, approved or cleared by FDA and have to be used in accordance with their label directions to make sure that the test results are as accurate as they can be.

We have extensive donor eligibility guidance that we’ve issued, and you can find that on our Web site if you’re interested in more information on that particular point.

We have current good tissue practices, which is obviously something that processors would be interested in. The GTPs, as we call them, address the methods, controls, facilities and are intended to focus on the prevention of communicable disease transmission.

Again, the purpose is to ensure that HCT/Ps don't contain communicable disease agents, aren't contaminated, and do not become contaminated during manufacturing. But we have designed the regulations to give manufacturers and processors flexibility to meet these goals by development of their own standard operating procedures.

We do have reporting requirements with respect to adverse reactions and HCT deviations. On adverse reactions, manufacturers or establishments are required to investigate adverse reactions, again, limited to communicable disease issues, and then report certain adverse reactions to us. Deviations as well are required to be reported.

There are also product labeling requirements, basic information about product, the information about the establishment that manufactures the products, storage information, some usage information; but also, if necessary, information about the communicable disease risks associated with the product.

Then there are inspection and enforcement provisions. These provisions are basic. They give FDA

the authority to inspect manufacturing establishments. Then there are some enforcement provisions that authorize FDA to take certain administrative enforcement actions in the event that we find a violation of the regulations.

So that’s the very basic overview of the regulations in their entirety. Now I’m going to talk about the regulations that really key on processing.

So what is processing? Our regulations define processing as any activity -- in the negative -- any activity that’s not any of the other things that constitute manufacturing.

So it’s not activity that’s not recovery, not donor screening, not donor testing, not storage, not labeling, not packaging, not distribution.

It does include testing for microorganisms, very important for HCT/P communicable disease safety; preparation; sterilization; steps to inactivate or remove -- I can’t say that word this early in the morning -- adventitious agents; preservation for storage; and removal from storage. So a wide range of activities that are a subset of manufacturing.

The basic requirements for processing and process controls are not surprising in light of the communicable disease focus of the regulations. The regulations specify that you should -- that processors must process HCT/Ps in ways that do not cause contamination or cross-contamination during processing and that prevent the introduction, transmission or spread of communicable disease through the use of the HCT/P.

Just want to remind everybody that, as we stated in the preamble to the final rule, these provisions don't require sterility. But we do expect processors to use aseptic techniques to control the risks associated with communicable disease agents.

Obviously this afternoon and tomorrow, the rest of this workshop will be talking a lot more about issues associated with sterility and what expectations are around sterility.

Then, pooling tissue from more than one donor is prohibited during processing. Pooling is what you might think it is, placing in physical contact or mixing in a single container tissues from more than one

donor for obvious communicable disease reasons.

In process control and testing, the regulations require that you must -- processors must ensure that specified requirements in process controls are met, and that the HCT/Ps during processing are controlled until the necessary inspection and tests or other verification activities have been completed and the tissues -- that it’s been confirmed that the tissue meets its requirements.

This means that processors need to establish or should establish mechanisms to control and monitor the process to ensure that the processing isn’t causing contamination or cross-contamination and does take steps to prevent the spread of communicable disease from the HCT/P.

Then as a corollary, the sampling of in-process HCT/Ps must be representative of the material to be evaluated. One issue associated with sampling of HCT/Ps is how to ensure that they’re representative. That’s an area that we’re always interested in, since there can be difficulties associated with that.

Process validation; fairly straightforward. With respect to HCT/Ps, process validation is focused again on communicable disease issues, but it’s not a new concept in the medical manufacturing world.

But if you can’t verify that your process does not cause contamination or cross-contamination and prevent the introduction, transmission or spread of communicable disease, then you have to validate and approve the process according to established procedures.

With respect to written representations, any written representation that processing methods reduce the risk of communicable disease by an HCT/P, including sterility or inactivation, must be based on fully verified or validated process. Again, that’s something that will be covered as we go through the panels today and tomorrow.

Then we get to what is validation and what is verification. As you can imagine, since we’re FDA, we defined these terms in our regulation. Validation is confirmation by examination and provision of objective evidence that particular requirements can be

consistently filled.

So with respect to HCT/Ps, you're just looking at whether your HCT/P -- the processing produces an HCT/P that consistently meets its predetermined specifications. This is an activity that is performed before you put your process in place and start your manufacturing. This is a prospective activity.

Verification, on the other hand, is confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. So verification that a process has produced HCT/Ps that meet specifications is performed after it's completed and needs to cover all of the products that are the subject of that process. So validation before, verification, during/after.

Not surprisingly, our regulations also address what processors should do if they change part of their process; fairly straightforward expectation, that if you change part of your process, you have to go back and validate or verify the change. This is obviously to ensure that any change does not have an

adverse impact elsewhere in your operation with respect to communicable diseases, doesn't change the risks.

Those are the regulations that apply specifically to processing, but there are also a series of regulations that apply across the board but are very important to the processing arena to ensure that communicable disease risks are controlled or eliminated. I'm just going to run through them because I didn't feel like it would be a complete overview without highlighting them.

We have regulations that deal with the requirements for facilities, including processing facilities. They address the facility itself. They talk about cleaning and sanitation requirements, setting up procedures for those two activities.

They specify the documentation and recordkeeping requirements and deal with control of the facility operations. So that's fairly straightforward.

Then with respect to the equipment that's used during manufacturing, including processing, again we have regulations that specify some baseline requirements. Again, to me, they seem fairly

straightforward.

You want your equipment not to cause further problems. So the regulations specify that the equipments needs to be of appropriate design, suitably located, and installed. Again, requirements with respect to cleaning, sanitization and maintenance and requirements with respect to establishing the procedures and schedules for those activities.

This also -- I want to emphasize -- applies to utensils as well as big processing equipment, the little things you use during processing as well. There are also regulations with respect to calibration.

On recordkeeping, there are recordkeeping requirements. It’s important to note that the recordkeeping must also identify the equipment that’s used in the processing of each HCT/P. That’s for traceability purposes, obviously.

Environment control, another very important aspect of controlling the processing operation to ensure that there’s not contamination or cross-contamination. We want the processing environment to be controlled so that it isn’t likely to

result in contamination of the HCT/Ps or of the equipment which could then contaminate the HCT/Ps.

So the regulations specify what to look at. The regulations do provide that -- depends on the environment and the operation and the tissues. So you have to assess what’s appropriate for your particular operation and your particular product. Things like temperature and humidity, ventilation and air filtration, the cleaning and disinfecting of rooms and equipment when you’re engaging in aseptic processing operations or aseptic technique and maintaining equipment used to control your environment.

Environmental monitoring, a corollary, important part of controlling your environment. We want to make sure that people monitor their environment to make sure that it’s not introducing communicable disease agents into the HCT/P or onto the equipment.

Then supplies and reagents, again, we want to make sure that what goes into the processing operation, the supplies you use in the processing operation and the materials you use, have to have been verified to meet specifications designed to prevent circumstances

that increase the risk of communicable disease. Reagents must be sterile where that's appropriate. If you're producing your reagents in-house, then you have to be validated or verified.

So that is the overview. We can move on to the much more interesting part of the day. Thank you very much.

DR. SOLOMON: Okay. Now that we've had the introduction, we're going to be starting Session 1. Session 1 will focus on the clinical practices.

We're going to have three speakers, surgeons who will talk about their expectations of the tissue that they use for their patients.

We sent the three speakers a set of questions to focus on. I'll just briefly review what those are.

The first is when receiving a tissue allograft in the operating room, what are the expectations and assumptions about its sterility and functionality? Would there be certain microorganisms that would preclude the use of the graft if it were not sterile?

Two, what are the data regarding the effect

of various processing methods on the function? And that two parts -- are there concerns that certain processes to decontaminate or sterilize the graft will have an effect on its function? Are there grafts that would not be used because they had been processed in a certain way?

Third, if the graft cannot be sterilized, what are the minimum expectations?

Four, is the graft cultured prior to implantation? If so, is this performed in the operating room? If the culture comes back positive, what actions do you take? Do you perform antibiotic soaks or other procedures on the tissue in the operating room?

We'd also like to talk about adverse reactions after the tissue's implanted. So what have been the experiences with infectious adverse reactions? Have any been attributed to the graft?

And a very important question, how do you evaluate potential infectious adverse reactions, meaning how do you evaluate if the tissue caused the reaction and how do you decide when to get back to the

tissue bank to alert them that there has been a reaction?

Next, do factors such as availability of a particular graft type or whether the graft is life-saving versus life-enhancing influence the decision to accept a graft?

Then in our panel discussion, we’ve laid out some preliminary questions and we hope that the audience will also have more questions for the speakers.

Would you implant an allograft that is known to be contaminated and why? When should a tissue be labeled sterile? Should labeling the package inserts specify the processing methods in more detail than they currently do?

So our first speaker will be Dr. Sam Doppelt. Dr. Doppelt received his medical degree from the University of Chicago School of Medicine. His post-doctoral residency in orthopedic surgery was at the University of North Carolina in Chapel Hill.

He was a clinical and research fellow in orthopedic surgery and endocrinology in the Department

of Medicine at Mass General. Dr. Doppelt serves as a chief, Department of Orthopedic Surgery at the Cambridge Health Alliance. Additionally, he is an associate orthopedic surgeon at Mass General Hospital.

His practice is in general orthopedic surgery and arthroscopic surgery. He is an assistant professor of orthopedics and medicine at Harvard Medical School.

Dr. Doppelt served in a number of positions in the American Association of Tissue Banks, including president. He has also been an invited member of the Blood Products Advisory Committee for the FDA.

For the past 18 years, he has been a consultant to the International Atomic Energy Agency for their tissue banking program for the use of radiation sterilization of tissue allografts.

He practices general orthopedic surgery and uses allograft tissue routinely.

Dr. Doppelt.

DR. DOPPELT: Okay. Thank you. Can you hear me?

Well, I'd like to thank the FDA and CDC for putting this very interesting program together and also

thank them for allowing me to present some of my thoughts regarding musculoskeletal tissue.

I want to spend most of the time addressing these issues, the questions that have been proposed to the three clinicians that are going to speak. But being a surgeon, I have to show a few examples to put things in perspective.

I'm sure you're going to hear more from Scott Brubaker about the total number of allografts that have been used, but according to the AATB survey, in 2003, there were almost 1.3 million grafts distributed.

You can see the various types: soft tissue, either bone-tendon-bone for ACLs, Achilles tendon for ACLs and PCL, fascia lata for all sorts of soft tissue reconstructions, demineralized bone, cancellous cubes, spinal grafts or proprietary preparation, traditional spinal grafts and large segment allografts.

The number is fairly large. It's been growing at about 15 percent a year. So according to my rough back-off-the-envelope calculations, we're probably at about 1.9 million pieces of tissue

distributed currently.

I don’t want to spend a lot of time on advantages and disadvantages of allograft tissue. But I would point out that for the autograft, there’s the issue of morbidity of the graft site where you don’t have that with allograft tissue.

We’re now seeing actually a number of patients who have had ACLs and they have another ACL on their other side that needs to be reconstructed. If they’ve had problems with donor site on one side, they actually come in requesting that the surgeon use an allograft and not take tissue from their other knee. Problems can be fractures of the patella, ruptures of the tendon.

One of the issues is that with an autograft -- for example, with an ACL, you can only take a certain amount of tissue, the mid-third, one-third of the patellar tendon, whereas with an allograft, you can use a hemi-tendon. You can use a much larger piece of tissue, which the net result is that there’s a larger cross-sectional area.

So when we get to the issue of is there a

problem with structure and strength, if you believe that to be the case, you can partially compensate for that by using a larger graft; and, of course, the issue of transmission of disease, which we'll hear a lot more about.

Now, some of the physicians early on who popularized the use of allograft tissue in orthopedic surgery were the tumor surgeons because here, for example, is a patient that has a lytic lesion in the distal femur and it turns out to be a giant cell tumor. And in the old days, you used to sort of curette this out and then pack it with bone. But you can see because of the irregular inner surface, it's very difficult to get all of the aggressive cells. So the best way to do it is to remove the entire thing.

But once you remove it, you've got to put something back that's functioning and here was a hemi-condyle. This provides two things. Number one, there's some structural integrity. Number two, there's a joint surface with viable cartilage cells. I emphasize viable.

Number three, there is some biology at work

here because you can see that it doesn't quite fit and there's a sharp step-off. But with time, this thing heals and part of this bone by creeping substitution is replaced by new host bone. So it's partly allograft and partly new host bone.

Here is an example of what I call an "oops" procedure. When you're doing a hip replacement and you're putting a stem down and you have to ream the canal, if you accidentally go out the cortex, that's a problem. You can use the strut allograft.

You can see here a strut allograft was placed and held in place with wires, and again you see some biology that over time this graft provides structural stability. It is osteoconductive and eventually a part of that becomes new living host bone giving additional strength to the construct.

Here was another patient of mine that had a similar loose stem. It has to come out. We thought we might do strut grafts. But it turns out when you got inside, there was like practically no bone left, very thin cortex, no way to reconstruct it.

So the whole thing was removed. Here's the

acetabulum. Here's the rest of the femur. The upper femur was completely removed. You won't see tissue like this anymore. This goes back maybe 15 years or more. But you can see there's some soft tissue here.

But here was the proximal femur that's being replaced with a step-cut to give rotational stability. Here's how it fits in the OR. Then afterwards, you can see here's the construct, a little flake of bone that we put on that was the patient's own host bone. But it provides structural stability and incorporation through creeping substitution. So you expect strength and you expect certain biologic properties.

This was another patient of mine who was riding a motorcycle, fell off the motorcycle on the highway and hit a guardrail which laid his leg open like a machete or a Samurai.

This is actually his femur which the distal end was cut in half. Here's a piece of the condyle. Somewhere in here his patella was cut in half and the upper end of the tibia was cut in half.

So basically his whole leg was filleted open. This is obviously not a very clean wound. Fortunately,

for him there was preservation of the neurovascular bundle. So that theoretically makes the extremity reconstructable because he's got a blood supply and he has functioning nerves.

The EMTs were kind enough to bring the other body parts that were left on the highway, but we decided not to use that. Initially, he was reconstructed here with a plate. The patella was put back together with wires, the tibia with plates and screws. The fracture was here.

The distal femur was cut in half. There was a huge defect here. So with a dirty wound, you don't want to throw in a lot of dead bone. But he was initially treated with antibiotics, made sure that the wound was clean. Then we came back later and packed this.

We expect that this bone will eventually heal. It will provide some structural stability because if it does not, this hardware will not last forever. The hardware will fail if you don't get bony reconstitution to absorb the stresses.

He did, in point of fact, at one point

fracture his plate and required a second bone grafting procedure with allograft again but did go on to heal.

Here is another patient who had another motorcycle accident years ago. He had a rod put in his femur. This was an open fracture. But he subsequently developed a chronic osteomyelitis with drainage. This had been curetted a number of times and treated with antibiotics, but he still had recurrent infection.

The disconcerting concept in orthopedics is once osteo, always osteo. So if you have an infection, it is virtually impossible to remove this by curetting or treating with antibiotics. The only thing you can do is remove the entire segment that has infection deep within it.

But then what do you do? Now you’ve got a femur that’s discontinuous. He was treated with methyl methacrylate and a plate initially until we could sterilize the bed with antibiotics for six weeks. Then we came back, took this out, put in an allograft.

You can see here there’s an allograft from here to here and then a rod. So this provides structural support but also biology. There is some

healing at the osteosynthesis site here, a little less so here.

In point of fact, he developed a nonunion and required a second bone grafting procedure but did go on to heal. So you're expecting structural integrity and certain biology to make this construct successful.

Here is just a cartoon with what we do with anterior cruciate ligaments when you have a tear of the anterior cruciate.

This is a PCL, the posterior cruciate. You need a soft tissue that goes from the femur through the tunnel, through the notch, and down into the tibia. One of the common ones is a bone-tendon-bone allograft, but you can use hamstring and so forth.

Again, if you're going to take your own patellar tendon, you can only take the central third. If you take more, you're going to rupture the patellar tendon.

So when you use an allograft, you can make a bone plug, number one, that is cylindrical. You can't do that in your own tissue. It's cylindrical so it heals faster.

Number two, this is a graft. The patellar tendon is flat and sort of in cross-section. It's a rectangle. But then you take a larger section of the tendon and you tubulate it and turn it on itself. So the cross-sectional area winds up being much more than you would have with your own one-third patellar tendon.

So again, when we get to the concept of if you believe that there is some loss of structural integrity, you may be able to partially compensate by using a larger graft.

So turning to the questions -- I wanted to go through that fairly quickly. But turning to the questions, when receiving tissue in the operating room, what are the expectations and assumptions regarding sterility? And unfortunately, there's a lot of sort of misunderstanding and misconceptions both by the medical community and by the public at large. That's one of the things that we're going to discuss.

But prior to 2001 and the CDC reports of allograft associated infections with the clostridia infection and the death of a patient, most people thought of grafts as being sterilely procured and

sterilely processed.

We use the term now aseptic, but the term sterile was always thrown around. If you look in the dictionary, what does sterile mean to the common person? Webster's definition is entirely free from germs of all kinds. That's great.

What's the definition of sterilized? To make free from germs. So the grafts are sterile and they're perfectly safe and there's no problem. That's basically what patients were told. It's sterile, no problem.

Then we find out in 2001, well, there is a problem. After 2001, what do you think? Well, we now know the tissue may be contaminated. From various reviews and so forth, the general concept is that ATB, accredited banks, were preferred because they were following at least some established rules. But still that doesn't give you any guarantee.

We also know from the LifeNet experience where there was distributed tissue that was processed and not processed, that the processed tissue did not transmit disease but the unprocessed tissue did.

So, number one, you want to use an accredited bank. Number two, processing is good because it's going to reduce your risk.

Well, how much does it reduce your risk? That's where you get into a little gray area because, at least for myself and some people in the -- most people now in the orthopedic community will divide things into three tiers in terms of risk level.

The low risk tissue is something, for example, that's all bone, cubes or morselized bone. It's heavily processed. There's no marrow elements. The banks now have an algorithm where they can measure the log reduction in bacterial contamination. So the risk here is very, very, very low.

Less so with the medium risk tissues that have soft tissue. Now you may have bone-tendon-bone, but there's still a soft tissue tendon, hamstrings. They are processed, but not as heavily processed as the all bone tissue. So there is some greater risk with that tissue because you can't process it the same way as an all bone piece of tissue.

The high risk is fresh tissue. If you have a

meniscus or osteochondral where it’s fresh, it may be in some culture medium until it can be implanted for up to three weeks or so. There’s really no processing other than washing and bathing in antibiotic solutions. You’re relying on your screening and you hope for the best. So there’s less of a guarantee with that kind of a tissue.

So what are the additional expectations regarding sterility?

Well, there have been a whole bunch of studies on different graft rates of infection and so forth. Here’s one that’s been quoted a number of times.

But it’s interesting in that for ACL reconstructions -- this was done in an outpatient surgery center reported by Crawford, et al. -- there are 331 patients. And of the patients that had allografts that were processed, but not as defined by the bank sterilized, they had a 4.4 percent infection rate.

Of the autografts, there were no infections and of -- I forget. There were about 45 or so

allografts that were, quote, “sterilized.” They did not transmit disease. There was no infection.

So processing again -- or sterilization using radiation seems to provide some additional margin of safety. However, in this study there were a number of things that were unaccounted for. You’ll find this in all of the studies because there are different banks being used, different grafts, different processing techniques.

But the general conclusion is and remains to be that sterilization and processing is of value if you’re talking about sterility and safety.

So what are further expectations? Processing is of value. The low risk -- if you label it sterile, people are going to say that means it is free of germs. Your risk is zero.

Unprocessed tissue is of greater risk. There are other alternatives to the clinician because of this greater risk. So, for example, in an osteochondral defect on the -- let’s say on your femoral condyle, you might want to use fresh osteochondral grafts, but that’s a high-risk tissue. How do you get around that?

Well, fresh frozen offers maybe some margin of safety but not much more; but popular, is called an OATS, an osteoarticular transplant, in which it’s like moving a golf tee hole. You take a core of bone and cartilage from one part of the knee that’s less important and transplant into the part and the weight-bearing surface that is more important because that’s where you’re going to develop arthritis.

So it’s an auto-osteocartilaginous autograft going from one part of the knee to the other. People would prefer to do that. If they have a larger defect, they make multiple plugs. It looks like a mosaic.

It’s not the -- it does work, but there are some problems with that as well. But people turn to other alternatives if you’re concerned about the risk of infection with the fresh tissue.

Continuing with sterility, in industry you define -- they use a sterility -- a concept of sterility assurance level. The sterility assurance level is usually 10 to minus 6 or less, which means that the probability that one product in a million will have a contaminating organism or agent.

The tissue banks now strive for the same or better. Most surgeons, however, think of sterility as an absolute. They don't think of it in terms of a probability.

But let's just see why that is the case. They don't have a concept of sterility assurance level. Let's take an orthopedic surgeon looking at national data that probably the average number of cases they're going to do is about 350.

So you do 350 cases. Let's assume that every case is an allograft, which, of course, isn't the case. Let's assume that the surgeon is going to practice for 40 years. He finishes training at 30, practices till 70. I don't know. It's pushing it, but 70.

So if you practice 40 years, 350 cases a year, that's 14,000 cases in your career. If you have 14,000 cases times the probability of 1 times 10 to the minus 6, in your career, in your lifetime of treating patients, you're going to have .014 infections related to this problem if you reach that sterility assurance level. So for practical purposes in their mind, the probability is zero.

Further expectations regarding functionality, for bone we expect the surface to be osteoconductive. It may not be very osteoinductive to induce bone but it is osteoconductive.

It’s not rejected because it’s mostly acellular and will demonstrate remodeling with what’s called creeping substitution, where some of the bone is resorped and then you make some more bone. Some is resorped and then you make some more bone. Eventually part of it becomes a new living host tissue. That’s good. As long as it’s living, it can respond to stress and heal microfractures and so forth.

For strut grafts, you expect it to maintain its mechanical strength and act as a support as I showed you in that oops procedure. But also, there has to be some osteoconduction because if it just sits there, then you might as well use a stainless steel plate rather than a bone. So you expect stability, mechanical strength and some biology.

For soft tissue grafts, like an ACL, you expect it to maintain certain tensile strength, close to normal, and also biology that the tissue will be

repopulated with the host fibroblasts, which will synthesize new collagen. And in point of fact, that eventually becomes the person's own real ligament. It's totally replaced, but it becomes theirs with their cells and their own produced collagen.

For a fresh frozen cartilage graft, like you saw in the first case, the tumor case, we expect some viability, but it's rather poor. It's usually about 40 percent or less.

The proteoglycans remain. But if the cells are dying, then it's not going to be as durable as host cartilage. So the bone may stay, but eventually the joint cartilage will start to disappear and won't be as durable. You'll develop some chondromalacia and loss of cartilage.

For fresh grafts such as a meniscus, you expect it to function much like a normal meniscus. It may although there are failures. You can get tearing of the meniscus. Maybe it's because some of the cells don't survive.

Now if you look at anterior cruciate ligaments, a normal ACL has 2160 Newtons to failure.

That’s the strength of it. If you take a 10 millimeter bone-tendon-bone that actually exceeds that so it’s a good graft, it’s an autograft. With quadriceps-tendon, you can get a little bit higher or close. With quadruple hamstring, you can get even higher.

If you take a 10 millimeter bone-tendon-bone allograft, you can actually increase the surface area, the cross-sectional area by using a larger graft. You can take a 10 millimeter bone, but you can have the tendon much larger. So you can actually -- by increasing the cross-sectional area, you can increase the strength. That’s what you do with the quadruple hamstring.

So if you believe that the tensile strength is impaired in some way by the processing or radiation, then there are some ways of overcoming that by making the graft a little bit bigger. You can do that with an allograft. You can’t do that with your own autograft.

So again, with functionality, demineralized bone, what are the expectations? Well, number one, you expect it to retain its osteoinductive potential. That’s what you expect.

Most people, surgeons, believe that it’s like taking a jar of mustard off the shelf. They’re all the same. So all preparations are the same.

What isn’t appreciated is that the bioactivity measurements are based on in vivo, usually mouse models or in vitro models. Many exist. There is no universally accepted model for measuring the bioactivity.

There is no assurance of the potency from batch to batch. So you can’t compare one company preparation with another. They simply aren’t all the same.

But when you’ve got it in the bottle and you use it, you don’t really know what you have. Okay? If it fails, is it the product or is it the patient? Because in all of these healing processes, one important part is the tissue bed, the vascularity and so forth.

So there’s a little conundrum here. Why did it fail? Was it the product or the patient?

So what effects do we have with processing methods on function? Well, first of all, processing

reduces the bacterial load. Each processing step, as you will hear later, can reduce the bioburden by a log reduction. The log reductions are additive. That's all good news.

But what are the adverse effects of the tissue if it's done too vigorously? Well, with bone without soft tissue, it's heavily processed but there don't seem to be many adverse reactions or adverse effects, at least from the current processing methods.

Soft tissues will be damaged if they're heavily processed and therefore less harsh methods have to be used. So your sterility assurance goes down, but you maintain the tissue.

For fresh, you can't process it much at all in any meaningful way, as I had mentioned before. So again, your three tiers of risks and processing levels.

What are the effects of radiation? Well, if you add -- it will add an additional log reduction to bacterial and viral contamination. That's the good news.

The bad news is that the D10 for bacteria is much less than that for viruses. So if you're trying

to get rid of the viruses, you're going to wind up cooking the tissue with radiation. So that's not going to work very well.

If you use higher doses of radiation, you will denature the proteins. That will have a negative effect on the structural integrity on the usual things, tensile strength, bending, rotation.

These bad effects are dose dependent. Most of the studies that are available, the tested doses range between 10 and 70 kiloGrays. Most common use in the United States is between 10 and 25 kiloGrays. So you're not getting up to those high doses. But this is the range that we commonly see.

What are the effects of radiation? To avoid adverse effects on strength, it's generally accepted that you have to have less than 18 kiloGrays is probably okay. Somewhere between less than 15 to 18 is probably okay with minimal effect.

However, we'll come back to this point that it also depends upon how it is done. I'll come back to that.

But use of low temperature radiation is

preferred because that can add an additional margin of safety when you get to higher doses. There’s less damage if you irradiate it with lower temperatures. Also, popularized, if you use cryoprotectants, that may also help protect the tissue against the denaturation and producing free radicals in the tissue while still killing the infectious agent.

I just use Clearant as one example. But they use 50 -- I think they’re still using 50 kiloGrays. They use some cryoprotectants. There are other methodologies out there as well.

So you can’t just say this was irradiated at this dose and compare it to somebody else without saying what are the other conditions. Was it low temperature? Were cryoprotectants used and so forth?

So it gets a little muddled. The surgeon has this piece of bone in the operating room. It says it was sterilized with radiation. So what does he know?

What are the generally held sentiments? Well, they vary. Depending upon what you know and what information you may know from the tissue bank -- and this comes back to the issue of how much information do

you want to have on your labels. But I want only irradiated tissue. It is safer with minimal adverse effects on mechanical strength. That's what I want.

But you talk to some other surgeons and they'll say I wouldn't touch that with a ten-foot cattle prod. I don't want irradiated tissue. It's more likely to fail.

If you go to the literature, you will find studies that say -- that support both points of view. What they don't -- but what you have to figure out is what are the conditions under which the radiation was done, the dosage, the temperature, the cryoprotectants and so forth.

Donor morbidity must also be taken into account. As I mentioned, we're now seeing a number of patients who are coming in for second and third operations and they actually often prefer allograft.

When you look in the literature, again, this dosimetry business is important.

So when you say you irradiated it with a certain dose, really what the tissue is seeing is that there is a range. It's not just a single dose, but

it’s a range. Different parts of the tissue have higher or lower exposure to radiation.

Depending upon how you do your dose mapping and dosimetry, a narrower range can be achieved which means you can use a lower dose because the whole range will be shrunk down toward what your target value for radiation exposure is.

Unfortunately, clinical studies on failure rates usually lump all of the irradiated tissue together irrespective of the dose, temperature, conditions, use of cryoprotectants. So you have a hodgepodge. You can’t figure it out.

There are clinical studies that support and refute the conclusions that irradiated tissue is more likely to fail. You can find any -- you’ll find whatever you want to support your point of view.

There are clinical studies. Then there are also biomechanical studies. The biomechanical studies are usually more likely to take these other factors into account because they’re controlled. It’s a prospective study. They probably do have some significant predictive value in terms of the strength.

So you can extrapolate from those studies to the clinical use. Again, 18 kiloGrays or less is probably -- under reasonable conditions will have negligible effects on the strength and is probably preferred. I would prefer to use irradiated tissue than not.

Again, I come back to the point that if you're creative and think about what it is you're doing in the operating room, you may be able to negate the negative effects of radiation by using a larger graft.

So what are some of the methods? Ethylene oxide used to be used. It was commonly used. It was associated, however, unfortunately, with synovitis due to ethylene oxide residuals and producing synovitis and inflammatory response, and eventually failure of the tissue.

So it's not really used anymore. As a matter of fact, ethylene oxide is hardly used for anything anymore because it's dangerous and there are other better sterilizing methods, even for instruments.

So are there concerns that certain processes to decontaminate or sterilize the tissue will have an

effect on function? The answer: Absolutely yes. The primary concern is that of radiation. It varies from very favorable to very negative.

In general, it’s viewed that processing methodologies are less of an issue and ethylene oxide is out. That’s history.

Are there grafts that would not be used because they were processed in a certain way? Yes. Again, it’s the same answer. Radiation, yes, no, it depends upon your point of view and ethylene oxide, absolutely not.

If the graft cannot be sterilized, what are the minimum expectations? This would apply primarily to fresh minimally processed tissues such as menisci and fresh osteochondral allografts. At a minimum, you would expect all final cultures to be negative.

What we also now know since 2001 is that you need to take into account bacteriostasis and fungistasis, which I think is now uniformly done but wasn’t before.

You’re expecting the cells to remain viable after transplantation. That’s your expectation;

otherwise, it’s not going to work.

Is the graft cultured prior to implantation and if so, where? Pre-implantation cultures, the answer is, again, a yes and a no. Some people do it and some people don’t.

Where would it be done? It would be done in the operating room when the graft is passed off from the circulating nurse to the scrub tech and then reconstituted. Being constituted means either defrosting and/or rehydrating before any antibiotics are added.

What would they do? They would take a surface swab. But we know that surface swabs are inferior to other methods of culturing. Bone segment immersion in media, direct inoculation, indirect washing with sonication or complete tissue immersion, or ultimately destruction of companion tissue testing, those are better methods than just a surface swab. But that’s pretty much all you’ve got.

What may not often be done is when the scrub tech is receiving the tissue from the nurse, they need to change their gloves prior to handling the graft

because the surgeon’s hands from the wound has contaminated, in the broadest sense, the instruments which are now in the scrub tech’s hands. Now he picks up the allograft.

So you talk about cross-contamination. He’s got to change -- he doesn’t have to change his gown, but he’s got to change his gloves to receive it, reconstitute it and take a swab.

It’s generally accepted that the results of in-OR cultures are difficult to interpret because even if you take a surgical wound that’s supposedly, quote, “sterile,” and you just willy-nilly send a bunch of cultures; you’re going to find some that are going to come up positive.

What does that mean? The patient did fine. There was never any clinical infection.

If you send a culture, the results are not going to come back for 24 to 48 hours at a minimum or possibly more. It could be three or four days, depending upon what you retrieve and what the bioburden is.

I’ve been down this road with our ID

consultants. You turn to the ID consultant and say is it real or is it a contaminant?

Now, orthopedic surgeons have -- for better or for worse, we have a lot of experience with wound and joint cultures where we see both false positives and skin contaminants. We often don't treat. Our ID people tell us these surface swabs, you got to get a deep culture if you want to really know what's going on.

So the question is -- the point is that some people do and some people don't. If you do -- if you come back with a positive culture, what do you do? Then you turn to your ID person and the answer is what do you do? You sit there and you scratch your head because now you've got this difficult conundrum.

Well, we started with antibiotics, which, elective surgery, you're giving the patients antibiotics. For virtually all surgeries, you're giving them antibiotics 24 -- starting an hour before the surgery and continue for 24 hours for a routine elective surgery.

What do you do? Do you restart the

antibiotics? First of all, you're restarting it maybe two or three days later. Now you're going to say what is it I'm treating. Am I treating something growing in a Petri dish or am I treating a clinical infection?

It's no small matter because if you make the leap of faith that it is an infection, then you are obligating yourself to a second OR procedure where you have to do a washout at a minimum. This becomes a big deal for a questionable indication.

So now you're sitting there. You're not sleeping at night.

Do you perform antibiotic soaks or other procedures on the tissue in the operating room? The answer is yes, usually with Bacitracin and polymyxin, which is the same solution that we often irrigate wounds at, at the end of the procedure.

The tissue is soaked from the time it's reconstituted until the time of implantation. The graft is trimmed and -- to make it fit and so forth. I suppose rather than taking a surface swab, you could send a piece of companion tissue. But that is not commonly done. If you sent it to our hospital

laboratory, they probably wouldn't know what to do with it. But it would probably be better than a surface swab.

So what's been the experience with infectious adverse reactions and have they been attributed to the graft? Most infections that we see are probably not allograft associated. Some are, but most are not.

There's a number of contributing factors to whether you get a wound infection or not. Number one, you can see from the joint reconstructions and the trauma cases and so forth, you're talking about the size of the wound; the amount of devitalized, avascular tissue; the length of the procedure.

We know from general orthopedic procedures, the longer it is, the more likely you're going to have an infection. Did you use the pre-operative antibiotics appropriately given 60 minutes before the procedure? Also now in the surgical infection programs in most hospitals, we're monitoring blood sugar. You want to keep your blood sugar under 200.

There's other variables post-operatively: bacteremia from pneumonia, urinary tract infections.

Hospitals are a scary place to be. There's a lot of bad bugs hanging out there.

So, unfortunately, there have been a number of allograft associated infections. When that happens, you can describe it only as a major complication and a catastrophe.

What kinds of infections are we seeing? For bacteria, there's been obviously the clostridia species, both gram positives and negatives and even TB; viruses, Hep B, C and HIV.

The end result may be disastrous. Now if you talk to the infectious disease people -- and I'm not an infectious disease person, so I'm just relaying what I've learned from them. But some bacteria are more sensitive to antibiotics than others. True.

But in bone, any bacteria, even the most feebleminded bacteria, can be a significant pathogen. I come back to this other principle of once osteo, always osteo. If you get a bone infection, you can never say that you are completely cured unless you had removed that entire segment.

If you treat by curetting, debriding,

antibiotics and washout, you may quiet the infection. There may be clinically no infection present, but 5 years, 10 years, it could come back.

So therefore, the only good bug is a dead bug. If you do a second operation, a washout, removal of the graft, IV antibiotics for 6 weeks and then further operations for debridement and reconstruction, the net result is you have really a poor outcome.

So for us, this whole business of infection is very, very bad. It’s very bad business.

Have there been -- further experiences, highly processed tissue is probably the safest. There are problems with high-risk -- with less vigorously processed tissue, as I pointed out.

So that leads people to think of other alternatives if your choice is high-risk tissue or what are my other choices. There are bone substitutes as osteoconductive fillers.

Rather than allografts, you can use plates and cables, stem prostheses, modular tumor prostheses and now Trabecular Metal for hip and knee reconstructions and using OATS procedures rather than

fresh graft and so forth. So everybody’s always looking for a better and safer mousetrap.

How do you evaluate a patient -- evaluate a potential infectious adverse reaction? The old motto in medicine: When in doubt, examine the patient. So start with a physical exam. You’re looking for pain, swelling and infusion of a joint and erythema and warmth.

Obviously, for a joint, you would aspirate the wound. You may have to do it more than once. You’re looking for cell count differential gram-stained cultures, routine and anaerobic.

This sounds pretty straightforward, but it’s not always. You talk to the ID people. You’ll be running around in circles doing more than aspirate. You're going to monitor their temperature, look at their sedimentation rate. The CRP is probably more helpful than the sedimentation rate.

You’re looking at the peripheral white count differential. That can even still be normal in serious joint infections. So physical exam, aspirates, cultures and monitoring the patient are going to help

you. But you're doing that all along, except the aspirates and so forth.

Do factors such as the graft being life-saving or life-enhancing influence the decision to accept the graft? Life-saving doesn't really apply in orthopedics because there are other types -- you can wait. You could do some other tissue, do a -- some modular prosthesis and so forth. So that doesn't really apply to us. Ours is sort of life-enhancing but not life-saving.

Life-enhancing is not a good enough reason to accept anything other than a graft that meets all the necessary criteria. There's absolutely no reason for cutting corners.

If the question was posed, would you use a contaminated graft? Absolutely not. There's no reason whatsoever to do that. There's other things that you can do.

For example, for ACLs, people were looking for bone-tendon-bone. You didn't have the tissue. They went to hamstrings. They went to Achilles tendon. Now they're using anterior tib and posterior tib. So

you can broaden your repertoire of tissues that you can use rather than accepting something that is contaminated.

So I would end by saying that there had been a lot of confusion in the orthopedic community. There probably still is. The things that need to be clarified are how do you define sterility; what kind of information does the surgeon need to have.

Is this something that you want to put on packaging labels so that they can make their own decision or is it something that they would have to take it upon themselves to call the bank to find out? How easy is that information?

So that's the background. Thank you.

DR. SOLOMON: Our next speaker is Dr. Richard Jonas. This is a *Reader's Digest* version of Dr. Jonas' CV.

Dr. Jonas received his MD degree from the University of Adelaide in South Australia in 1974. He received a master's in 1994 from Harvard.

He did his residency in general surgery at the Royal Children's Hospital in Melbourne and then

fellowships in cardiovascular, cardiothoracic surgery at Brigham and Women's, Children's Hospital.

He was a professor of child surgery at Harvard and the associate director of the cardiothoracic residency training at Brigham.

Currently, he is professor of surgery and pediatrics at George Washington University, chair of cardiovascular surgery at Children's National Medical Center and the co-director of their heart institute.

He does a surgical and peri-operative management of congenital heart disease and acquired congenital tracheal abnormalities.

Dr. Jonas is a consultant to our circulatory system device panel, Medical Devices Advisory Committee at CDRH.

He's a member and has held office in various professional societies and was president of the American Association of Thoracic Surgery. He's on the editorial board of numerous journals.

His major research interest is what support techniques can be used during cardiac surgery to maximize intellectual development and minimize

neurologic injury in the patient.

He’s participated in numerous clinical studies funded by NIH. He’s co-authored and authored approximately 300 articles and various letters, books, editorials, reviews and book chapters.

Dr. Jonas.

DR. JONAS: Thanks, Dr. Solomon, for that very comprehensive introduction.

It’s a pleasure to be here. I appreciate the invitation to participate in this most interesting workshop.

I am a clinical cardiac surgeon, basically, and work here at Children’s National Medical Center, which is just a few miles from us here in Bethesda, and undertake around about 400 to 500 congenital cardiac procedures per year, predominantly in children, but also in adults with congenital heart disease.

It’s something of a sort of geographical accident really that my interest in cardiac allografts – and relates to the fact, as Dr. Solomon mentioned, that I’m originally from Australia. I also had the opportunity to work in New Zealand for two

years before I came to Boston.

It was in New Zealand that a lot of early developmental work on cardiac allografts was undertaken by Sir Brian Barratt-Boyes. I was a little disappointed when I got to Boston to find that allografts were not being used at that time. They had gone through, as we'll see, an area of -- a period of enthusiasm, but at that time there was less enthusiasm.

What I'm going to do for you is to review the history of allograft development, to review why materials in general, how they're used in cardiac surgery, and, once again, emphasizing that my perspective on this is as a congenital cardiac surgeon. There is about one congenital cardiac surgeon to at least 10 or 15 adult cardiac surgeons.

We'll look at some specific applications of allograft tissue, preparation of allograft tissue from the clinician's perspective, and then address the specific symposium questions.

It's appropriate in this Nobel season that it's nearly a hundred years since Alexis Carrel was awarded the Nobel Prize in medicine for this pioneering

work in transplantation of blood vessels in the development of surgical techniques for actually transplanting blood vessels.

He was really ahead of his time. It was probably the absence of antibiotics that meant the clinical application was not really possible pre-World War II. It was during World War II that Robert Gross, working at Harvard and Children's Hospital at Boston, was the first to clinically apply vascular allografts.

He did that for a repair of coarctation of the aorta, a narrowing of the aorta which he resected and in some cases could not do a direct anastomosis and needed some vascular substitute.

This, of course, was pre-Dacron and other synthetic vascular grafts that were just about to be developed in the 1960s. So during the 1950s also, there was quite a bit of vascular allograft work done, for example, in Korea during the Korean War.

With the beginning of open heart surgery in the 1950s, independently Barratt-Boyes in New Zealand and Donald Ross in the United Kingdom developed the

concept of replacing the aortic valve with an aortic allograft. Ross extended that idea to the use of an allograft as a right ventricle to pulmonary artery conduit.

In 1984 with the development of cryopreservation, as I mentioned, there was a renewed enthusiasm in allografts in the United States which had really gone out of favor for the previous 10 or 15 years. Various applications have been used since that time.

So there are a number of applications in cardiac surgery where we require a biomaterial of some sort. These include valve replacements, conduits and various patchplasties.

We do, like the orthopedic surgeons, have a lot of choices other than allografts. These include synthetic choices, xenografts, autografts and tissue-engineered options are on the horizon.

When I was in Boston, we were doing a lot of work in our lab on the development of a tissue-engineered heart valve in which seeding of the patient’s own cells onto an absorbable polymer could

ultimately result in a valve that would have growth potential and have the ability to avoid thromboembolism and therefore any coagulation.

Dr. Toshi Shin'oka, who was our fellow from Tokyo who was doing a lot of this work, is now at Yale continuing to lead this research. He does have a *New England Journal* publication of the use of tissue-engineered patch as a patchplasty that has been performed clinically at Japan at least, not yet in the United States that I'm aware of.

Now the history of cardiac valve replacement goes back to the 1960s. It was in 1960 that the first successful valve replacements were undertaken. Initially, they mainly looked like the ball-and-cage valves that you see at the top. But there were many, many variants, including these tilting disk valves.

The Bjork-Shiley valve was popular for a time. The most popular valve for the last 30 years has been the bileaflet St. Jude prosthesis that remains the most commonly used prosthesis.

There's a long history of problems with mechanical valves. This is the Braunwald-Cutter valve

that was developed here at the NIH actually. The basic theory behind it was great. That was that if you covered the metal struts with the Dacron fabric, then this would encourage tissue on-growth and endothelialize and prevent thromboembolism, and allow you to avoid anticoagulation.

The only problem was that the Dacron wore down over time and then had a sandpapering effect on the silicon ball which got smaller and smaller and then would acutely leave the cage. That was uniformly a fatal event. So a lot of these valves had to be electively replaced.

All mechanical valves have some sort of a Dacron sewing ring to fix them in the valve annulus and that Dacron can excite a fibrous reaction that builds up a pannus that contributes to accelerated stenosis of a mechanical valve. Of course, with children who are growing, they are going to become stenotic every more rapidly with this pannus accumulation.

So mechanical valves then have a whole lot of disadvantages, including poor hemodynamic performance in smaller sizes in particular, a risk of

thromboembolism even with Coumadin and anticoagulation which, of course, can be difficult for a family to manage in a child. There are the risks of the Coumadin itself and the prosthesis does not grow.

Glutaraldehyde-treated porcine valves mounted in a metal or plastic stent covered with Dacron fabric became popular in the 1970s and essentially completely took over from allografts during the 1970s and early 1980s.

However, with time it became apparent that glutaraldehyde treatment, particularly in children who are rapidly calcifying long bones, also resulted in rapid calcification of the xenograft tissue. This valve was in place in the mitral position for just 18 months before it required replacement in an 11-year-old child back in the 1970s.

So xenograft valves have also had poor hemodynamic performance in smaller sizes, rapidly calcify, exacerbated by glutaraldehyde fixation, and do not have growth potential.

So allografts then have been applied for aortic and pulmonary valve replacement for quite some

time. The technique that was originally developed by Ross and Barratt-Boyes was the so-called subcoronary freehand implant.

So here the aorta is being opened. The aortic allograft is being trimmed down. Now the patient’s valve is being excised. The calcified stenotic aortic valve has been excised. The allograft will be slid down inside the patient’s own aorta.

The allograft is then inverted on itself for a first suture line. Then a second suture line is run to essentially recreate the patient’s own valve, but it is supported by the patient’s own aorta.

Now the Ross procedure is an autograft valve procedure that was quite popular in the 1980s and much of the 1990s, but has also fallen into a disfavor more recently. This also illustrates a root replacement technique that can also be applied for an allograft to replace the aortic valve.

So this is a big operation. It involves excising the main pulmonary artery and the pulmonary valve, the so-called pulmonary root. The patient’s own coronaries are harvested with a button of aortic wall.

The aortic root has now been excised.

If you were going to use an allograft, you could place an allograft into this position. But here we're seeing the autograft Ross operation where the valve is being sutured to the left ventricular outflow tract. The coronary arteries must now be implanted.

Of course, you've now got the problem of reconstructing the patient's right ventricular outflow tract. So here an pulmonary allograft usually is applied of reconstruction in that location.

Now, allograft valves do have a lot of advantages, the low transvalvar gradient and extremely low, almost zero risk of thromboembolism, and therefore stroke and endocarditis, a very low risk of paravalvar leak and they are silent.

On the other hand, they do have disadvantages. The insertion is clearly a lot more technically demanding than placing a stent-mounted mechanical or bioprosthetic valve. There are the problems of logistics and availability and having to wait for the thawing and rinsing rather than simply taking it off the shelf.

The durability does vary. Initially, in the 1980s with the reintroduction of allograft valves, it was hoped that durability would be very much better, certainly for the adults, than the xenograft valves.

But with the development of some of the more recent xenograft pericardial bovine stent-mounted valves and other xenograft options, allografts are not really viewed by the adult surgeon as having any durability advantage relative to off-the-shelf xenografts.

It would be my sense -- and I don’t have numbers. I’m not really sure how you could get them exactly across the country and across the world. But it would be my sense that allografts for adult aortic valve replacement is now very, very much less popular than it was 10 or 15 years ago.

I’m not sure that a lot of the current era of trainees in adult cardiac surgery are even going to learn the techniques that are required.

It’s also clear that the durability of allografts, just as we heard about for orthopedic implants, are affected by a number of variables that

really haven't been very well defined and include such factors as donor age and HLA matching, time of collection after death, method of sterilization, method of storage and the presence of viable or non-viable cells.

Now, in contrast to the adult experience with aortic valve replacements, allografts as conduits remain very popular for the congenital population. But once again, to put the numbers in perspective, we do around about 25, 30,000 pediatric operations a year in the United States as against about 450,000 adult open-heart procedures.

Conduits are applied for a number of the congenital conditions, including tetralogy of Fallot with pulmonary atresia, the Rastelli operation that we'll see in a minute, and truncus arteriosus.

Here then is the Rastelli operation. This child has transpositions. So the aorta's coming from the right ventricle. The left ventricle is connected to the pulmonary arteries. There's a stenosis under the pulmonary valve.

What we're doing here is baffling the left

ventricle to the aorta. We need to connect then the right ventricle to the pulmonary arteries. Some sort of conduit is needed for that with ligation of the main pulmonary artery.

Here then is a bioprosthetic conduit, once again, popular in the 1970s and early 1980s in the United States. So this is a tightly woven Dacron conduit containing a xenograft valve.

The disadvantage of these conduits is that they must be constructed from very low porosity, very tightly woven Dacron. The peel that forms inside such conduits, a fibrin/fibrous mix, does not adhere well to low porosity Dacron.

So you get repeated microdissections, repeated thrombus formation and fairly rapid accumulation of an obstructing peel within the conduit. You combine that with the disadvantages that we already saw of the xenograft valve that is inside such a conduit with early calcification, particularly in younger children, but also in young adults.

So for that reason, the aortic allograft conduit or pulmonary allograft conduit remains the

choice of the majority of congenital surgeons.

So once again, there are new xenograft and other options constantly appearing to challenge that position.

Allograft conduits ultimately failed, as any non-growing conduit does, in the child through outgrowth, which is both a diameter of becoming inadequate as well as the longitudinal growth that stretches out and thereby further narrows a conduit.

You can get calcification that’s so aggressive that you get nodules that project into the lumen and stenose the conduit. The valve leaflets are the last to calcify, but they can become rigid and stenotic.

You can get compression by the sternum of proximal anastomosis. Some allografts even shrink without growth with a fibrotic process that may be immune-mediated.

Pulmonary allografts are thinner and less prone to calcification than aortic allografts. A number of retrospective series have suggested a greater durability relative to aortic allografts. But we’re

still only talking perhaps 4 or 5 years for a 1-year-old who has one of these conduits placed as against 3 or 4 years for an aortic allograft when it must be re-replaced.

There's some risk of aneurysm formation particularly at systemic pressure. This has also been found for the Ross autograft operation that these can become aneurismal.

This was a study that I did back in the late '80s, published in the early '90s, in Boston, where we looked at pulmonary allografts at systemic pressure because I was interested, as we began to use cryopreserved allografts, as to how these would hold up, and undertook these translational research studies in chief and did confirm that there was a small risk of pulmonary allografts becoming aneurismal.

It was extremely rare to see a problem clinically. We did have one or two cases of pulmonary allograft rupture after the Norwood operation for hypoplastic left-heart syndrome, which is an example of an allograft being used as a patchplasty.

Just to illustrate that for you briefly, the

hypoplastic left heart syndrome was really the last big challenge for the congenital cardiac surgeon. The Norwood operation was developed in the early 1980s.

We suggested the use of pulmonary allograft tissue in this application. So here then is a chart that has aortic atresia. A newborn who has an aorta that is generally around about 2 and a half millimeters in diameter but it can be as small as 1 and a half millimeters in diameter.

To reconstruct this, we divided the main pulmonary artery and fillet opened that tiny ascending aorta and then used some allograft, which can either be an aortic allograft or a pulmonary allograft, to reconstruct the neoaorta, essentially turning the pulmonary artery which is connected to the functional right ventricle.

A child usually doesn’t have a left ventricle. That then becomes the single ventricle pumping to the body as well as to the lungs. Allograft tissue is really done extremely well in this setting.

Just reviewing, my perspective on the history of allograft processing, in the early years the

collection of cardiac allografts was generally at autopsy in the United Kingdom and in New Zealand.

When they were reintroduced in the United States, it was initially collection in the operating room from brain-dead, heart-beating donors. But gradually cadaver collection, of course, developed, as it was apparent that we simply weren't going to have enough grafts if we limited it to brain-dead, heart-beating donors who were also not suitable for heart transplantation.

Of course, this introduced a variable of warm ischemic time. We were interested in that issue. This was a study published in *Circulation* in 1991 where we looked at and compared cryopreserved aortic allografts and the time from donor death, and did not find that warm ischemic time or cold ischemic time influenced the durability in a sheep model over a few months at least for what that information is worth.

Now, transportation to the processing center of the undissected heart and great vessels is generally done on ice. There's been some variability as to whether this has been in an antibiotic or whether it

has been in just a balanced salt solution, which was certainly the way that we originally had did it. This contributes to cold ischemic time.

Allograft dissection when I was in New Zealand was performed by the surgical residents. So you had the opportunity to do this pretty frequently and dissect out the aorta from the heart that would arrive in the middle of the night from an autopsy in another part of New Zealand.

So here there is the dissection, the finished allograft including the aortic arch, the arch vessels, and the ascending aorta, and the homograph aortic mitral valve.

We then place the allograft into antibiotics. A typical mix in the early years was penicillin, gentamycin, streptomycin and amphotericin that was used by Donald Ross, Barratt-Boyes and others.

Barratt-Boyes in New Zealand had quite an interest in developing an optimal antibiotic solution and came up with the CLPVA mix working with his microbiologists and his histopathologist Lois Armiger.

The Barratt-Boyes concept of an allograft is

that you wanted to do minimal injury to the ground substance into the ultra-structure of the allograft and encourage the on growth of recipient cells. It was believed that on-growth was what was really mainly responsible for the durability of allografts and that the donor cells soon died and were of little importance.

There were alternative points of view, as we will see. But his antibiotic mix was developed to optimize on growth of recipient cells and in growth of recipient cells. Generally, the exposure was for 48 hours. But, of course, there’s been a lot of variability about duration and temperature. A lot of it has been proprietary as far as I know in this country.

We also became aware that antifungal agents had been removed by some of the processes through dealings with the New England Organ Bank. There actually have been remarkably few reports of bacterial contamination in children receiving allografts.

But there have been occasional reports of fungal infections. I’m sure this is an area that will

be discussed during this workshop.

Allografts storage then, the tissue culture medium is the -- with no antibiotics is one method of storage at 4 degrees for up to 4 to 6 weeks. Cryopreservation, as I mentioned, was reintroduced in the mid 1980s. There are some storage techniques like storage at the temperature of dried ice or freeze drying that clearly did result in structural damage.

Robert Gross actually in Boston did a lot of work and published in the *New England Journal* in 1945 with his first case report of a successful coarctation repair, the fact that if you store a dog allograft in a water stored at minus 72, the temperature of dry ice, then almost all the animals died from hemorrhage and the graft had broken down because of structural failure.

On the other hand, storage in a balanced salt solution at 4 degrees, he was able to sacrifice all the animals and the majority of these aortic grafts looked excellent as he describes them at the time of sacrifice.

Now, cryopreservation with controlled rate

freezing, you certainly know a lot more about than me. But it’s my understanding that the addition of dimethyl sulfoxide as a cryoprotectant, preventing ice crystal formation is an important part of this. Of course, that subsequently needs to be washed out.

Cryopreservation, we can put cryopreserved and fresh allografts in a sheep study in the 1980s and did not histologically see that there was any adverse effect of leaflet integrity and conduit function in that study where we worked with the New Zealand pathology team as well.

This was a challenging surgical preparation that placed these allografts between the right ventricle and the pulmonary arteries, which was done without cardiopulmonary bypass, just by placing a side clamp on the right ventricle, which is generally not recommended in humans.

This is some histology of the cryopreserved aortic allograft wall illustrating the calcification that you will see in a young sheep and certainly see in young children as well. But you also see that in a so-called fresh allograft, antibiotic treated but

stored at 4 degrees.

Now, transportation to the hospital is another area where there can be variability and maintaining an optimal temperature for transport, how much time you can spend plus or minus 196 degrees if you're not close to some liquid nitrogen, I don't believe is very well defined.

There are a number of different proprietary thawing and rinsing protocols that are used for valves. Certainly, in the early years, we did see some valves that were too rapidly thawed in hot water and came out cracked. You don't need much of a crack in an aortic valve leaflet to have a serious problem with aortic regurgitation. There are various proprietary methods for rinsing out and cryoprotecting.

There have been some controversies over the years. Once again, Barratt-Boyes believed that what was really key was the preservation of ground substance and ultra-structure and on growth of recipient cells.

Another point of view espoused by O'Brien from Brisbane in Australia, which was another big allograft center worldwide, was that donor cell

viability was the key to durability.

This controversy really led to a major about-face with the introduction of the so-called SynerGraft. I think it was in the late ‘90s where Fred Schoen from the Brigham in Boston came up with the concept that donor cells were really the focus of the calcification that’s seen in the aortic wall; that all you had to do was to remove all donor cells. And so I understand a detergent-like process is used to remove DNA and cellular fragments.

The so-called SynerGraft is marketed as a cardiac allograft that is completely free of donor cells and therefore likely to have greater durability. So we went from one end of the spectrum to the other in terms of the importance of donor cells.

Of course, the presence of live donor cells reignited the question as to how important is rejection, which is the first question that every parent asks you when you tell them you’re going to implant some allograft tissue.

The real answer is that we really don’t have a good handle on it because there’s such variability as

to endothelial cell viability versus fibroblast viability; fresh thawed, the old historical allografts that were all stored at 4 degrees versus the newer generation of allografts that are stored and cryopreserved.

There have been studies. Charles Yankah and Bill Lind did studies in inbred rats in the 1980s that did document that you could get accelerated skin graft rejection if you placed an abdominal aortic allograft into a rat.

There have been some retrospective studies that have suggested that if you can ABO match allografts into children, then you'll get greater durability. But once again, you're talking perhaps an extra year out of 3 or 4 years.

The logistics of just having the right size in your liquid nitrogen freezer outside your operating room on a given day, for a given operation, for a given child, let alone being able to ABO match that, it's really pretty frightening.

The majority of cardiac surgeons have not been in a position to be able to ABO match even if they

wanted to.

Just to cover from a clinician's perspective, the symposium questions that were put to us. I'll be happy to go into more detail. But once again, I'm not a microbiologist.

I've had exactly the same experiences as Dr. Doppelt in going to the ID people and saying well, what does this mean. They turn to me and say well, I don't know. What does this mean?

So it's an area that really hasn't been very well defined, either by good clinical studies and certainly not by any translational laboratory-type studies.

What are surgeon expectations regarding sterility? I would guess if you ask the majority of cardiac surgeons -- certainly adult cardiac surgeons who don't work with allografts all that much -- they would just assume that all allografts are completely sterile, that they don't have any bacteria in them. And if they have any at all, then they would be rejected.

Certainly, that's not an unreasonable

expectation considering the cost for what we pay for allografts these days. They have become extraordinarily expensive. In the majority of cases, about double the cost of mechanical valve, for example. To some extent as we all become more cost-conscious, that’s been driving the development of various xenograft alternatives because the cost has become so great.

But I guess the assumption has been, well, if they cost that much I guess they must be very carefully monitored bacteriologically. I know a lot of thought and effort does go into that.

But for the majority, I would say congenital cardiac surgeons do have an understanding that there is a treatment with various antibiotics and antifungals and that the risk of contamination should be extremely small.

The fact that we’re implanting these tissues into a highly vascular area in young children who normally have very good immune systems and good livers, kidneys and once again, highly vascular area is probably the explanation as to why it’s been

exceedingly rare to see important clinical infection in allografts.

In fact, if you have an adult or child who has active bacterial endocarditis, or if they have a staph infection that’s destroying their aortic valve, they have an abscess that’s burrowing into the ventricular septum, then the valve replacement of choice in that setting is to use an aortic allograft.

They have a pretty good track record of healing in that setting, certainly much better than a mechanical valve, which is very likely to end up with a paravalvar leak and prosthetic valve endocarditis, which can be exceedingly difficult to manage.

So in 60 years of clinical application, there have been very few examples of bacterial contamination. Once again, there have certainly been reports of fungal contamination.

But I think for the average surgeon, the incidence is so low that, as Dr. Doppelt has said, you can practice for a lifetime and operate on 5 or 10,000 children and you’re never going to see a single case of documented allograft infection.

Once again, as with the orthopedic experience, there is a sense out there that irradiation and gas sterilization resulted in denaturation of collagen and ground substance. So it’s my understanding that these techniques have not been used for sterilization of cardiac allografts since the 1970s.

Minimal expectations, well, we’re really dependent on everybody who’s in this room to help to establish those minimal acceptable levels of contamination. Presumably there are some specific bacteria and -- I do believe that fungal contamination, after receipt at a processing center, has pretty uniformly over the years been a reason for rejection of an allograft.

But whether that’s in print -- and as I say, a lot of information regarding processing we learned early on was held in a proprietary fashion as we dealt with a number of the early tissue processing centers through the New England Organ Bank.

Certainly, it’s assumed that appropriate viral screens have been performed of all potential

donors to hopefully eliminate the risk of HIV or hepatitis, a blood-borne infection.

The graft is pretty much routinely cultured I think in most operating rooms by snipping a small segment of the allograft. I'm not sure why we do it because, as Dr. Doppelt said, when we get a positive culture, which is not all that uncommon, the general response is to disregard it.

One has to look at the patient and establish whether there are any signs of systemic infections, which not uncommonly we'll see in a child who's got a bit of a wound infection and a bit of a fever. We treat them with antibiotics for that and that generally will clear up.

It's not uncommon for a newborn to have a transient bacteremia. They have multiple lines. They have an umbilicus that has an umbilical line. So having a staph cultured from bloodstream, which in an adult I think would probably be a pretty serious thing, is not all that uncommon in the pediatric setting.

Generally, it's handled by a child without difficulty. It certainly doesn't automatically lead to

bacterial endocarditis, infection of an allograft.

So we would use the response to a positive -- we would use a positive culture in terms of identifying an optimal antibiotic if the child were to go on and develop an infection. We would treat as for any bacterial endocarditis with 6 weeks of antibiotics.

But so far in hundreds, if not thousands of allografts that I've inserted, and certainly the volume of cardiac allografts, is a fraction of the number of orthopedic implants that are undertaken. I'm certainly impressed to see the number of orthopedic implants that have been done around the country, but it's not a common operation amongst cardiac surgeons even amongst congenital cardiac surgeons, not what we would call a common operation.

In general, we do not do antibiotic soaks. We've got a cross-clamp on. The heart's ischemic. The heart-lung machine is running. We're generally standing there pushing the scrub nurse to get the thing thawed and rinsed so we can put it in because we can't measure and size until we've removed the valve and exposed the heart.

So the processing time actually can be important to us in terms of which proprietary center we'll choose because if we really have a lot of time pressure, then we're going to choose the quickest thawing and rinsing protocol. So there's really not time to do further antibiotic soaks.

As to the overall experience with infections complications -- and you can go back 20, 30 years and find some reports from the UK and so on. But in the contemporary literature, there's just exceedingly few reports, if any, of bacterial infection of cardiovascular allografts.

They heal successfully in the setting of bacterial endocarditis. Once again, presumably the washing and the blood supply to the mediastinum are responsible presumably for the low risk of infection.

Essentially, all cardiac allografts are life-saving. You cannot live without an aortic valve. You cannot live without a main pulmonary artery.

So none of these are what I would call life-enhancing, though once again, there are choices. We do have bioprosthetic choices other than allografts.

There is an extremely low probability of important infection resulting from contamination. It's really durability is what helps us to decide. And as I say, cost is also coming into it.

So in conclusion, allografts are amongst the most widely applied biomaterials in congenital cardiac surgery. But it's a fairly small field and not all of our cases require biomaterials.

They have a reputation for greater resistance to bacterial infection than alternative biomaterials. We're really dependent on processing facilities to ensure that allografts are supplied free of fungal and viral contamination and a minimal bacterial level.

Thank you very much.

(A recess was taken.)

DR. SOLOMON: Our next and last speaker is Dr. Richard Kagan. Dr. Kagan was born in Springfield, Massachusetts. He obtained his Bachelor of Arts from Case Western Reserve in 1970 and his M.D. degree from St. Louis University in 1974.

He received his residency training in general surgery at University of Illinois Hospital in Chicago,

before joining the University of Illinois faculty as a staff surgeon in Cook County Hospital Burn Unit.

Dr. Kagan joined the staff of the Shriners Hospital for Children and the University of Cincinnati as an assistant professor of surgery in 1988 and has been director of the university hospital’s adult burn center since that time.

Dr. Kagan was promoted to associate professor of surgery in 1994 and professor of surgery in 2003. He was named assistant chief of staff of the Shriners Hospital in 2000 and became chief of staff and chairman of the burn division in 2004.

His interests are in acute and reconstructive burn care, tissue banking, skin substitutes, burn center reimbursement and acute and chronic wound care.

He has authored over 130 abstracts, 65 articles and many book chapters on burn injuries and tissue banking.

In addition to these responsibilities, Dr. Kagan is a medical director for AlloSource and a past president of the American Association of Tissue Banks from which he received the Jeanne Mowe

Distinguished Service Award in 2006.

He is currently president of the American Burn Association and also serves as chair of the organization's Burn Registry Committee, and as the ABA representative to the AMA's CPT and RUC advisory committees.

I don't know what those are but -- so Dr. Kagan.

DR. KAGAN: The surgeons will know what those committees are.

Thank you very much for having me here today.

In accordance with what Ruth had advised me to talk about, to not talk about, I'm really going to confine my comments to allogeneic skin substitutes and not talk much about the synthetics or the xenografts that are available for use.

The ideal properties of skin substitutes have been described for probably 30 or 40 years in that the substitute should rapidly adhere to the wound, be semipermeable to water and oxygen much like normal skin, limit bacterial colonization and proliferation on the wound -- and this is particularly important in the

care of burn patients -- be somewhat elastic and durable so it can fit over irregular surfaces and ideally that it should be sterile.

The most commonly used allogeneic skin substitutes this day and age are human allograft skin, a product called GammaGraft, which is also allogeneic skin which is irradiated and Alloderm which is a dermal remnant, if you will, of where the skin that is harvested that is removed of the epidermal layer and lastly, human amnion, which was used quite extensively until about 15 or 20 years ago but has fallen really off the radar screen for quite some time partly because of difficulty with being able to recover as well as earlier requirements with following the early tissue banking requirements for retesting of living donors, since human amnion typically comes from living donors. So for that reason, there are very, very few organizations that even consider using human amniotic membranes in this country for wound care.

Over the years, the benefits of allograft skin in wound management, particularly in burns, have been identified. As I said earlier, it does definitely

reduce evaporative water loss and protein losses through wounds, which in the cases of patients with extensive burn injuries can be extremely important.

It also prevents tissue desiccation. That's also very important because oftentimes this is applied over an excised wound where all of a sudden there is absolutely zero protection for the underlying tissues. If left open to air, they will desiccate or become even more heavily colonized.

As such, the allograft will also suppress spectra proliferation, and while not necessarily maintaining the wound in a sterile environment, it will at least minimize the amount of contamination on the wound.

As we've known it for many, many years, when we wash the burn wound, manipulate it in any way, or even provide a shower or bath, that the incidence of traj (phonetic) bacteremia in these immunosuppressed patients can be in the range of 35 to 40 percent.

So there's constant risk with doing what we have to do every day to manage these patients. It's also been associated when applied to partial thickness

wounds, which are as painful or more painful than essential sunburns just so I can make it relevant for many of you -- that applying the cadaveric skin or a skin substitute will reduce pain by covering all the exposed millions of nerve fibers.

It’s also been found to stimulate in the vascularization in an underlying wound bed. This is also particularly important in the case of acute wounds, infected wounds, or even deep wounds where you don’t want to utilize the patient’s own skin grafts at a time when you can’t be assured of the success of graft take, particularly in the case of patients with extensive burn injuries where there is limited donor site availability.

You hate to take a graft and then have a zero percent take and have to wait a number of weeks until you can reharvest that graft site.

Some early studies that we did at our tissue bank a number of years ago looked at the value of procurement cultures in skin banking. Our pre-prep cultures, not surprisingly, showed that there is an awful lot of bacteria that you can get off the surface

of the skin merely by swabbing it.

What we’ve learned over the years is that if you take a swab of a normal person’s skin and it comes back no growth, then there’s a problem with your culturing technique because we all have bacteria flora on our skin.

Perhaps more importantly, what we learned was that the prep was very effective and that in our final packaging -- which in those days we were determining those final cultures based upon mincing of the skin and doing quantitative analysis -- was that by crushing and mincing the skin, we actually recovered bacteria that were lying within the sweat glands and with the hair shafts deep beneath the surface of the skin. So we had a much better yield of what was truly colonizing the skin, at least in terms of bacterial identification.

A few years later, Dr. Jan Pierce and his crew in Salt Lake City at their skin bank looked at procurement and processing cultures. There was no growth in both the procurement and processing cultures in over 98 percent of all skin recoveries at their institution, with there being only about 2 percent in

the procurement cultures and 1 and a half percent or so in their processing cultures.

When they broke down those that had growth, there was actually a very small number with gram positive organisms, which you'd expect to be heavily colonizing the skin in a normal situation, very, very little gram negative in the processing cultures. Surprisingly, there was growth of clostridium and then there were various other bacteria that in many cases were merely skin contaminants and not considered virulent pathogens.

So over the years, there have been a variety of indications for using human allograft skin in a traditional sense, probably the most significant of which is excised burn wounds and not necessarily extensive burn wounds like those in excess of 60 or 70 percent body surface area. But even those that might be smaller where there's a very, very deep wound and even after excising the tissue, the surgeon is unclear whether or not there's clear viability of the wound bed, or perhaps where the patient is very sick and you don't want to now create a donor site that doubles the

wound size and you really just want to get temporary coverage, let the patient get better by eliminating the wound and focus on the other issues such as inhalation injury, respiratory distress syndrome, other organ failure.

We've also used it traditionally to cover widely expanded autograft. This was popularized in the early '70s actually at the Shriners Hospital in Cincinnati by Drs. McMillan and Alexander, who used techniques to widely mesh the skin so it would cover a much larger area of the body and thereby allowing the epidermal cells to migrate across the large gaps.

The problem was that these grafts were so widely meshed that they were concerned about the large denuded areas that were left open. So they were applying allograft on top of that with the hopes that this would promote epithelization from beneath, provide a cover to the wound bed, prevent desiccation, and eventually that that allograft would be rejected once the autograft grew underneath it.

This technique has been largely abandoned in this country just because most of us these days are not

performing widely expanded autografts. We have other techniques and other means of covering the wound through other types of agents.

We also have used it traditionally at our hospital for exfoliated skin disorders. These are disorders where typically due to drug reaction the patient will slough anywhere from 40 to 90 percent of their body's epidermis. It's the equivalent of a large second-degree burn.

These patients have the same risks. They also generally come in with pre-existing co-morbidities for which they are being treated initially. Getting a temporary wound cover to reduce evaporative water loss, prevent bacterial proliferation and also reduce wound pain has been very, very important in having a high survival rate.

As I said before, we've used it many times for testing the wound bed for autografting. In some centers, they've used it as a dermal template for the later application of cultured epidermal autografts.

In our experience, a lot of the surgeons now at burn centers, who find their hospitals beds filled

with many patients from necrotizing soft tissue infections, that we're using it a lot for those kind of wounds as well to provide temporary wound coverage and prepare the wounds for definitive management.

Over the years, the LA50 or the burn size that would kill 50 percent of all burn patients has changed dramatically. Back in the 1940s, the LA50 was a 35 percent burn.

You can see from this slide that there have been a number of innovations over the years from the development of fluid therapy for resuscitation, the development of topical antibiotics in the early to mid '70s, aggressive nutritional support, which essentially the burn centers have been leaders in for many, many years -- to what we're now able to do with aggressive excision of the burn wound.

But the rate limiting step in that was that if we were going to excise the wound, we just couldn't leave all the exposed tissue open. We had to have a means of temporary coverage if we didn't have enough skin.

This has led to our use of the skin

substitutes in this category and, hopefully, the development of new permanent skin substitutes through tissue engineering techniques.

So one of the things that has come up over the last 10 or 15 years is the burn surgeon's preference for using fresh skin. The reason that we like fresh skin is it has enhanced engraftment. It actually vascularizes on the wound bed as well as an autograft does whereas in the past we've noted that cryopreserved skin did not do that.

We found that it did it so very rapidly so that at five days post-grafting, if you put it side by side with an autograft unless there were color differences or marking differences, you may not even know which one was autograft and which one was allograft.

Clearly, by becoming vascularized, it becomes a part of the patient's body on a temporary basis. It eliminates the bacteria. It's the patient's own temporary skin.

The issue that came up many years ago following FDA's initial rule was the issue of what do

we do when we need to get the skin to the burn center and we don't have all the results back. So the exception-release, which was necessary, really applied to non-availability of autopsy results, if one had been done, and non-availability of culture results because you just don't get those within two or three days.

We've always had the serologies. We've always had the history and physical, but we did not have the culture results in many, many cases.

This picture shows the difference between a cryopreserved allograft skin on the chest of a young infant. This is at 5 days. You can see a little bit of epidermolysis here.

This is another infant who had also a severe burn. Here's a tracheostomy. These are two of his nine chest tubes.

So our goal that day was to get the burn off, eliminate that aspect of the inflammatory response and control the wound without creating a donor site which would be another inflammatory wound on the patient.

You can see there's a big difference in the appearance of the two. This one if you press your

finger on it, it blanches just like an autograft does.

In this particular case, these grafts were applied as sheet grafts to get complete coverage as opposed to a mesh appearance where there would have been exposure of the underlying tissue.

We actually studied this at our institution, both the burn center and the tissue bank, and compared our results with refrigerated and cryopreserved skin.

Not surprising, we found that the take rate at 10 days, that skin that was still vascularized and adherent in the wound bed was at least statistically significantly greater. Albeit, 90 percent to 98 percent may not make a huge clinical difference but clearly the fact that it was vascularized in our minds made a huge difference in our use of it.

From a clinical perspective, our use of fresh skin is depicted on this slide just at our Shriners Hospital where we treat children with burn injuries. You can see that the yellow bars which was fresh skin clearly are heading upwards.

If we had our choice, there would be no orange bars at all as we move farther and farther to

the right. The problem is matter of timing as well as the number of recovery agencies that can actually maintain fresh skin.

Ultimately, though, the goal would be find means of cryopreserving the tissue and maintaining viability so that we didn’t have to go through exception-release and we could always have viable skin rather than calling it exception-release or fresh skin.

We also just in the last year looked at some of our results with the use of skin and actually worked closely between the burn center and the tissue bank as to the issue of safety of allograft skin on our burn patients.

When we looked at those donor tissues that were culture positive, we found that there was about 14 percent but only 2 percent of those actually had pathogenic organisms. We found zero adverse patient outcomes in our patients treated at the Shriners Hospital.

We also just recently, with our microbiologists, reviewed our fresh and frozen allograft outcomes in patients. While you can see some

differences in the fresh and frozen for pre-antibicrobial positive cultures, those differences were not statistically significant.

The post-antimicrobial treatment in the tissue bank cultures were about the same. Not surprisingly, in the burn patients, the recipient sites, before we put the skin on, were far more contaminated than were the allografts that we were using. We found zero incidence of common microbes between the donor and the recipient.

Again, these are things that we kind of always felt but finally we had some data to prove it. This actually is in the process of being published in the *Journal of Burn Care*.

Just to point some of the things that I've mentioned to you pictorially so you can have some idea, the widely meshed autograft concept is on top of the excised wound bed, you would place a 3:1 or 6:1 mesh autograft. Then on top of that an allograft that might be meshed 1.5:1 or a much narrower mesh to provide wound coverage.

Again, this is something we were doing in

Cincinnati quite a number of years ago. I can't remember the last time we've done it. It's probably more than 15 years.

As far as the exfoliated skin disorders, this is a young teenager who came to us with the skin slough following treatment with an antibiotic for a sore throat. He ended up with about 80 percent skin slough.

We actually brought skin over from the skin bank, anesthetized him in his room, washed him down, covered him from head to toe with allograft skin. Here he was about 2 weeks later completely healed.

Again, this helps with pain. It helps with healing. It closes the wound. It helps manage all the critical situations related to multi-system organ failure.

This is probably one of the great saves we've ever had. This was a patient who had a ruptured abdominal aortic aneurysm. He was being treated in the surgical intensive care. This was an 80-year-old man.

Here all of his intestines are exposed. When we were called, he was being treated with Saran Wrap. This is about 20 years ago. Literally Saran Wrap was

being applied to his abdomen.

His fluid balance was totally impossible to manage. He was ventilator dependent.

We brought over some skin from the skin bank, meshed it 1.5:1, placed it on the wound, did a little bit of debriding over the intestinal walls every day until we got adherence.

Literally in the matter of a week, this patient was able to have his fluids managed and weaned from the ventilator just with this temporary coverage.

So following that and getting a little bit better, we took him to the operating room, autografted him with him own skin, had a hundred percent take on that wound bed which most general surgeons would not be able to achieve. He left the hospital, expectedly developed a hernia, and a year later came back and had his hernia fixed.

So there’s a lot that you can do to prepare wound beds with allograft as well as help take care of the patient’s other critical conditions by eliminating the wound.

The two allogeneic skin substitutes that I

had mentioned earlier are GammaGraft and Alloderm. I just have a couple things to say about those.

GammaGraft is an irradiated allogeneic split-thickness skin graft. The company claims sterility for it although I've not seen any specific literature to support that. They also claim that it's cytomegalovirus-free, which for some burn centers is an issue.

From a clinical perspective, however, one has to understand that the majority of the skin donor population is CMV positive. When you work in a pediatric burn center, most of your patients are CMV negative. But ultimately, most of us are going to end up CMV positive in our lifetime with or without a burn injury.

So the issue of CMV positive seroconversion really is one that has been debated in the burn literature quite extensively. Quite frankly, there's never been any morbidity or mortality associated with CMV conversion during the course of burn treatment.

Alloderm is an allogeneic dermis. It's the one that I'm most familiar with. There are a number of

other companies that are processing allogeneic dermis where they may or may not be using proprietary techniques to preserve the basement membrane.

Alloderm was originally designed to be used in burn care so that we could take very, very thin split-thickness almost epidermal grafts and apply it onto that basement membrane.

Unfortunately, a lot of the studies in that area did not really pan out all that well. So the company took its product in a different direction. That’s really where it’s taken off.

The indications for use of allogeneic dermal substitutes is largely in plastic surgery. It’s used in scar contracture release soft tissue defects, nasal septal defects, cleft lip repairs and scar revisions.

But what’s even more remarkable is its use in various other indications. These are just some of them. As surgeons are learning more about what allogeneic dermis can do, they’re using it more and more.

It’s been difficult to get to the general and plastic surgeons for them to identify where it might

have its greatest benefit. But the list probably one year from now will probably be two slides of double the amount of data.

I’ve highlighted the amount of repair of abdominal wall defects because I think that this is probably one of the major areas where surgeons have realized the benefit of this.

I’ve borrowed this slide from some of the folks at LifeCell who I’ve known for many years. This points out some of the differences between their allogeneic dermis and the synthetic substitutes that have been available for surgeons for many years.

We talk about abdominal wound defects. Imagine that patient that I presented to you with the ruptured abdominal aortic aneurysm and the need for more immediate closure.

If at the time of the repair they had put on something, a temporary, and perhaps when the patient was better a couple days later or stabilized, they were able to totally close that defect permanently with an allogeneic dermis, all the steps that I took later on might not have been necessary. He might not have

needed three more operations staged over the course of a year.

With this, they found in a study of 150 patients, collected from a multiple-trials group markedly reduced incidence of hematomas or blood clots under the wounds, much lower incidence of dehiscence or breakdown of the wounds, lower infection rates and markedly less recurrence than our currently available synthetics.

Trauma surgeons nowadays, after they're doing these life-salvaging laparotomies, are very quick to use these types of products to restore abdominal wall continuity. There are even reports of women who've had such usage actually going on to having normal abdominal pregnancy and a normal vaginal delivery with this Alloderm in place.

In my mind, the important considerations in selecting a skin substitute are number one, its safety. Number two, its rate of engraftment if that's what you're using it for, its permanency if that matters. Ultimately, it's cost-effectiveness.

There are a number of other skin substitutes

that I will not address whatsoever that are exceedingly expensive. I will give you an example. Integra skin substitute, for example, is about $12,000 to $15,000 a square foot.

If you apply this on a wound and it's contaminated and you don't get a hundred percent take, you have to regraft with something. So that money is gone. The company doesn't reimburse you if the product fails or if the wound that is contaminated.

So let me get to the FDA's questions.

When we receive allograft skin in the operating room, what are our expectations, assumptions about sterility and functionality? Well, as burn surgeons, we don't expect the skin to be sterile. As a matter of fact, we know that the wound we're putting it on is far more contaminated than anything else.

The skin that is going to be transplanted should not contain pathogenic or virulent organisms that could compromise patient safety by colonizing the wound. These are patients who are typically immunocompromised. They have no wiggle room. They cannot take a second hit beyond their initial injury.

In my mind, if we're going to be using it to cover an excised wound, the skin needs to be of sufficient viability and quality to vascularize and provide that temporary cover. It's got to do what the surgeon expects it to do.

What organisms would preclude use of the graft if we knew about them? We'd do back to the AATB standard where the burn surgeons have worked very carefully with the skin council at the AATB to identify those organisms.

We haven't gotten so specific on staph aureus to say it can't be MRSA. It's any staph aureus, group A strep, enterococcus, gram negatives, clostridium fungi.

These are the kinds of bacteria that can kill our patients. They have enough to deal with with their own microbial flora and the flora in their room or in their unit. Adding things from the outside is a death knell.

What are the data regarding the effects of processing methods on function? Most of the questions regarding skin processing have not been answered.

It’s not because we haven’t tried. For over 25 years through my involvement in the AATB and my work with the Shriners Hospital, we’ve tried real hard to get some of these projects funded. We can’t. Nobody thinks it’s serious enough.

We’ve worked real hard with expert grant writers to make sure it’s not our grant writing or the details in our proposal. Most people just don’t think it’s worth investigating.

So we’ve not been able to determine the optimal nutrient media for maintaining skin viability or the ideal antimicrobial agents to be used and/or their concentrations.

We haven’t been able to identify the ideal cryopreservative agent or the concentration for it or even the ideal methods of cryopreservation. All we’ve been able to show is that some are equivocal to others, but we haven’t been able to set essentially the gold standard.

Some of the issues related to gamma radiation and high concentrations of glycerol on sterility and tissue integrity have not been studied.

So are there concerns that the processes to decontaminate or sterilize the grafts may have an effect on graft function? There's no doubt.

At present the grafts are processed in a manner that adversely affect skin viability, most often used for superficial burns.

Again, it really comes down to what are you going to use it for. I can use essentially tanned skin that's treated in 95 percent glycerol if I'm applying it over a skin wound which I expect to epithelialize spontaneously.

If I'm applying it to a graft that's been applied to an excised wound bed where there's exposed subcutaneous tissues, then I have to expect that I will get adherence and vascularization.

Which grafts would not be used if they've not been processed a certain way? Well, generally, we would use all the grafts except for those that are mechanically unusable in the operating room.

Our preference for certain allogeneic skin grafts typically depends on the nature of the wound that we're treating and/or the processing method. So

it’s surgeon preference most of the time. That’s dictated by what kind of a wound are we treating.

Our reasons to discard a graft in the operating room are epidermolysis of the graft, where the epidermis is already separating from the dermis. That suggests that it’s a quality issue possibly related to the preservation technique, possibly related to something else that’s been done in the processing methodology.

Grafts that are too thick or too thin, if we do decide to run them through the mesher -- even if it’s a tiny split hole of what we call a 1:1 mesher, where there’s really no expansion but just small pinpricks placed in the graft -- if it’s too thick, it won’t go through the mesher. The mesher will totally destroy the graft. If it’s too thin, the graft will tear apart like wet tissue paper.

If there’s loss of integrity of the container or package or gross contamination of the skin container, we’ll discard the skin.

Typically, at least in our hands, and that may be something unique to our hospital, we take a look

at the container before we actually look in the basin on the nurse's back table. We want to see is the solution that the skin came in murky. What does the skin feel like? We actually touch it ourselves before we bring it on to the operative field.

If the graft cannot be sterilized, what are the minimum expectations? This again goes back to the AATB standard. We don't want pathogens to be added to what we're already dealing with.

Is the graft cultured prior to implantation? If so, is it done in the operating room? I would have to guess that most surgeons do not culture the allograft skin either in the back table, in the dish that it comes over in at all.

I can't imagine that -- you have to understand where we operate. Most burn surgeons are operating at a room that's set at about 90 to 95 degrees Fahrenheit and about 30 percent humidity. That means that everybody in the room doesn't want to be there except for the burn surgeon and the patient.

Anesthesia is taking a break every 5 minutes. The nurses are dying. People come in -- we used to

wear underwear under their scrubs. They walk into our room. They go out. They come back almost naked.

It's a very uncomfortable environment, but it's the best environment for the patients. So generally, there isn't added time taken to culture the skin.

If it were to be done, we'd probably take the culture from the site where the allograft skin is going to be applied. That would be done in the operating room.

But again, if we were going to do it, we would culture the skin prior to the implantation, not before we remove the dead skin. But when we get that healthy wound bed where we've controlled all the bleeding, the fat essentially, we would swab that. We know that that's almost for sure going to be no or minimal growth because we just threw all the bad stuff in a bucket.

So in my mind, that's a fairly clean wound bed but clearly not sterile.

If the cultures come back positive, what do we do? Well, if the results were known pre-operatively

and the organism was not pathogenic, we'd probably just go ahead with the procedure. If the organisms were virulent, we'd probably cancel the procedure or use an alternative skin substitute.

Many times with these patients with the extensive burn injuries, they got to go when it's time. So you have to go to alternative techniques if necessary.

If the results aren't known until after the operation's over, the transplanting surgeon will review the culture results and make a decision.

The nice thing about burn centers is it's extremely rare that we consult infectious disease folks. That's because, historically, infectious disease people come up and look at the culture sheets and treat what's on the piece of paper, and don't look at the wound, and don't see is this patient merely colonized or is this patient infected.

The only person that can tell if the patient is infected is the surgeon with many years of experience. So it would be extremely unlikely that the patient would be returned to the operating room for

removal of the allograft skin if the culture had come back positive, whether it had been applied to the surface of the wound or whether we had implanted it into a wound in the case of some of these dermal substitutes.

We might employ systemic or topical antibiotics as well as heightened surveillance, looking for a wound infection.

But again, in burn care, most of our patients are going into antibiotics soaks anyways following the application of the graft. It's been shown to actually enhance penetration of these antibiotics through the skin grafts and thus end up with a much better graft take.

Do we perform the antibiotic soaks? As I said, on the tissue, no. That's already been done at the bank. In my mind, they've already been exposed to antibiotics.

The patients with burn injuries who undergo the grafts whether it's allografts or autografts, at least in our institution, they're getting some type of a slurry or some type of an irrigation of that wound

bed with antibiotics. They're very effective against gram negatives and gram positives.

We do use some topical antifungal agents. Unfortunately, they're not terribly effective. The ones that are effective are extremely toxic. So fungal infections in burn patients, particularly those with in excess of 80 to 90 percent body surface area burns, are inevitable and they're very difficult to treat.

I think allogeneic skin products that are actually implanted are not treated in that manner. Those that will be implanted under the abdominal wall, in soft tissue defects will probably be treated in much the same manner as most plastic and general surgical procedures with one dose of pre-operative antibiotics and close follow-up. They generally would not be treated with even 24 hours of antibiotic coverage.

But we do do that prophylactic systemic antibiotic approach that has been advocated by American College of Surgeons and others.

What have been our experiences with infectious adverse reactions? Have any been attributed to the graft?

I would say that most of the reports of wound contamination from skin substitute grafts, particularly skin allografts, you have to go back more than 30 years to find reports in the literature, well before the development of AATB standards and quality skin banking as we see it nowadays.

The very few reports of infectious complications attributed to allograft skin, again, you got to go back to 1994 when Dr. Kealey from Iowa reported on some concerns about CMV seroconversion. But there have been no studies, as I said before, proving that this is a clinically important issue.

The only report of HIV transmission in *Lancet* in 1987, if you look very carefully at that report, the recipient had more risk factors for HIV than the donor.

They'd actually done the skin transplant before they even had the results back from the pre-implantation testing. So, in my mind, that's an example of what can happen if you don't do anything.

How do we evaluate a potential serious infectious adverse reaction for systemic infections? We're just concerned about a seroconversion. Not only

when we test the patient immediately at the time of our being informed of a sero positive result, we'll also bring these patients back at 3 and 6 months for follow-up.

That's one of the good things about burn patients, is we have to follow them for long periods of time anyways. They're not difficult to track down. If anything, they want to come more often than less often. In addition to that, we contact the patient's primary treating physician or pediatrician.

As far as the wound infections, we get cultured sensitivity at the surgical site infection, if indeed there is one. Although I can tell you in burn care, the standard of burn care is that cultures are done of the wound beds generally twice a week in every burn unit generally to monitor the patients' flora and the burn unit flora. So we keep Johnny's bugs on Johnny and Sam's bug on Sam. We make that there's very little cross-contamination.

Comprehensive testing of the recipient donor tissues would be necessary to determine whether or not the transplanted tissue was a source of viral

transmission. But I can tell you in my 25 years experience, we have not seen this once. That includes all the hundreds of blood products that these patients typically get throughout their hospitalization as well. We've not seen a single seroconversion in any of our patients or a clinical case of hepatitis.

What about the availability of graft types and whether the graft is life-saving or life-enhancing have -- this is one where I think for many burn cases it's really not life-saving.

It gives us a chance to save the patient's life but because it's not permanent I don't think you can call allograft skin truly life-saving. It gives us an opportunity to save the patient's life.

So yes, when we are using fresh skin allografts where we don't have all the results back at the time of transplant, it's very valuable. It's very important for us as a life-enhancing or perhaps one step in the life-saving process for extensive third-degree burns.

But as I said earlier, it does require exception-release. We work very, very carefully

between the skin bank and the burn surgeon so that both sides know what the exception-release means. We actually include that in our hospital in the consent process as well to the families so that they understand that these are the same risks that one would have with a blood transfusion.

But there’s also a no because for a variety of other allogeneic skin products for elective surgery, cosmetic surgery or management of a chronic wound, the availability issue may not be as critical. Those procedures can be put off, be done on a little more elective basis and risks don’t have to be taken.

So with that, let me just conclude by saying that, in my opinion, allograft skin has probably been one of the major reasons why we’ve been able to change mortality in burn care over the last 20 years.

It’s good comprehensive surgical care on a team, understanding the fluid management, ability to control the wound very early on.

Nowadays if a burn center hasn’t completely excised the entire third-degree burn wound within the first week of admission to the hospital, something’s

not right because that's the standard of care. When you do a procedure like that, you've got to provide immediate wound coverage and then get on to planning how you're going to provide ultimately long-term permanent wound coverage.

If the patient dies from an infection as a consequence of the wound and the flora that has been allowed to get out of control, that is quite commonly the source for the multi-system organ failure that kills our patients.

So with that, I'll conclude. I guess we'll go on to the panel and try to get to everybody's questions. Thank you.

DR. SOLOMON: Could the speakers please come up?

Also, if you need cards to put your questions on, just raise your hand or if you have cards with questions, pass them to the aisles.

One of the things that I think we can conclude from the three talks is that the surgeons are putting a lot of trust and faith in the tissue bank to provide them with a good quality product.

Judging by the number of infections that the surgeons are seeing, the banks must be doing a fairly good job at doing that.

Let’s see. I think when we asked the question to you about how do you evaluate the potential infectious adverse reaction occurring in your patient, most of you explained you would look for a clinical infection.

But I think what we had in mind more was let’s say you have that rare clinical infection. Can you discuss how you would go about trying to decide whether it was the result from the tissue or not, and at what point you would alert the tissue bank that such an event had taken place?

DR. DOPPELT: Well, let me just say that before 2001, if there was an infection, probably more likely than not, it would have been blown off as it was related to the patient or -- and it wasn’t allograft associated. So now there’s a much greater heightened awareness that it may be allograft associated.

Again, early on in the discussion about allograft associated infections, many surgeons were not

bothering to report it to anybody because they didn't think that that was what the problem was. Now, of course, they would. As you know, the CDC had to actually sort of solicit people to respond to any potential problems.

So at this point, if there's a question of an infection, you would go through the clinical things to sort of determine that. You would have to communicate directly with the bank to find out if that same organism was present pre-processing. I'm assuming that all the terminal cultures would have been negative.

So to be allograft associated, you have to have presence of that organism early on on that tissue and then retrieve that same organism later after implantation to make the connection. So there would have to be a discussion with the tissue bank. I am assuming that that would occur without hesitation.

DR. KAGAN: I think the burn centers, or at least units that are accustomed to taking care of patients with acute wounds, probably have a pretty good protocol for routine surveillance of the patient's bacterial flora.

So I would think that if they found something unusual that was new in a post-operative dressing change that was not there prior to that, they would begin an investigation and contact the tissue bank.

DR. JONAS: I think it’s a similar response from a cardiac point of view. Assessment of bacterial endocarditis on a cardiac valve is relatively routine.

The advantage, I think, of having cultured the allograft in the operating room is that might be a link back in a patient who was subsequently proven by echocardiography to have a vegetation on a valve, consistently positive blood cultures.

Then if that linked back to an organism that was cultured in the operating room and that linked back to an organism that was cultured on the pre-processing screen that was performed at the tissue bank, then obviously, you would have reasonable confirmation that it was an allograft-induced infection.

In fact, in about the only report that I was able to find of an allograft that was implanted and proved to be infected, it was possible to trace it back to the pre-treatment screen at the tissue bank. That

organism was then gone. It was a fungus following treatment.

I think that report subsequently led to fungus becoming an absolute contraindication even on a pre-processing screen. But, as I say, there's probably a lot of you in the audience who know a lot more about that than me.

DR. DOPPELT: One other thing, I think it's important for someone from the hospital, the surgeon or someone else to report -- number one, to report it to the tissue bank to try and sort out the infection in that particular patient. But also it lets the tissue bank know that there may have been a problem. They can track the other tissue that was implanted or quarantine the remaining tissue until they sort that issue out. That's number one.

Number two, hospitals these days take infections very seriously. They have an army of people on their infection control group. If the surgeon doesn't call the tissue bank, one of these other people will.

In our hospital, we have a woman who's like a

dog with a bone. I mean she won't let go until she figures it out. That's actually good news.

DR. KAGAN: There's another place I think where hospitals have responsibility where at least a number of years ago they weren't accepting it and that's in the operating room.

Right now, the operating room is the pass-through place. But if tissues are stored in the operating room, right now they really only come under the aegis of the JHACO in terms of requirements for proper storage conditions and the like.

While the skin when it comes in, for us, literally gets delivered at the front desk and is brought immediately back to the room, there's not much opportunity for things to happen.

In the case of other bone products and other things that may be sitting on a shelf where some things require refrigeration -- but there is some burden for the hospital to not only do it but to monitor that just as we would do in a tissue bank.

DR. SOLOMON: Okay. Again, I think you fairly well explained when you see a tissue that's

labeled sterile, your expectations of what that means. So I don't think we need to discuss that anymore.

We've got a fair number of questions.

Dr. Kagan, would you like to --

DR. KAGAN: This question is what's the definition for viability for skin cells. Is it a number like 100 percent or less? That's the first part of the question.

Nobody's really identified what that number needs to be. Again, an area for research. I think about 30 years ago Randy May, who was one of the early researchers in skin, threw out a number like 75 or 80 percent. But I'm not even seeing that published anywhere.

I don't know that anybody's ever determined how much viability do you have to have measurable in the skin to ensure vascularization of tissue. This is something we've tried to do a little bit of research on the side within our Shriners Hospital.

But we haven't been able to come up with a number because generally what you're doing is you're taking a small piece of skin. You're subjecting it to

various types of in vitro testing, but you're not taking an entire piece of skin. So that's something that's on the horizon possibly for us to get some answers to it.

The follow-up to that is is there any data on average viability following cryopreservation. I would have to say that's the same thing. Nobody's really done that research.

The last part of the question is at what point is viability of fresh skin compromised, how many days post-recovery. That's a tough one to give you a specific answer about.

I think there are two things that happen over time. Number one, the skin is more likely to become contaminated because it's growing in nutrient media, which is ideal for the metabolism of the cells in the skin, but it's also great for metabolism of any contaminants that might be in the nutrient media.

While we tend to keep it at refrigeration temperatures, I can tell you that Steven Boyce, who's the PhD that grows our patient's skin in the culture laboratory, has that skin growing at body temperature,

at 37 degrees Centigrade, which is ideal for growth of tissues.

So we get into that balance of do you want to keep it cold to minimize contamination, but which isn't as good for cellular metabolism, or do you want to have it warmer and run the risks of contamination.

As a matter of fact, one of the people that reviewed our Shrine grant who as a burn surgeon a number of years ago -- we even proposed doing testing for the viability of keeping the skin at 37 degrees. His comment was it'll get infected so you won't be able to do the study. Then he turned down the grant.

So again, it comes down to a knowledge base for those people that are reviewing these grants to understand what's the purpose in it and not necessarily going along with their preconceived biases.

DR. JONAS: Cardiac allografts contain some DMSO and residual antibiotics from processing. Does the culturing method being used at implant and in the clinical laboratory take these substances into account?

I don't believe it does. I'd be interested to know just how much residual DMSO present after what

seems to be endless washing processes that go on as we wait for the tissue to become available.

But no, I'm not aware that our lab does anything special other than just, I believe, treat the tissue as though it was excised tissue that we would be asking for a culture result on.

Having said that, it's interesting that it's not uncommon to get positive cultures back. Whether that's from contamination in the operating room -- and certainly we are at fault in changing gloves for the surgeon and changing gloves for the scrub tech. It's certainly possible that contamination occurs in the operating room.

So the answer is no, nothing is done.

DR. DOPPELT: Okay. I have a question here. It says when using ethylene oxide sterilization, if the ETO residual is very low, do you still worry about synovitis?

So it's a little bit harder to answer because I guess it depends upon how sensitive is your assay for ETO residuals. It's my understanding that in these grafts you always have some ETO residuals. It's

impossible to remove all of it. So then you're at risk.

What is -- how much do you have to have a meaningful synovitis? I don't think anybody knows that, but the correlation was that with the ETO, the grafts had a reasonably high failure rate.

So at this point, I would just say you cannot completely remove all of the residuals. Therefore, it just isn't safe to use.

DR. KAGAN: I've got a question.

Why would clostridium presence on skin grafts be a problem in light that the clostridium is an anaerobe and the skin is in an aerobic environment?

First of all, clostridium can be a fairly invasive organism. It requires the use of an additional antibiotic that we would not like to subject our burn patients to unnecessarily.

Thirdly, these are immunocompromised patients. While you may think that they are aerobic, these patients frequently are severely acidotic and fluid compromised, have shifts in blood flow to the skin.

You probably don’t know this. When a person goes into shock or drops their blood pressure, the organ that has blood flow turned off to it first is the skin. So when we have patients who are having difficulty with fluid management, the last thing that we can afford to have happen is to have the skin become an anaerobic organ.

That and the fact that with the continued risk for cross-contamination, despite all efforts to maximize hand washing and the use of gowns and gloves, we just don’t want a bug like this anywhere on our unit, period.

While he’s reading, I’ll get to this next one.

With changes regarding donor testing for amniotic membrane donors, do you think amnion clinical application for burns will increase?

My gut says no because I think burn surgeons think well, I’ll replace skin with skin. When we take a piece of 4 inches wide, 3 feet long skin to cover a wound, it’s very natural for us.

I do see a potential increase for management

of partial thickness wounds, particularly smaller ones. I can see it increasing a lot with chronic wound care, possibly.

But I don’t think that it would necessarily take the place of allograft skin in the management of patients with extensive burn injuries.

DR. DOPPELT: Okay. Here’s a question on ACL grafts. So for the functionality for the grafts for ACL reconstruction, how much weight would the sort of sub-failure properties hold versus failure properties given that sub-failure for activity mimics activities of daily living?

So I guess the question is how strong does the graft have to be so that it doesn’t fail. So here’s the point. In people who are -- you’re doing ACL reconstructions in people who -- the clinical indications are younger, active and symptomatic with instability.

An older person -- let’s say older being relative but over 45, sort of a couch potato. Older isn’t 40. I see some grimaces there.

But older and less active, you’re not going

to be doing an ACL reconstruction. So if you're not involved in sports and you're just walking and doing stair climbing and usual working for most of us, you don't need an ACL to get by with activities of daily living.

It's if you're going to go skiing, you're playing sports and you're more aggressive, that's when you need the ACL reconstruction.

So for activities of daily living, it's not an issue. You started out with -- the patient started out with a normal ACL and blew a normal one. So you can still rupture an allografted ACL. The re-ruptures occur usually within the first year because you have to give it time to sort of repopulate with cells and start forming some additional collagen.

Surgeons may choose to protect the graft in the first year using what's called a functional knee brace. So you can return to sports early. The graft doesn't see all the forces but is being partially protected.

The bottom line is that if the graft has a strength of over -- the failure strength is over

2200 Newtons, then you probably are going to be okay because that was the strength of the original graft. You can achieve that with most of the allografts.

But the failures are -- the reasons for the failures can be complex. It may be the tissue that fails, but more likely, it may also be the methods of fixation.

So if you look in the orthopedic literature, there’s a million different mousetraps. Every company’s got another way of trying to fix this thing because it’s not only the biology and the strength of the graft but it’s also the physical ways in which you fix the graft.

So there’s a host of reasons for failure. But the bottom line is if you’re up to the strength of a normal ACL, 2200 Newtons which most of these are, it’s probably not going to fail of that.

DR. SOLOMON: Okay. Here are some general questions that weren’t directed to anybody in particular.

This would be a two-part question. Part of it’s my question and part is this one.

But, Dr. Kagan, you mentioned that you looked at the container to see if it was murky. But how often do surgeons actually look at the label and the summary of records and things like that when the tissue comes into the OR?

If you do look at the label, is there enough detail in the information? In other words, would you like to know more about the processing methods than is currently described in the package insert?

DR. KAGAN: Well, for me, it’s a little different situation than it is for most because I’m a skin banker. And I’m a medical director of a skin bank so I know what to look for.

My guess is most burn surgeons just turn around and say give me the skin just like the orthopedic surgeons turn around and say give me the bone or give me the tendon.

Most of my interest when I’m in the operating room is to look in the box and see is it fresh or is it frozen because oftentimes the bank isn’t able to tell me in advance which one. I know what I’m requesting, but it’s kind of like peek inside the package and see

what they brought over.

Usually if the tech is smiling -- if they happen to actually bring it over from the tissue bank, if they're smiling, it's fresh. If they got a frown on their face and they're backing up a little bit, they think I'm going to yell at them because it's frozen, then that's the situation.

It's me. I think I would be probably the one burn surgeon in this country that actually looks at the murkiness, or absence thereof, of the nutrient media that the skin comes over in.

My review of the records is as probably as cursory as the rest. Again, it's because we use one tissue bank. I happen to be a consultant to that tissue bank and I've written most of the rules for how they do what they do. So my expectation is they follow the rules that are written.

If we had to obtain skin from a number of skin banks and I had less confidence in that process, then I might be a little bit more prone to look at summary of records, et cetera.

But with us having essentially a sole

supplier, it’s less of an issue for me.

DR. DOPPELT: Well, for orthopedics, I think it’s a little bit more complicated. I think in most hospitals they’ll have certain rules like you need to purchase the tissue or acquire the tissue from an AATB accredited bank so you have some assurance that they’re following rules.

Having said that, different banks process things in different ways. Some surgeons, as I pointed out, may have very strong feelings about I want it irradiated, I don’t want it irradiated and so forth.

The problem is that the surgeon doesn’t actually do the purchasing. He just says I’ve got a case and this is the equipment I need, i.e., I need an allograft. It’s up to the purchasing agent in the hospital who actually submits the request.

Although the surgeon may think that the tissue is coming from a particular bank, depending upon availability, it may actually come from a different bank. It’s still an AATB accredited bank. However, they may be doing it in a different way.

So in our hospital, we have a group of people

that are orthopedic scrub techs that are the only people that scrub on the orthopedic cases. So for us, they know what we want.

But in other hospitals where the scrub tech is -- they're doing OB one day and orthopedics another, you may wind up getting a graft past you that wasn't what you thought it was going to be or come from the bank that you thought it was.

So that's one issue that many surgeons, if they're familiar with these issues, would want to have that information.

I think on the label it's helpful, obviously, to know if it's irradiated, it's not irradiated. When you get into other technical issues in terms of how it was processed, I think most of the banks now are using processing methods that they understand it can't be too harsh for the tissue and so forth. But at least for radiation, that would be an important point.

DR. JONAS: Yeah, on the cardiac side, I certainly do look pretty carefully at the label. But the information I'm really looking for is the exact shape and size of an allograft. My choices to which

allograft to use will be determined by how good a fit the given allograft is for the reconstructive project that I've got in mind.

There's really not a lot of information that I look at and perhaps it's on the labels. But there's no detailed information about pre-screening bacterial cultures. That would not be something that would influence my choice.

I do think that while we're on this area, though, it's important to remember that with small cardiac allografts there really is an availability issue.

We are very much limited in sizes that are available. It's not at all uncommon for us to have to use sizes that are not optimal simply because we don't have a range of sizes available.

Our earlier discussions with the New England Organ Bank and some of the tissue processing facilities was stimulated by that lack of tissue. When we found that 40 or 50 or 60 percent of small allografts were being discarded because of what seemed to me a miniscule amount of contamination, I for sure would be

prepared to accept some degree of contamination to have an optimal shaped homograft rather than having to go to a synthetic alternative where that synthetic tissue, I know, has a real risk of getting post-operative infection, whereas the allograft has an exceedingly low risk, probably even with some degree of contamination.

So I do think it’s important that we balance out here for cardiac tissues reasonable continuing availability and yet also a reasonable standard in terms of contamination.

DR. KAGAN: Yeah, I’d echo that comment. I remember a number of years ago our pediatric cardiac surgeon called me because of his frustration with not being able to get an ample supply of sized valves. His issue was again with the tissue bank that had essentially a zero tolerance type of policy.

DR. JONAS: Right.

DR. KAGAN: And yet when we discussed it, I said you need to get more involved with the tissue bank medical director because not all these things are dictated by FDA. It’s the bank that implements some of these policies.

In some ways, the burn surgeon community, which essentially developed skin banking, has had a grassroots influence on skin banking policies from the outset.

While it may not be reasonable for orthopedic surgeons to develop those kind of relationships with musculoskeletal tissue banks, it may be more reasonable for cardiac surgeons to take a greater interest in working with the medical directors of the heart valve banks to look at some of these issues and develop policies that make clinical sense and not just tissue banking sense.

DR. DOPPELT: Can I just add one other thing? In regards to labeling, there’s one other point here. It was brought up at the last -- for those of you that were at the last AATB meeting, there was some discussion about it.

Because of the -- when some bank or some procurement organization is doing something that’s a little bit shady and these are -- you read it in the newspaper and they’re not AATB accredited. I don’t know. They’re just some sort of shady organizations

where body parts are moving across the country.

That becomes very high profile and raises the question by a lot of surgeons as to, well, maybe I need to know more about the tissue. So they're saying -- what we heard at the AATB was that some of the surgeons -- the banks were getting questions from the surgeons. "I want to have more information on the label."

For example, if you're going to use a strut graft, that you need to have a certain biomechanical strength, is this coming from someone who's a 75-year-old female who has significant osteoporosis or is this from a 25-year-old male who died in a motor vehicle accident?

Related to the previous question of an ACL and the strength of the tissue, is this someone was elderly and the tissue in general may have been poor in terms of tensile strength?

The problem is that, in reality, the accredited banks, the reputable banks, have very reasonable criteria in terms of how they're going to use their tissue. If it isn't suitable for strength,

they're not going to use it for that type of a graft.

But the confidence of some of the laypeople and in the medical community has been shaken by some of these things that you read in the newspaper which brings people to the table asking questions. “Well, give me more information like about this and that.”

I go back to if it’s a reputable bank, you have to have confidence that they have professionals doing this. Probably the surgeon’s input into his decision about this, that or whatever is probably not going to bring additional information that the bank director hasn’t already thought about.

DR. SOLOMON: Going back to what Dr. Jonas mentioned, so you would accept a pediatric heart valve if it were the correct size that you needed even if it were not sterile. Is that what you’re implying?

DR. JONAS: Well, I think we need to develop a better understanding of pre-antibiotic treatment contamination, post-antibiotic levels of contamination that would be acceptable and subsequent clinical sequelae.

Right now I think that the bar is very, very

high is my understanding. Once again, I'm not directly involved with these sorts of decisions.

But I was appalled to find that there's so many of the pediatric donors were being rejected because of pre-antibiotic treatment screens that were positive.

It seemed to me that in the early years of development of the allograft it was assumed that essentially all allografts were going to be contaminated. They were collected at autopsy in a completely unsterile fashion.

The goal was to reduce the bacterial load by the antibiotic treatment which was one at a hundred percent of allografts. Then they were implanted with apparently an extremely low risk of subsequent clinical consequences.

So I'm not involved with tissue banks. Perhaps I or other members of our field should be. But certainly pre-treatment total rejection seems to be unreasonable.

DR. SOLOMON: Another question: How often would you do serology testing on a recipient and would

it be both and after the implant?

DR. KAGAN: Well, I can tell you for burn patients, in 25 years I've never upon admission tested a patient for serologic tests unless there was a history or unless they came from a foreign country where we didn't necessarily have testing and there was potential risk to the staff in the hospital.

But in this day of universal and standard precautions, we treat everybody like they've got some viral or worse disease.

But from a clinical treatment perspective, I've never tested anybody when they're admitted to the burn center for a variety of things. I would only test them after they've developed clinical signs and symptoms of something like hepatitis or something of that nature.

DR. JONAS: It's the same for cardiac.

DR. DOPPELT: Yeah, I would say for orthopedics it would be the same. We don't test for the serology.

But in the previous example of notifying the bank if the bank called, then all the other surgeons

that had used tissue from a donor that there was a question of transmission of disease, obviously, you would be following that particular individual.

DR. SOLOMON: Along those lines, and maybe not quite related to processing, but in terms of being able to track a tissue to a recipient and find that recipient, do you -- what is your experience with the return of the recipient cards and also documentation in the patient's record of the lot number and things like that?

DR. DOPPELT: For orthopedics, first of all, JHACO was mentioned before. So all the hospitals are following -- have logs where they have a tissue log of who -- where the tissue came from, the identification number and what patient received it. That's kept in the operating room.

In addition, in the patient's chart, there is entry as to what tissue was implanted and its identification number.

So if somebody were to call us up and say what's the tissue identification number for this particular patient, we would be able to find it easily.

In terms of is the information going back to the tissue banks, I think that has been a common problem, a common deficiency. The banks will ask that that information be sent. They may even call about it, but they don't often get it.

DR. KAGAN: In burn centers, at least in my experience, there is a tag being placed into the patient's -- the paper part of their chart at least, very similar to what's being done by blood banks that provide the tracking number. So if it were necessary to take information about the patient and track it back to the tissue bank, that would be easily doable.

As far as do we routinely send information back to the bank on the card or whatever, I'm a very unique situation. The skin bank is two blocks from my hospital. Somebody, who I know their face, comes over and generally puts it in my hands or in the hands of one of my associates.

So they're generally actually right outside the operating room door. They know which patient it is. They actually get the information and have it in hand before they even leave.

That’s a very unique situation. Most other places have to ship. There’s a big distance between the one and the other.

Patient information has to be kept under tight surveillance. You got to be careful who you say the skin’s going to be used for, even nowadays. So there are some HIPAA type issues that might preclude passing information over the phone.

Plus sometime when you get the skin and you put it in your own refrigerator or your freezer, you won’t know until it’s time to use it which patient you’re going to use it for as well. In those situations I think the hospital and the physician managing the patient have to take some responsibility for notification although I wouldn’t think it’s done terribly frequently.

DR. JONAS: Well, once again, it’s JHACO that mandated that the information be reported in the patient’s chart.

I know our operating room records and OR nursing staff are certainly very careful about following all of the JHACO regulations these days.

I’m assuming that the tissue preparing facilities are getting the information because I sometimes receive from the tissue companies requests for information. “What was this tissue implanted? We don’t know exactly what operation was done. Please let us know.”

So I have the sense that it’s being tracked pretty carefully today. Now that certainly wasn’t true 15 or 20 years ago. But today it’s done very, very carefully.

DR. SOLOMON: Okay. This next question, I think we touched on it but maybe it requires a little more depth.

They say it’s very hard to establish a causal relationship between a post-op infection and an allograft. Are there any standards established or recommended by the infection control department of your hospital, or in your opinion the best tests and/or investigation to confirm the causal relationship between a post-op infection and the allograft?

DR. DOPPELT: Well, it is difficult. Obviously, you’re going to mentally go back and ask the

question was there a break in sterile technique. You would know that during the case or at the end of the case, in which case you probably spent some sleepless nights.

But in the absence of that, again, I would just say you have to rule out other sources of infection. Either you would go back to the operating room and make sure that the sterility runs for all the instruments were appropriate and so forth.

Then go back to the bank and what you're looking for is some pre-processing culture that has the same organism that you retrieve as a post-operative infection.

If you recall in the reviews in early 2001 to 3 or so forth, some of the -- if you want to call it a root cause analysis of how the infections occurred, there was release of one tissue that was allegedly irradiated or thought to be irradiated and was accidentally released not having been irradiated.

There were some failures in the processing methodology in those days where it wasn't quite done the way it was intended to. That has all been

corrected.

But -- so you have to go back to the bank and find out if there was some organism early on in the processing of that tissue.

Short of that, I'm not quite sure what else you could do.

DR. KAGAN: I would agree with that at the skin side.

Part of our responsibility as clinicians and as users of tissue, particularly in a burn unit, is to educate the infection controller who is involved in monitoring. I'll give you an example.

We have a patient who has an 80 percent burn. He had allograft applied. He just got autografted for most of his wounds this week. He now has a culture on his wounds positive for aspergillus which is a fungus, an opportunistic infection.

She did not come to me right off the bat and say check the tissue bank because that's way down on the radar screen knowing what we know. She's actually contacted the environmental people in the hospital first to find out about construction and air ducts.

So she’s looking based on the organism and where is it most likely to come from and not starting with things in the wrong place. Again, if she had no knowledge of what we do with skin banking and the like, and the processes that occur, she might figure well, the one thing she doesn’t know anything about would be the first place she’d go.

I would agree with Sam. It’s not been unusual at all for us to find a skin graft measure that we used on Friday that we asked to use in the operating room on Monday was sitting down there, and we thought it was sterilized, and the whole weekend it didn’t get sterilized.

So that’s another place where every once in a while you’re going to be surprised to find that’s true. But sometimes the OR processing team just doesn’t do what they’re supposed to do in a timely fashion.

DR. JONAS: Yeah, I agree that the causal connection is difficult to establish.

We can have situations where a child might have a cardiac arrest in the first 24 hours post-operatively, is placed on ECMO support. The chest

is left open for three or four days. The child's then taken off ECMO. Then multiple cannulas going in through an open chest wound for several days.

This is the sort of patient that's likely to end up with a mediastinal-type infection. Interestingly, they really are very resistant to getting sternal osteomyelitis. Even if they do have a purulent mediastinitis with an allograft in positive, I've seen the allograft be resistant to infection and to be able to heal up a purulent mediastinitis and get the sternum closed with no long-term sequelae.

So it's difficult to -- in a circumstance like that, say, well, the reason this child got a mediastinitis was because of contamination because from the bank originally.

Clearly, as has already been said, if you can trace an organism from the pre-screen, the post-screen, the low level of post-treatment positive culture and then a positive culture in the operating room and you have a consistent organism, then that would be reasonably convincing.

But, as I say, the sorts of situations where

we see infection are like the ones that I described.

DR. SOLOMON: Does it also depend upon your experiences when you perform surgery and don't use an allograft in terms of the frequency of post-op infection and the particular organism, if you feel like the rate with the allograft is approximately the same rate as without, you might attribute it to other things?

DR. DOPPELT: Right. I think the -- if you look at the total number of allografts that have been used in the last ten years and the total number of allograft associated infections, the overall rate is very, very low.

As tragic as even one infection is, the overall rate is pretty low, lower than in actually routine orthopedic surgery.

So again, in orthopedics to get an infection, there are many, many other variables such as the extent of the wound, devitalization of tissue. There's lots of reasons why people can have an infection.

Sometimes you can't even find a reason. It just is.

DR. SOLOMON: Okay. It’s 12:15.

Does anybody want to come to the microphone to ask a question?

Okay. Then I think we’ll adjourn. You have an hour and a half for lunch, so we’ll reconvene at 1:45.

(A lunch recess was taken.)

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

DR. KUEHNERT: We've reached the time to start the afternoon session which is Session No. 2, Pre- and Post-processing Cultures for Microorganisms: Usefulness, Reliability and Validation.

My name is Matt Kuehnert. I'm from CDC. I'm the moderator for this session.

We have a number of questions for the speakers to address. So I'll just go through them in order.

For Scott Brubaker, who I'll be introducing in a moment, the questions are what are the current AATB standards with regard to use of pre- and post-cultures such as presence of a discard list? How did AATB decide on these standards, particularly where there is controversy?

Next, are there updates on new standards, guidance relating to culture methods and validation of processes?

What is recommended to actually culture? How is culturing performed? What is done with the results in terms of evaluation?

Next we'll have Martell Winters addressing what are the current culture methods; what are the concerns and challenges with these methods with respect to usefulness, reliability and validation; and what is considered best practice and how can the industry do better.

We have Arjun Srinivasan addressing the following issues: Based on investigations of infectious disease transmission by tissue, where have current pre- or post-processing culture failed? Can recommendations be made for particular methods or combinations of methods?

Then we'll have a panel discussion including comments on questions regarding challenges and concerns with current culturing methods and what new methods for obtaining a representative sample are being developed.

I also have some other thoughts on the panel discussion, which I'll present at that time.

So for the first presentation, I'd like to introduce Scott Brubaker.

Scott has held the position of chief policy officer at the American Association of Tissue Banks

since August of 2004. His duties are to be a liaison with the FDA, CDC and other professional organizations, nationally and internationally, for standard setting and regulating bodies for cell and tissue banking.

He also serves as the office liaison for the AATB Standard Committee, the Scientific and Technical Affairs Committee and the Physician's Council. He was at LifeNet before he joined AATB.

I can attest that he's a busy guy. He works very hard with us on tissue safety issues.

Speaking for the Workshop Planning Committee, I'd also like to thank him for all the help in putting together the agenda and rounding up speaking and panel discussants, so a special appreciation for that.

Scott.

MR. BRUBAKER: Thank you, Matt.

I want to thank FDA as well. It is always a pleasure working with the FDA folks in putting together some presentations and workshops. So we hope to do more in the future.

I'll be giving just a brief overview of the standards and guidance that was requested. These are

the most recent ones that we've got out there. We do have a lot of history, of course, in standards setting. There has been changes through all of these different standards. But I'm not going to go through the history, just address what's currently in place.

You've seen the questions before, but, basically, what I really wanted to do as well was to provide you with some of the controls that we address for recovery operations, which do affect the cultures that are coming in and the processing and all the validations as well.

First, we do screen the donors for suitability to rule out clinical evidence of active infection at the time of death. Thorough physical assessment can also reveal some risk factors that you might find on the body that are related to an infection.

We have time limits for recovery that have been in place for many years. These are based on body cooling guidelines. There are specifics I don't go into here.

Just to give you an overview, for cardiac

tissues and vascular tissues -- but for vascular, it's based on this body cooling as well as perfusion times for vascular tissues. For cardiac, subjecting the tissue to a cold isotonic sterile solution ends that requirement.

There is no body cooling, refrigeration. Recovery must commence within 15 hours if there is no cooling. If there's body cooling within 12 hours of asystole, recovery must commence within 24.

We do have situations with cadaveric donors where sometimes there'll be some body cooling followed by none. So we address that by not allowing 15 consecutive hours.

Asystole definition for us I think is very, very important. We believe the asystole definition as well offers control because we do get specific in here in that if the death was not witnessed, which can happen very often, asystole is defined by the last time known alive. That can then -- then the cooling parameters are also part of all that. So that requirement I think does offer some level of control.

I do want to mention, too, we have a guidance

document that we created a few years ago and updated once. Just briefly, that's there.

We do control the site of recovery. We have new standards and guidance that were issued in May for that.

We expanded our aseptic techniques for recovery as well. Isolation draping, recovery zones now are -- there's 13 of those that are now identified. Also, selecting and documenting the sequence of recovery can help in evaluating the cultures that you do obtain from recovery pre-processing.

This was the form that's also used. It's required that all of this is followed. I do want to mention just one thing. You can probably see it in your handout pretty well. That's why the handouts are printed larger so you can read the small print.

But the recovery area in a pre-recovery evaluation, I just want to mention number 4. The recovery area has a controlled closed air flow system. This means there is no direct access to the outside of the building from the room at any time during, before or after tissue recovery.

The examples that are given are like doors, windows that can open, fans, air conditioners, et cetera. So we're trying to really control environments where recoveries do take place by all of these parameters that are listed there.

Also, aseptic techniques are required. This is a lot -- or found in Section D. I'm not going to go through all of those. But when you look at levels of safety, it's really we were trying to mimic what happens in an operating room and that kind of environment.

AATB Bulletin No. 702, this is the only slide that's not in your handout. I thought I would put this in there because years ago there was an issue of sharing of results like the pre-processing results or recovery culture results. We've mandated that now in this new standard that was published back in January actually.

The tissue bank that recovers cells or tissues must share tissue recovery or pre-processing culture information with all tissue banks to whom tissue from shared donors was sent. In other words,

that information was shared with all the processors.

The processors do report back that information individually, but that information needs to be shared. So every processor will be looking at all of the culture results that have been taken.

If a donor is determined to be unsuitable for any reason, that information must be shared with the recovery agency from that particular processor, and that information is shared with all of the other processors. So everyone has the same information.

There could have been some investigation done by one processor that the other one didn’t do or didn’t notice could be done. That information is now shared if the donor is found unsuitable, another level of safety.

So the first charge for this presentation is what are the current AATB standards in regard to pre- and post-cultures, discard lists? How do we decide upon that? Yes, there was a little controversy.

Like all of our standards, we do develop them from shared experiences of our tissue banks, best practices. They have been shared for many years and

consensus agreement by members.

We do get information not only from our councils. Like Dr. Kagan mentioned, the Skin Council, they developed those list of organisms for our standards. They have a lot of input, of course, those who are on the skin council and end-users as well in that situation.

We also rely on task forces. We had a sentinel events task force. After the clostridium death occurred back in 2001 associated with an allograft, a sentinel events task force was organized. They came up with some recommendations as well that we did implement.

The standards committee, of course, looks at all of it. It’s finally approved by the board of governors.

Now, organism discard lists, there was some controversy when we put these together. It’s dependent on the validations by each organization, and what they’re capable of doing, and how they’ve done their validations. There can be different levels of I guess feelings about if an organism is pathogenic to them or

not because of their process and what they validated.

First, let’s look at short lists and why are there short lists. Disinfection, there can be different ways and methods applied for disinfection. We’re going to hear about some of those today and tomorrow.

The antibiotic mixtures will be different, incubation temperature, exposure time, rinses and so forth. So different banks will have experiences and different experiences with specific organisms.

As we get into contamination or pathogen reduction and elimination, which could be sterilization as well, the methods would differ as well, too. You can see the different ways that tissues can be treated in different steps that can all each reduce the level of contamination that might be present originally.

Validation methods have evolved in recent years. That’s one, really, of the basic reasons that we’re here today, is that they really have evolved and they’ve improved.

Microbiological tissue cultures, this is in the K2.200 series in our standards. This one addresses

pre-sterilization and pre-disinfection cultures. The kinds of tissues are -- if it’s not a general standard applicable to all tissues, you’ll see the specific tissues listed off to the left. This is for cardiac, vascular, musculoskeletal, osteoarticular and surgical bone donation as well, which really doesn’t happen in the U.S. anymore.

But each individually recovered or packaged tissue intended for transplant, a qualitative pre-processing culture shall be obtained prior to exposing the cells or tissues to antibiotics, disinfectants or sterilizing agents. The medical director must review these cultures prior to release of the tissue.

Individual tissues with culture results showing clostridium and strep pyogenes shall be discarded. Any other individual tissues from the same donor that were recovered under conditions that could result in cross-contamination must also be discarded unless they can be treated with a validated sterilization process.

So basically, there were two organisms picked

that would always result in discard for all of those tissue types. There was an original list that started out with ten. It was reduced to these two because there was a lot of concern that well, wait a minute, we have validated processes that we know we can get rid of this organism or that organism. So there really does need to be a consensus for standards when you look at what's reality and what does work.

Skin is separate. As we've heard today, it's separately -- it's viewed differently by clinicians as well.

You can see -- I'm not going to go read through all of that. But there is a pre-processing skin culture that's required from representative anatomical areas. That's a little bit different than the other tissue types.

It is a separate zone. Those results are actually looked at individually compared to the rest of the donor because we do know that skin is not sterile.

Now going on to final and pre-packaging cultures, I do want to read some of this, too, here that's underlined. All cells and/or tissues to be

released for human transplantation shall have representative microbiological cultures obtained.

Now, this has changed over the years. There was a shall back in 1994 I believe. A couple of years later it was an if. Then it was should. Then it went back to shall again over a period of time.

Now, it does say you have to do that unless dosimetric release has occurred by a validated process according to E1.044.

Appropriate final packaging cultures, aerobic and anaerobic shall be obtained. The results shall meet established parameters, defining acceptable packaging cultures before cells or tissues is released for transplant.

It does go and talk about a batch as we move on. I wanted to show you what dosimetric release was about as well as a batch or you can read it probably better in your handouts.

But dosimetric release is cells or tissue release based on dosimetry instead of sterility testing. If you go to the *American Heritage Dictionary,* you'll see that dosimetry is the accurate

measurement of doses, especially of radiation, which I'm sure you all know.

A batch is identified as a specific quantity of cells or tissue intended to have a uniform character and quantity within specific limits, which is produced according to a single processing protocol during the same processing cycle precluding mixing of cells or tissue from two or more donors. This is not pooling. This is just mixing like different donors in a freeze dry run.

Now, batches can contain lots. I wanted to mention that. There is a definition for lot that that's there.

But here as we move further into final and pre-packaging cultures, I do have on this slide that -- and this was the question earlier -- cardiac tissues. There must be representative cardiac and vascular tissue samples that are retained for fungal growth. That has been the requirement for quite a long time.

Again, I won't go into all of the detail here. You can see the skin organisms that have been

presented already. One of the reasons skin has organisms here as well, and pre-processing and post-processing, is due to the fact that fresh skin can be released on the -- not a final culture -- or actually the final culture is the pre-processing culture.

Sterilization and disinfection, E1.040, had been referenced in one of the previous standards. This one’s very important as well. Individual processing facilities shall establish, validate and document antibiotic regimens and microbial surveillance methods.

The SOP, of course, should have a list of organisms which would necessitate discard. Also, the list shall be based upon not only the category type of tissue but also the method by which the tissue was processed such as cryopreserved MS tissues that cannot be sterilized and can only be disinfected. So we realize that those are issues with different tissue types.

Cardiovascular. That group agreed upon these three. I can tell you that when we did review this with the five heart valve processors at the time, there

were different lengths of lists. One had about 24, another one had 18, and other ones had less. So these were the three that they wanted to agree on. Actually, in reality, there are longer lists than this out there.

For skin, you'll see that again -- for disinfection of tissue for this culture, you'll see the same listing for skin.

Now, describe the new standards and guidance related to culturing methods and validation of processes. Then I lumped number three in with this because they do relate. What's recommended to be cultured? How is culturing performed? What's done with the results?

All these revisions did appear in the eleventh edition. There were some that were published back in 2004.

We changed our definition for sterile and sterility assurance level. Actually, sterility assurance level is the one that changed.

We used to reference 10 to the 6 log reduction here. It was changed to the probability of a single viable microorganism occurring on a product

after sterilization. Other speakers will get into this in more detail. But we basically did take some of the definitions that were already out there and applied them to our standards. As you'll see, the reference to ANSI and AAMI standard here.

Same with sterilization; we didn't think we should differ in our viewpoint of what's already out there in other industries.

Non-terminal irradiation, that's the term that we created for radiation processes that are applied after recovery and before further processing. So we used to call it pre-processing radiation. When you irradiate something, you're processing it. So we changed that to non-terminal irradiation.

Terminal sterilization, bioradiation is addressed here. What's underlined in red and blue is very important. The sterilization dose used must be validated and supported by data. And SAL shall be selected and the sterilization dose must be shown to be capable of achieving that SAL.

Validation methods used may include, but are not limited to, bioburden based methods, again

referencing AAMI ISO, or it may be based on a group of pre-selected organisms. Again, other presenters will get into this in more detail.

Sterilization by other methods is basically similar wording to what you've seen already. This table for -- someone mentioned residuals for ETO. This table -- that is there sometimes and not -- has been in our standards for many, many years and is I think -- there are other references out there that we got those numbers from.

Disinfection by chemical agents, of course, that occurs. You saw that on an earlier list mainly from musculoskeletal tissues. You can see we require validation for those procedures as well.

Other disinfecting agents, again, require validation.

We know that guidance -- we need to have more guidance for our banks to follow to make sure that we've got standards set for the way things are cultured, how you look at those results. So we have a draft guidance ourself in mind. Our standards committee has just mandated that we do this. We'll be

seeking an outside consultant help to produce it.

But tissue culture methods do require validation. You should have an ongoing bioburden monitoring program. There should be assurance of adequacy, which is basically the sensitivity and specificity and recovery studies, also known as BF testing, should be part of that process.

Also looking at environmental monitoring, making sure that the program is where it should be. Process validation program in its entirety should be addressed. Microbial surveillance linking all of the cultures of the above together prior to release is probably going to be the topics that we address.

This could lead to more changes in standards. Standards are constantly changing with more knowledge that we do gain.

I just wanted to mention that we’re working with AAMI on this technical information report, which might be mentioned later in another talk. But this is regarding sterilization of health care products, guidance on radiation, and sterilization of human tissue based products.

We've had input to it. Our membership has. This is a great document. I'm sure we'll reference this in our guidance and our standards when it's released. That should be soon.

I just wanted to mention, too, a little bit more about AATB Guidance Document No. 2. You can see the title "Prevention of Contamination at Recovery." The focus on the culture results, this helps to answer some of the questions.

Discard organisms covering all tissue types, I mentioned clostridium and strep pyogenes. Again, maybe that will change in the future. I don't know. Other tissues for this donor you have to discard unless they can be treated with a validated sterilization process.

We do know the limitations to the culturing methods that are being used today that are available. Again, tissue establishments who determine final donor suitability may consider that more organisms fit this classification. They do that.

The culturing methods, I mentioned pre-processing cultures can be done in different ways

for different tissue types. Filtered culturing method is done for cardiac and vascular. You can actually quantify those results as well. Those cultures are done by the tissue bank before processing and right after some rinsing, but before the tissue is subjected to antibiotics.

So that’s a more sensitive method. So is fluid extraction method for other tissue types, but it’s very labor intensive to do that. That’s often done, as you’ll hear probably. I hope someone will cover this. That’s done as part of your validation process to make sure your process is working the way you want it to.

Swab culturing, we do know that that has low accuracy, sensitivity and reliability. Many studies are out there that we reference for that. That’s why we look at the other cultures that are acceptable or negative when there’s a nearby tissue that cultured positive for clostridium or strep. That’s our concern right now.

Again, there are basically two processing methods. I think you all know that already.

Disinfection and sterilization, this piece discusses the two general processing methods, the relationship to assurance of controls that should take place not only at recovery but also during processing and associated culture results that could be pathogenic.

Just some other standards that are related. We do have standards to make sure that the microbiological testing in the laboratory is approved and that they're following the procedures as they should.

We're developing actually a guidance document and a form for banks to use to audit their testing laboratories who run infectious disease testing as well as microbiological testing. But that's a little down the road.

We do have standards for transport medium, selection of growth medium. They should maintain viability of aerobic and anaerobic bacterial and fungal organisms.

That's all I have. I'm sorry for the -- my Mac isn't strong enough apparently. So if my boss is here, he needs to know that.

Thank you.

DR. KUEHNERT: Great, thanks, Scott.

So next we have Martell Winters, who's going to be speaking on challenges and concerns regarding current culturing methods.

Martell Winters has been at Nelson Laboratories for 13 years and has spent most of that time heading the radiation sterilization group, which is responsible for bioburden testing and radiation validation studies.

He's currently a study director and consultant for Nelson Laboratories and serves on many AAMI sterilization working groups. He is also on the AAMI standards committee and is the liaison to the AATB Standards Committee for AAMI.

In 1995, he received his registered microbiologist certification and also has a certification as a specialist microbiologist in consumer products and quality assurance microbiology.

Come on up.

MR. WINTERS: Okay. I am pleased to be able to be here to join this event. I'm excited for the

opportunities we're going to have for discussion. I'm pleased with the presentations that have been shared already. I just kind of went through the questions I was given. I'll be answering those as we go along here.

I broke up the culturing methods into two general types. These culturing methods are all being done in one form or another. But some are a little more old school and some are perhaps a little more new school. So I'll cover the old school or the current school first.

The first of these is swab cultures. Scott already mentioned these, that primarily swab cultures are qualitative. They're used primarily in recovery cultures. They're also sometimes used in pre- or post-processed tissue as well as sometimes even post-sterilization or post-sterilized tissue.

Then one of the other primary culturing methods that are commonly used are what's often referred to as a destructive test, which is a test for sterility. In these situations, the tissue is immersed in growth medium or sometimes a rinse aid, or an

extract of the tissue is added to growth medium and then incubated for growth.

Some of the culturing methods that for some people who are a little on the newer side are -- swab cultures can be done quantitatively where you're actually getting a number. So in this instance, you're doing a swab followed by removing the microorganisms from the swab and filtering or plating the organisms got off.

Another type of culturing method is a bioburden test. We're actually performing an extraction of the tissue and then enumerating the microorganisms in that extract solution.

I'll go into a little more detail on all these pretty soon here. All of these culturing methods are performed usually on usable tissue or tissue which might be called companion tissue, or tissue that would normally be discarded either because it was the wrong size or shape or something like that.

So let's talk in detail about the qualitative swab cultures. The benefit to the qualitative swab culture is that's very easy and fast to perform. It

requires minimal materials and equipment, minimal training and it has very long historical use. So it's very -- everyone knows about the test, and everybody's used to it.

The cons of this test are that you can get variable results based on the technician. The recovery efficiency is low, meaning the percentage of organisms that you will remove from the tissue with the swab can be and often is quite low. It also gives you a presence or absence of microorganisms. It does not give you a number. It just gives you what's there.

So I want to talk a little bit about presence and absence data. From a scientific standpoint, presence or absence data, or plus or minus, or positive or negative results, generally speaking, are actually very -- there's very little usefulness that you can get out of that kind of data because you don't know if that positive was – if it was positive because of one CFU or 10,000 CFU because from a scientific standpoint, both the type and the number are significant.

Generally, almost no scientific decision can really be made with presence/absence data except for

discarded tissue. Generally, that's what we've been doing most of the time which is not a terribly scientific approach.

A more scientific approach would be an approach related to log reductions and/or sterility assurance levels. But in order to be able to gain any information to be able to take a log reduction or sterility assurance level approach, you must have the knowledge not just of the type but of the numbers that are present.

So let's talk validation of a qualitative swab culture for a little bit. Validation. The first issue is the efficiency of removing the microorganisms from the tissue with the swab.

Scott Brubaker mentioned there are a number of articles that have been written on this, but, generally, it's quite low. It can range between 5 and 20 percent, so a quite low efficiency.

Then you also have a second efficiency issue of removing the microorganisms from the swab and of the growth media. I've seen or heard of situations as easy as taking that same swab and wiping it onto some type

of growth medium, or dipping it in there and swirling it around a little bit. So generally, that type of recovery is also very low.

So you actually end up with a compounded recovery efficiency problem because you're trying to remove organisms first in the tissue and then from the swab. If both of those are low, the combined recovery efficiency can be extremely low.

Now, if you use some dissolving swabs or perhaps vortexing or things like that, that can assist in removing the microorganisms from the swabs.

One issue of validation of these types of swabs is that person-to-person variability in technique can add a lot to the variability of the swab culture in general.

One thing that needs to be addressed in validation of a qualitative swab culture is the issue of aerobic versus anaerobic methods as both would be important in many cases.

So if you talk about the destructive test for a little bit or sterility test, a sterility test is used in a variety of instances. I've seen it used in

recovery cultures. I've seen it used in the post-processing cultures and in post-sterilization cultures. Often it's performed on tissue which otherwise would be usable, or also on companion tissue.

The benefits to the destructive tests or tests of sterility is that it's obviously the best sensitivity. You're not removing the microorganisms from the tissue. The tissue itself with its inherent microorganisms is being placed in the media. So it has the best sensitivity.

It also has a long historical use. Many banks have been doing this type of testing at different stages for a long time.

The cons for a destructive test, if you're talking in recovery cultures and post-processing cultures, is that the tissue normally has to be discarded after testing. Also once again, it gives you a presence or an absence which is not a number.

Now, there is one exception to presence/absence data being beneficial. It's called an MPN or a most probable number test. I'll go into that pretty soon here.

One of the cons for destructive tests post-sterilization is that it shouldn't be done. That's my opinion and I'm going to go into more detail on that as well. But my opinion is based on sound scientific principles, I believe.

So let me go into that. So post-sterilization tests for sterility, again, don't do it. If you truly have a terminal sterilization process, then your terminal sterilization is achieving some form of sterility assurance level.

One of the more common sterility assurance levels used is 10 to minus 6, which is a 1 in 1,000,000 probability of a non-sterile unit. So if you test 10 samples for sterility after sterilization, what does that mean? Ten samples can give you a sterility assurance level of 10 to the minus 1, which is a 1 in 10 probability.

So statistically, you're performing a test at 10 to the minus 1 and trying to show that it verifies or validates a 10 to the minus 6 sterility assurance level. But yet you have this 5 log discrepancy between what you're testing and what you're trying to prove.

Now, if you have no validation of the sterilization procedure you're using, I suppose it's better than nothing. That's the only time where perhaps it has any relevance at all from a scientific standpoint, is if you have no validation at all of your sterilization process.

So tests for sterility -- so validation -- and I realize I put post-sterilization up there. But whether it's used for post-sterilization or if it's in the context of recovery cultures or post-processing, either way the validation for those is, first, is aseptic performance of the test is critical.

So validation or qualification of the technician, and that would be to assist in removing as much as possible any potential for false positives, which would be due to contamination during testing.

Bacteriostasis fungistasism, or BF testing, has become a very commonplace word as we go through these discussions. I'm very pleased to see that that is the case. Obviously, that is critical.

Package or container validation is also

critical. If there is no data present regarding the packaging of the product regarding its integrity after sterilization or after shipping, then a positive test of sterility after sterilization could be due to faulty packaging rather than due to the inability of the process to perform its function.

Again, invalidation for a test is truly you must address the situation, both aerobic and anaerobic methods. I'm not saying that in all these validation procedures you must do aerobic and anaerobic testing every single time always and forever. But I am saying that it must be addressed at least.

So let's go in detail on BF testing. It is very common for certain types of tissue to contain residual antibiotics from processing. What this results in is what's called a false negative. It is a test sample which should have been positive because there were living microorganisms on the product, but due to some inhibitory factor in the solution, the living microorganisms did not grow in the solution.

This will validate that the media type and the media volume neutralizes any inhibitory residuals.

That is critical. It's critical to note both the media type and the media volume as changes to the media type or the media volume, if you're going smaller in volume, can negate any BF testing that you've done.

The primary document that is followed right now for the BF testing is called the validation test in USP 71, which covers the tests of sterility. The validation test is a portion of that chapter.

In essence, you're inoculating the media with the tissue in it or with whatever sample you're testing in it with less than 100 CFU of specific microorganisms. You're seeing if those organisms that you had, if they are able to grow in the presence of your test sample.

So in performing BF testing on your product, you may find, and you probably will find if you're doing it, that neutralizers may be need to be added to the media for certain tissue types.

USP 1227 has some commonly used neutralizers. There are other references as well. That's one of the more common ones.

So what you'll likely result in is that for

some of this testing, you may end up with non-standard media. You may end up with normal soy, which is TSB, a normal thioglycollate broth. It may not pass a BF. So you may end up with a modified TSB or a modified thio.

Now, another option is dilution, which would be to use larger bottles and more media. Oftentimes, by sheer dilution you can neutralize inhibitory factors coming off of a product.

So let’s talk about quantitative swab cultures. Quantitative swab cultures are a step up from qualitative. It’s a rather small step because of the issues with swab cultures, but it is a step up.

Pros are they’re similar to the qualitative swabs, which it’s easy and fast to perform, minimal materials and equipment, minimal training and long historical use. The cons, once again, is similar to qualitative swabs which is that you can get the variable results based on technician and the low recovery efficiency.

Now, a bioburden test, the pros to a bioburden test is that it is a quantitative test. It is much easier to get better consistency. The shaking

procedure can be done using mechanical means. So there are fewer issues with variation from technician to technician.

A bioburden test can be designed so that you can still use the tissue afterwards. The test sensitivity can be adjusted depending on how you set up the bioburden test. Cons are it is much more intensive regarding materials and equipment as well as training.

Validation of a bioburden test, again, we get to recovery efficiency, which is similar to the swabs. Again, you are removing the organisms from the tissue. Now, in a normal bioburden test, you’re immersing the tissue into a solution. So the recovery efficiencies are often much better.

There are two approaches that can be taken to recovery efficiency. The first is exhaustive rinse, which means that you are taking the same piece of tissue and you’re performing several extractions on it. After each extraction, you assay the solution to determine the quantity of microorganisms.

Now, in the case of tissue, exhaustive rinse often does not work very well because the natural

bioburden on tissue is frequently quite low. When the natural bioburden is fairly low, then the exhaustive rinse data can be extremely variable.

So the inoculated product is often the approach that needs to be taken with tissue because of that low natural bioburden issue. In an inoculate product situation, you are adding microorganisms through the tissue and then performing the extraction.

The concept there is if there were microorganisms on the tissue, can I get them off. You might find that as you add microorganisms, you can't get them off.

So the reason recovery efficiency is so critical with both bioburden and with swab testing is that you might be getting low counts but it might be because you cannot get the organisms off. There might be a large number of organisms there, but the test method is not removing the organisms.

Neutralization is another issue with bioburden testing. This applies to other methods as well. USP 1227 recommends a 70 percent recovery. There's some discussion if that's going to change to

50 percent recovery being the accepted percentage.

Now, neutralization should not be confused with recovery efficiency. The USP 1227 was never intended to be a required percentage of removing organisms from tissue.

The concept here is once I get organisms into the solution and I filter them, can I quantitatively recover a high percentage of what I put in there or of what is in there. So usually, you are -- if you're performing a filtration method, for example, for bioburden, usually, you are performing the extraction of the tissue, putting some of the solution into a funnel and then oftentimes you're rinsing that. You're filtering that solution, rinsing the filter several times, adding more sterile solution, and then inoculating that solution with the microorganisms and filtering that.

So the idea is if living microorganisms end up on the filter after the filtration process, can they grow. Oftentimes, you'll find that filtration of solution with a large quantity of residual antibiotics or other chemicals, those antibiotics or chemicals will

often get trapped in the filter.

No matter how many times you rinse them, some filters have a great capacity to grab on to inhibitory factors and not let them go. So you may end up looking at alternative methods or alternative filter types, such as nylon, for example. Once again, you need to be addressing both aerobic and anaerobic methods.

Most probable number, this is best applied in situations where the bioburden is consistent. So if one is going to apply an MPN approach to your testing, it is best to have a lot of data which would be quantitative data to demonstrate that when you do get microorganisms from your tissue, you're not getting large variations.

For example, your ranges should be something closer to zero to 3 CFU per unit as opposed to zero to 30. Also, you have some data to demonstrate that from batch to batch. This is best to use post-processing. But from batch to batch post-processing, you are also getting very little variation from donor to donor.

Now, once consistency can be demonstrated and the MPN approach can be applied, it's much more

sensitive than bioburden testing because again, you're not removing the microorganisms from the tissue.

So essentially, you're performing a test of sterility on non-sterilized tissue. Once again, don't forget the BF. That would still apply.

Then the MPN calculation is very simple. It's the number tested divided by the number negative. You take then natural log of that result.

So, for example, if you test 20 samples, you get 14 negatives, which are 6 positives. Then the calculation will be natural log of 20 divided by 14.

Now, it's extremely common in applying this calculation for people to put the number positive and the denominator rather than the number negative. So it's critical to remember that.

The MPN testing can be an extremely powerful tool is used properly because -- the main reason is that you can accurately resolve bioburden down to less than 1 CFU. You can accurately resolve bioburden down to something as low as .1 or an average of .2 CFU per individual tissue type. That's because of its extreme sensitivity. Since you're not removing microorganisms

from the tissue, you're testing the tissue itself.

Typical use of culturing methods. So this is kind of a generalization based on my experience. I should mention I've never been an employee of a tissue bank, but I have been extremely involved with the tissue bank industry for the last six years. So I think what I've put here is a fairly accurate general representation. However, my ego is not so easily damaged that if I'm wrong someone can point that out to me.

So first, often what's performed is recovery cultures. Generally, those are required. Those are usually qualitative swabs, not a number. The microorganisms are compared to some type of categorization which is usually determined by each tissue bank. So they look to see if each organism that comes up in that swab culture fits under Category A, B, C or 1, 2, or 3 or whatever they call it.

Often, the organisms which are recovered in the recovery cultures will determine what type of tissue processing is to be done. For example, certain organisms come up in recovery culture that might go

through radiation pre-treatment before it’s processed. It might go through different processing steps, more harsher processing steps, or might be used for validation or testing purposes rather than for actual release for transplantation. They’re also often used to determine if tissue should be discarded.

Post-processing cultures are often performed. These are again usually a qualitative swab culture or often a destructive test, which would be an immersion test for sterility.

I see two different things happen here. Sometimes if anything pops up, then they will be discarded or reprocessed or put through sterilization, or sometimes, depending on the type of microorganism that comes out, the tissue may be discarded or reprocessed or terminally sterilized.

Then often post-sterilization cultures are, in my opinion, unfortunately frequently performed as well. Usually, it’s a test for sterility. Oftentimes, any positive test in that situation means a fail. So the tissue is often either discarded or it might be moved over and used for validation and testing

purposes.

So that’s kind of a general idea of what I’ve seen and what I understand is fairly common. Perhaps not every single one of those steps is done by every tissue bank, but I know that those are quite common.

So if I look at these culturing techniques from a scientific standpoint, what do these tell me? Well, first thing is that testing each batch both before and after processing is uncommon in any other industry for validated process. You’ll see I underlined for a validated process because that’s critical in this comment that I’m making.

Many people say that tissue banking is different and that there is much more bioburden variability in product or in tissue beforehand. However, those that have done fairly extensive trending and validation work have seen that processed tissue actually is quite consistent and quite low in bioburden, which means that tissue banks are just great at microbial reduction.

Compared to cotton gauze, for instance, and other things that are used in other industries such as

the medical device industry -- the cotton gauze, for example, can be extremely variable in quantity and type of microorganisms. Even those situations, they have validated processes that they use for processing or for sterilization and testing of every batch is just not done.

Again, from the outside looking in, if I look at all of this testing that’s being done both before and after processing, and oftentimes even after sterilization, this tells me that either I feel like I don’t have a validated process or I don’t trust my validation just because of the sheer quantity of testing that’s being performed.

Of course, tradition plays a role in this as well. This is what we’ve always done, and this is what we’re doing.

So one of the questions that I was asked was how could the tissue industry improve. I don’t think any of this is new necessarily to those of us who have had these types of discussions for the last couple of years.

So the tissue bank history generally shows

excellent results with regard to reduction of contamination.

Obviously, certain improvements could increase tissue yield. For example, if scientific improvements are made to the process or validation is performed, which would allow you to not perform as much destructive testing, for example, or it would allow you to use tissue that right now is being discarded due to additional steps, that might be added to the processing steps.

What must be in place in order to be able to change things from how they are now? If we wanted to increase the yield of tissue, if we wanted to get a little more flexibility in the organisms that might show up, or the quantities that might show up, what things must be in place?

First of all, validation of BF would be critical for all tissue types. This would just demonstrate that each test is getting proper results.

In my opinion, a shift from organism types and plus or minus to an organism count, scientifically, there’s a lot of power in knowing the quantities of

microorganisms as opposed to only knowing the types.

For sterilization, this comment has been made before a little bit that generally speaking there's no correlation between organism pathogenicity versus organism resistance to the disinfection or the sterilization process. This is especially the case in sterilization processes.

So it's necessary to understand this and actually believe that this is the case before this type of shift can be made because sterilization processes, generally speaking, the organisms, which are of most concern from a clinical standpoint, are extremely easy to kill from a sterilization standpoint.

For sterilization, again, an organism count as opposed to plus or minus; there is power gained in having that knowledge from a scientific and from a sterilization standpoint.

Other things, proper validation of processing steps. I'm just going to refer to Joyce Hansen's talk tomorrow about that.

Improvements to processing steps. This might not be the case across the board, but in some cases

validation will tell you what can be optimized, improved or perhaps even removed. You might find that a step that you're doing in the processing provides little or no microorganism reduction.

Proper validation of sterilization. Again, I'll defer to Joyce Hansen's talk tomorrow.

AAMI TIR 36 is the document number for that document which Scott referred, to which AAMI has developed, with some help from some tissue folks, regarding radiation sterilization of tissue. That will be given the number AAMI TIR 36.

It provides guidance on microbiological methods for tissue. Much of what I've described here regarding issues that you need to deal with in bioburden testing or in sterility is included in that document.

So once I get all this in place and I have this thorough understanding of my process, what does that give me? So now I know I have a greater knowledge of my incoming bioburden. I know both the numbers and the type. I can trend that data over time. I have a greater knowledge of my processing capabilities. Log

reductions are provided.

Much of post-process bioburden, which is what you'll likely find if you're doing bioburden testing on post-process bioburden, you'll likely find that much of the post-process bioburden may be environment in nature rather than clinical in nature because you're so efficient at removing or killing the microorganisms that came in on the tissue, what ends up there is microorganisms from handling.

It gives you knowledge of sterilization capabilities, what the true capabilities of your sterilization process are. Also related to that, a knowledge of how much overkill is in the process. Based on a more thorough understanding of the overkill and the process and the sterilization capabilities, advantage can be taken of that knowledge and perhaps tissue that you are currently discarding could be used based on better understanding of the vast overkill which is in the process.

All this allows for application of what I call routine process monitoring rather than routine product monitoring. This is what is much more common

in other industries which -- and I know that we can’t always compare, for example, the device industry to the tissue industry. However, certain concepts certainly do apply.

One of the concepts which, in my opinion, does apply is that routine process monitoring is extremely common in the device industry and routine product monitoring is very uncommon especially on a batch-to-batch basis.

It’s not necessarily going to be easy. If some of these are new concepts or new issues to implement, then it requires an increased understanding and application of these scientific principles. But it’s also not rocket science. These are concepts which can be fairly easily understood, but oftentimes implementation can be quite a discussion.

Like I said, some people already understand and use these principles in the tissue industry. My intent here obviously is not to reduce quality, as I feel that these concepts would not reduce quality but that implementation of correct scientific principles will actually assist you to better understand and apply

quality to the tissue rather than reduce the quality.

So what next? So based on some of these concepts or thoughts, these are not things that I'm saying we should just go out right now and make these changes. But with all of these things in place I've been talking about, what might be options?

One might be not to perform recovery cultures on every single donor. Obviously, we should always be screening for viruses. But from a microbiological standpoint, recovery cultures -- we might realize that recovery cultures are not as beneficial as we thought they were.

Perhaps not performing any post-processing cultures on every single batch. Perhaps allowing for use of tissue, which is positive for clostridium or other microorganisms, perhaps only in a situation where you only have terminal sterilization in place.

I know that the thought or the concept of releasing tissue which you know, for example, had clostridium on it when it was recovered may seem impossible. Maybe it is. I don't know for sure. But what I do know for sure is that from a scientific

standpoint if you have a knowledge of a number of clostridium microorganisms on a tissue, you can without a doubt extrapolate a sterility assurance level down to a minus 6 or even better.

So those concepts can be applied to any type of microorganism, any type of bacteria or fungi. Just things to think about, just kind of opening the door here. But based on a good knowledge of processing and the sterilization, these types of things would be possible.

That’s all I have. Thank you.

DR. KUEHNERT: I think now we have a break scheduled. So let’s take that. We’re a little bit -- running a little early. So do we want to stay on schedule or actually try to get a little bit ahead?

Why don’t we break for 15 minutes by my watch?

(A recess was taken.)

DR. KUEHNERT: We have one more presentation to go in this session. It is the CDC perspective on current culturing methods. It’s going to be given by Arjun Srinivasan.

Arjun is a medical epidemiologist at the Division of Health Care Quality Promotion at CDC.

Before coming to Atlanta, he was at Johns Hopkins, where he was the associate hospital epidemiologist and has acquired plenty of experience in the investigation of outbreaks as well as infection control, work on multi-drug resistant gram negative pathogens, device related infections and, of course, infections related to organ and tissue transplantation.

I just want to say personally that it's a pleasure to have Arjun as a colleague at CDC and that I look forward to his perspective on these issues.

Arjun.

DR. SRINIVASAN: Thanks, Matt.

Thank you all. Thank you for having me. I think this is a really fantastic workshop. I've already learned a lot. I think today and the rest of the discussions today and tomorrow will be extremely helpful.

I also want to thank a few folks. I want to thank Matt and Scott Brubaker both for taking a look at these slides and helping me make sure I was covering

all the topics properly and also Matt Arduino in the CDC environmental microbiology lab, who has done a tremendous amount of work on culturing tissues for all of our investigations, who also helped me with this.

I have no financial disclosures. However, I'm required by law to tell you that the findings and conclusions in my presentation don't necessarily represent the views of the CDC.

So what I'm going to try to address in giving you what I perceive as the CDC perspective on current pre- and post-culturing methods is a discussion of some of the benefits and limitations of pre- and post-processing cultures of allograft tissues, primarily based on our perspective of doing investigations of reported adverse events and infections.

Then I'll end with some suggestions to optimize the use of pre- and post-processing processing cultures of allograft and try to address those questions that were posed to all of us.

So I think from our perspective there are several benefits of pre-processing cultures. I've

summarized the ones that I think are most important here. I think they add information to the overall donor assessment. They provide information on recovery practices. They can reveal indicator organisms that we've heard a little bit about that might require special action. They can also help our processing procedures.

I'm going to talk specifically in detail about one of our investigations here. That is how pre-processing cultures can help with donor assessment.

This is an investigation that many of you are probably familiar with. It was a Group A streptococcus investigation that happened in 2003. The case was of a 17-year-old previously healthy male who developed a surgical site infection following an anterior cruciate ligament repair with a tendon allograft.

He was eventually taken back to the OR and cultures of his wound, his blood and the explanted tissue all grew streptococcus pyogenes or Group A streptococcus.

An investigation was conducted. Of course, one of the initial steps in any investigation of a

possible tissue allograft associated infection is a review of the donor history. This donor was a previously healthy man in his 30s. He had died in February of 2003.

About three weeks before death, he had undergone a cervical spinal fusion for degenerative disk disease. Three days prior to his death, he had presented to an emergency room with a diffuse rash that was thought to be a medication reaction. He was given some medications for that and sent home.

Three days later, he returned to the ER complaining of back pain, nausea and vomiting and unfortunately died soon after coming to the emergency department.

An autopsy was done which showed the rash again along with potentially toxic levels of a muscle relaxant and an analgesic medication. In the autopsy report, the coroner attributed the death to an overdose of those medications.

In doing the tissue trace-back, we found that the donor had been recovered by a single tissue recovery organization. Several tissues were cultured

at the time of recovery and all of those tissues that were cultured at that time grew Group A streptococcus.

Tissues were then sent to two different tissue processors. At one of the processors, 14 of the tissues that they received were cultured and again 14 of them grew Group A strep. This was out of I think about 20 that they cultured.

The tissues were processed and then recultured. The post-processing culture showed no evidence of organisms, so the tissues were distributed.

When we got involved in the investigation, we did a variety of microbiologic and pathologic testing. We performed microbiologic testing on some unprocessed tissues from that same donor of recalled tissue samples that had been processed. We were also able to obtain an archived serum sample from the donor to culture and also were able to obtain the autopsy tissue samples for our pathologists at CDC to examine.

We were able to recover Group A streptococcus from the unreprocessed tissues, also from the donor’s serum sample. We didn’t recover any Group A strep from any of the recalled tissues, however.

Our infectious disease pathologists performed a number of special stains and were, in fact, able to demonstrate the presence of Group A streptococcus in the skin, blood vessels and lung of the donor.

We did a molecular typing on the isolates of Group A strep that we recovered at CDC and compared those to the isolates of Group A strep that were recovered from the infected patient, and found them to be genetically identical, indicating that there was a link between the organism causing the infection in the recipient and the tissue allograft.

So, in retrospect, when you put all these findings together with the clinical history that the donor had presented with -- the rash, the nausea, the vomiting -- the most likely cause of death was actually in retrospect streptococcal toxic shock-like syndrome.

In fact, the coroner upon reviewing all of our findings actually changed the cause of death on the death certificate because he felt fairly strongly that this was a more accurate reflection of the cause of death.

Now, again, hindsight’s always 20/20. The

fact that so many of these pre-processing cultures grew Group A streptococcus was, in fact, a pretty good indication that there was a disseminated infection in the donor.

So what are some other potential utilities of pre-processing cultures?

I'm sorry. So in conclusion, I think that this investigation at least demonstrates that pre-processing cultures can add information on the donor's infectious disease status at the time of death. I think everybody now is very well cognizant of that.

Pre-processing cultures are, in fact, part of the donor eligibility determination. We re-review those cultures to see if, in light of the clinical findings, those cultures tell us additional pieces of information.

What are some of the other utilities of pre-processing cultures? Well, one is that they can be a good assessment of recovery practices.

Tissue recovery, as all of you know better than I do, is a complex operation; pun intended. It has to be done in a timely manner, as Scott has told

you. Many different people might be involved. Recovery environments, though we do our best to control them, can be a little variable. Careful technique has to be maintained for this long procedure.

So pre-processing cultures can help us detect some problems in our tissue recovery. For example, if there is contamination due to insufficiencies in the recovery environment, we might see an abundance of environmental pathogens that suggest that problem.

Likewise, if there are problems with recovery technique, we might see particular microorganisms that suggest that there are problems with how those tissues were recovered.

Another utility of pre-processing cultures is again, as you've heard, the ability to detect certain special organisms, or indicator organisms, or highly virulent organisms, whatever you want to call them. I think we'd all agree that there are certain organisms that have special implications.

Some of them are hard to remove. They're a spore form of organisms, like clostridia, that are a little bit more difficult to remove than some

vegetative bacteria.

Fungi might be more difficult to remove because they require antifungals as opposed to other antibacterials.

There are also other organisms. As you've heard, Group A streptococcus is highly virulent. I'll suggest that not only is it highly virulent but it's also special because I think even a single culture of Group A streptococcus is suggestive of a disseminated infection in the donor, and therefore special attention might be required if you recover Group A streptococcus on one or more samples of your tissue.

I think when we recover these organisms, the pre-processing cultures are very helpful. It helps us in two different ways. First of all, it helps inform our process and decisions. If we get an organism that's hard to get rid of, we then say well, we need to treat this with a more rigorous method of pathogen reduction.

Likewise, it helps us also with donor evaluations. If we see several pre-processing cultures growing Group A strep, we might go back and re-look at

that clinical history and say maybe this is a sign of a systemic or disseminated infection.

Pre-processing cultures also help us assess our processing methods. They can be a quality control of the pathogen reduction methods. They help us know whether our pathogen reduction procedures actually removed the organisms.

If we don’t do pre-processing cultures, at the end of our procedure we don’t know if our processes removed pathogens or if there were no pathogens there to begin with.

Likewise, I think pre-processing cultures can help assess the overall tissue processing procedure. They’ll help us understand if organisms were perhaps introduced during the processing procedures.

Again, if we don’t have any information from pre-processing cultures, we don’t know if the organisms that we get on a post-processing culture were ones that were not removed by the pathogen reduction method, or were organisms that were there all along, or maybe they were introduced during the processing procedure and suggest that we might have actually contaminated the

tissues.

There are, of course, some limitations and unanswered questions. Let me just discuss these briefly here for pre-processing cultures. I'll do the same for post-processing cultures.

I want to touch on a few of these. Again, Martell touched on this one. Pre-processing cultures are generally not quantitative. There's some unanswered questions with respect to how many cultures we have to obtain to have optimal yield, how they should be done and when they should be done.

So they're not quantitative. So what are some benefits that we might gain if we had some quantitative? Well, if we knew the microbial load of contamination pre-processing method, could we then choose our reprocessing pathogen reduction methods based on the results of pre-processing cultures?

For example, we're talking about pathogen reduction methods that might be damaging to tissues. Could we preferentially choose less damaging methods if we knew a tissue was contaminated with a certain organism load? If we knew something had 10 to the 3

organisms, could we choose methods that perhaps are not as damaging to the tissue as one that was contaminated with 10 to the 6? Without quantitative information, we don't know. So we have to treat everything with the higher level.

Another potential benefit to some quantitative pre-processing cultures would, of course, be to help define the common range of microbial loads on tissues. That kind of information across the spectrum of all tissues might help inform our targets for our pathogen reduction levels.

So those are some potential benefits that we can gain from quantitative pre-processing cultures, but I'll readily acknowledge that you can make the counterargument does it really matter. Quantitative cultures, as we've just heard, are very time consuming and they're also more expensive.

Right now, we have a failsafe in place. So irrespective of what methods we use, failure to remove organisms should be detected by our post-processing cultures. So do we really need to spend the time and money to do quantitative pre-processing cultures? If

we get rid of all of it, does it really matter how much was there to begin with?

How many pre-processing cultures is enough? We need, I think, more information to tell us how many cultures we need to obtain to have the highest likelihood of finding disseminated infections in donors if that’s one of our goals.

What methodology do we use? We’ve just heard an excellent description and discussion of some of the various methodologies that are out there.

Pre-processing cultures can, of course, be done in a variety of ways. Swabs and rinses of tissues and also potentially destructive cultures of some sample of what’s recovered. Each of these might have different applications and has pros and cons.

For example, when you’re talking about swabs and rinses, on the plus side, these are maybe good for assessing surface contamination. They might be good for monitoring recovery practices where we’re worried about surface contamination. We can culture lots of the tissues. You can swab many, many tissues when you’re doing this.

The con, of course, as you've heard, is recovery is highly variable with these types of cultures. You can't at all detect internal contamination because this is a surface swab.

What about destructive culture methods? Well, these are better in terms of providing the overall microbial pictures because you get a sense of both what's outside the tissue and what's inside the tissue. So you get the vast overall microbial picture.

The downside is, of course, it's maybe not as helpful for monitoring your recovery practices because you can't culture that many tissues. If you want to use these tissues to process, you can't destroy too many of them.

The other issue, of course, is how many do you need to destroy. What's the number of tissues that you would need to quantitatively culture or just perform destructive cultures on to get a representative sample of contamination within that recovered tissue?

I think when to obtain them is another issue where there are some unanswered questions. Cultures can be obtained at the time of recovery. They can also

be obtained at the time that the tissues are received at the tissue bank. I think there are again advantages to both scenarios.

When we obtain cultures at the time of recovery, we might improve our yield for ensuring the growth of some pathogens that might be present in the donor. I think this is especially true for hard-to-grow organisms, for things especially like clostridia where our lab will say if you really want to recover clostridia, what's key is inoculation as close to the time of sampling as you can get. So at the bedside ideally if you can do it, but the sooner you do the culture, the higher your yield for something like clostridia.

However, on the flip side, the recovery environment is not nearly as controlled as the tissue bank environment. So if we do our cultures at the tissue bank, we might have less risk of contamination because the environment is much more controlled.

Another advantage of doing these cultures at the time of the tissue bank is that it might be better for assessing contamination that occurred during

recovery because it would allow for some incubation time. So anything that's introduced at the time of recovery would have 12 to 24 hours, some time to divide and multiply, so we might be more likely to detect contamination that occurred during recovery.

We turn now and spend some time talking about post-processing cultures. So I think, as we've all heard, there are some definite benefits to post-processing cultures. I think that's been our experience as well.

They do provide a final check for pathogens. They can assess the efficacy of pathogen reduction methods, especially when they're paired with pre-processing cultures. They can help identify possible tissue contamination during processing.

I think these first two are fairly self-explanatory. So let me provide a little information about the use of post-processing cultures for this last scenario through one of our investigations focusing on contamination during processing.

In September and November of 2006, CDC was

notified to two cases of chryseobacterium meningosepticum surgical site infections in patients who had undergone tissue allograft implantations. Now, both of these tissues had been processed by the same tissue bank. However, they had both come from different donors.

So what in the world is chryseobacterium meningosepticum? That's the first question I asked when they called me to tell me about these cases. It's a waterborne gram negative organism. It's widely distributed in the environment. It's found commonly in tap water.

If you look at the literature, it has been reported as a very rare cause of neonatal meningitis. It's been a rare cause of outbreaks in health care. But as best we could find in the medical literature, there's been no past associations with tissue allograft associated infections.

We performed cultures of some unimplanted tissues from both donors that were sent to us at CDC. Our lab was able to recover chryseobacterium meningosepticum from tissues from both donors.

We were able to obtain an isolate from one of the patients that was involved. We did pulse field gel electrophoresis, comparing the isolate from the tissues and the isolate that we had from the one patient.

You can see here -- but they were considered to be highly molecularly related. There was almost a 92 percent similarity between the specimens from the tissues and the isolate in the patient. In our experience, that indicates that there’s a fairly strong genetic link suggesting that the bacteria had, in fact, come from the tissues and infected the patient via that route.

The tissue bank did a thorough root cause analysis. During their investigation, they discovered that their sterility failure rate at one of their processing facilities had increased between February and August 2006. They found that to be important information because the tissues in both cases had been processed in May during this time.

Chryseobacterium meningosepticum was one of the organisms that had been identified during that period. The first sterility failure for

chryseobacterium had occurred at the end of February 2006, so it is in this same time frame that the tissues had been recovered.

So based on the microbiology, a thorough environmental assessment was done with a special focus on water sources. As I've told you, this is a water loving organism. Chryseobacterium meningosepticum was identified in the clean room drains and traps of the sinks.

So here I think the post-processing cultures provide a tremendous amount of very useful information in guiding this root cause analysis. First of all, it helped identify there was a problem. There was an increase in sterility failure rates that would not have been detected had they not done post-processing cultures.

It helped pinpoint the likely etiology. The pre-processing cultures did not show the organism. The post-processing cultures did, which suggested that the organism was introduced during the processing procedures and helped refine the investigation for where they were going to look.

Finally, it really helped hone in on the ultimate source. We knew this was a waterborne organism and so they focused their investigation very thoroughly on water reservoirs within the facility and indeed were able to find the organism in some water sources in the facility. So clearly the post-processing cultures helped guide this investigation in a number of very important ways.

What about limitations and unanswered questions for post-processing cultures? I think there are a few. The methodologies can be challenging. There are some questions about what the optimal methods might be.

So in order to discuss some of the methodologic challenges, I'd like to tell you a little bit about how our lab processes cultures when we're asked to perform one of these investigations. We do destructive cultures of the soft tissues and we destroy the tissues in different ways.

If it's a tissue that's soft enough to grind in a tissue grinder, our lab will use a sterile tissue grinder to do that. If it's a tendon or something that

they can't grind up, they macerate it with a scalpel in order to try to destroy the tissue. For bone which is much more difficult to destroy, they immerse it in broth and sonicate the bone.

Depending on the pathogen reduction method that was used, they develop then a strategy to try and neutralize any residual antimicrobials that might be present. This is done, as you've just heard, using a USP standard whether; they'll either use dilution or they'll use neutralizing buffers.

But this is one of the reasons why, for those of you who've been involved with us in investigations, we always ask you what was the method that was used. We need the specifics of how you process these tissues because we need to know those specifics in order to figure out how best to neutralize any residual antimicrobials that might be present. The sample's then, of course, incubated aerobically and anaerobically.

The importance of neutralization was, I think, another item that we discovered during our investigations. Again, it's certainly not news to the

members of this audience, but I'll just present our experience with the importance of neutralization. This, of course, comes from the clostridial infections that were published in 2004.

The post-processing culture method that had been used to release these tissues was the culturing of a companion tissue. This companion tissue was processed in parallel with the allografts. After the processing, the companion tissue was then placed in a culture media which was agitated and then aliquoted into standard blood culture bottles.

In talking with the folks who did the processing of the tissues, it was determined that about 1 ml of the processing antimicrobial solution was probably carried over with the tissue.

Well, our lab then went and did some studies looking at various amounts of antimicrobial solution carryover and how that might impact the results of cultures done in standard blood culture bottles.

As you can see from this table in the paper, if you have about 1 ml of carryover into a standard blood culture bottle of this pathogen reduction

solution, antimicrobial solution that would be used, we were not able to reculture any organisms when we inoculated a culture with 100 spores of c. sordellii. This is again the USP standard that’s used to assess bacteriostasis.

So the carryover of a single ml of solution might have been enough to impede the growth of those cultures and make them falsely negative.

What was interesting is that even in charcoal containing blood culture bottles, the yield was still very, very low. It was 1 out of 3 or 33 percent. So neutralizing this antimicrobial solution did require some additional steps with dilution and some different buffers. But it lends credence to the importance of neutralization in ensuring that post-processing cultures are accurate.

So some of the methodologic challenges -- our destructive culture methods, they worked very well for us. But they’re not very good if you need to use that tissue later on. So they’re not widely applicable. We can’t do this on all tissues.

So what is the ideal culture method of

post-processing cultures? Is it potentially a swab culture of all processed tissues? That has some attractions. As you've heard, it's simple to do. It covers all the tissues. You can culture every single one of them if you wanted to because you don't have to destroy any of them, but it's only going to address surface contamination. It's not going to give you the total microbial picture of the tissue.

Well, what about destructive cultures of randomly selected prepared and processed tissues? So let the tissue go all the way through, make the bone-tendon-bone allograft and then take some of those samples and do destructive cultures.

That could be attractive because it simulates all of the manipulation that's going to happen. Clearly, manipulation, as we make our allografts, introduces the possibility of contamination. If we can simulate all of that manipulation, we get the best picture of what might be on the tissues.

However, this has a lot of practical drawbacks. It's probably unrealistic to try to culture one of each type of allograft. We're going to end up

wasting so much tissue. Tissues are very precious. We don't have an abundant supply of them. We don't want to waste processed tissues by culturing too many of them to try to get this information.

What about destructive cultures of processed companion tissues? This perhaps is a nice compromise because it gives you the power of a destructive culture, so you get that full microbial picture but it doesn't require destruction of too much tissue.

Again, this is nice because it does allow for simulation of the processing steps, so it would be helpful in detecting processing contamination. In general, these companion tissues go exactly in parallel with their other tissues, so we get a good sense of contamination that might arise as the tissues are moved forward.

But we don't exactly know, I don't think, how many, what types and what sizes of companion tissues might be needed to be 100 percent representative of all the tissues that we're trying to culture.

So I want to spend the last few minutes of my presentation talking about some of the data that might

help us answer some of these unanswered questions and move this field forward.

I want to point out, as Scott has pointed out to me, that in many cases, some of this data exists. It exists in abstract forms. People have done small studies. But what I think we need to do is get all of our data together, look at the aggregate of the information that we have, see where the holes are, and figure out what more needs to be done.

I think we need studies looking at the amount of contamination that’s present in various tissues under a variety of different circumstances and using various different culture methods. We need to look at what happens to pre-processing cultures if there are variations in recovery practices and if there are variations in donor medical conditions and donor specific conditions. We might need some animal studies looking at tissue contamination and disseminated infections and how best we’re able to detect that.

What would that tell us? Well, I think data from these types of studies would really help us define the spectrum of potential tissue microbial

contamination. Again, there's a huge variation out there. What we need is a good handle on what's the lower limit, what's the upper limit, what's 95 percent, and where are our confidence intervals? What represents a standard deviation or two standard deviations above and below what we would expect to see?

Having that kind of information I think is incredibly helpful in helping set microbial targets for tissue processing methods. If we want methods that address the vast majority of contamination, we have to know what the vast majority of contamination is.

It's going to help us how to guide -- how to optimize the yield of pre-processing cultures because we can compare different methods. That's going to help us select the optimal methods.

With respect to post-processing cultures, I think some studies looking at different methods for post-processing cultures under various circumstances would be helpful, looking at various pathogen reduction methods, looking at various levels of contamination. Again, this might help us guide the selection of optimal methods for post-processing cultures.

Again, I'll point out this is probably data that exists in different places. What we need to do is bring it all together, see where the holes are, and decide what additional studies need to be done.

So what can we say about failures of these types of cultures? I think we live in an age -- if you've seen these reports now, how we can't ever tell our children that they fail. So failure now is really all about perspective, right? No one is an absolute failure.

That's certainly, I think, the case if you take different perspectives on tissue culturing. On the one hand, you could say that our investigations demonstrate that our pre- and post-processing cultures don't fail. They work very well.

In our investigations that I've just told you about either the pre- or post-processing cultures were positive for the pathogens that caused the infections. So in a sense, the pre- and post-processing cultures did not fail. They were able to detect what was there.

However, in each case the tissues were released and implanted. So there was a failure in the

sense that the tissues were released and got out, but it was not because there wasn't a way to detect what was there.

So I think we have to acknowledge that and remember that failure can be caused for a variety of reasons. Failures can be microbiologic. In the clostridia example, there was a false negative. We would say that's a microbiologic failure of the method because there was not appropriate neutralization of the carryover of the bacteriostatic agent that was used.

But failures can also lie in our interpretation of the data. We could in retrospect look back and say that there was a failure in the Group A streptococcal investigation; not of the culturing method but of our actions based on those culturing methods.

I think when we talk about failures, we also have to remember that failures might be silent. Infections are certainly underreported. I think we all feel that way. There are probably more allograft associated infections than we know about. Some of these may, in fact, be due to failures of our pre- or

post-processing culturing methods. We don't know because we're not able to investigate them.

Another issue is if an infection is reported and review shows that the pre- and post-processing cultures were negative but no additional culturing of those tissues are done. We don't know if that represents a failure of the cultures or if that's proof that the organisms were never there to begin with.

So I'm going to end by making a shameless plug for this type of ongoing communication and collaboration. As you've just heard, in the AATB standards, communication between tissue banks is indeed critical. Tissue banks may have different pieces of information but all of that information really feeds into the same purpose. It's helping evaluate donor eligibility.

If we all share our information from our pre-processing cultures, we can have the best overall microbial picture of the donor to guide eligibility.

Likewise, I think continued communication at forums like this one, between tissue banks and clinicians and those of us in public health, are going

to help us improve the overall safety of an already very safe tissue supply.

I'm keeping with the theme of keeping us just a little bit ahead of schedule. So I'll get Matt to come back up and we'll have the panel discussion.

Thank you all very much for your attention.

DR. KUEHNERT: I'd like to ask the speakers to come up.

In addition, we're going to have some other people on the panel. While I name them and introduce them, if we could have my question presentation up, that would be great. Then I could go over those again.

So in addition to our speakers, we have David Fronk, the vice-president of Regulatory Affairs and Quality Assurance from CryoLife; Deborah Schafer, quality control manager and tissue processing at Community Tissue Services; and Chad Ronholdt, who is the director of research and development at AlloSource.

So the panel discussion is for, first, comment on questions in the session regarding challenges and concerns with current culturing methods. Also, what new methods for obtaining a representative

sample are being developed? I think we've heard some perspectives on those.

I also wanted to just add some additional thoughts for discussion, which I think was addressed by Arjun somewhat, which is sort of taking a step back and saying what do cultures tell us and why do we do them, and sort of dividing things into pre-processing and post-processing cultures but also in recipient cultures.

So I think most people think about the processing cultures as really being geared towards patient safety but particularly towards a particular recipient who is receiving that tissue and provides information on safety and efficacy of that tissue, but also provides information on processes surrounding that tissue, on recovery data concerning the tissue donor, as we heard about, and about the recovery environment.

Post-processing cultures, I think, give information also on the individual tissue and on recovery data, but also on the efficacy of the processing method and on the processing environment.

Then the final issue, which we may not think

about as a post-processing culture but still is very important, is the culture of recipient clinical infections, which perhaps is the most informative but the least well-characterized of post-processing cultures.

I know that’s not the focus of the workshop, but I think it something that we have to keep in mind, that that really is a lot of times the most critical data that we have about the failure of processing and gives us information that we can learn from and how we can encourage clinicians to provide that information; because even if it’s not clinically significant in their patient, it might be clinically significant in another patient who gets tissues from the same donor.

So with that, I wanted to go back to the questions.

The only other obstacle we have to overcome is the mikes. Do we have the mikes on at the table? There we go.

While we’re addressing our AV challenge here, why don’t we -- concerning the challenges and concerns with culturing methods, maybe we can have folks

sequentially come up and just give their views on what the challenges are.

Who'd like to start?

Do you want to -- yeah, go ahead.

We also have, of course, the index cards that have come in with questions and maybe that will be under that category. Okay, great.

MR. BRUBAKER: The question is, it appears that the AATB standards no longer include a requirement for 10 percent destructive testing or 100 percent swabbing. Is this true? If so, why the change?

If you look at our standards in my presentation, this would be referring to K2.220, final pre-packaging cultures. It states now that all cells and/or tissue to be released for human transplantation shall have representative microbiological cultures obtained. Then it goes on to refer to, unless dosimetric release has occurred by a validated process according to E.1044.

I remember when this change was made, I think Martell, you were on the standards committee. You can probably address the scientific reasons why.

I know that 100 percent swabbing as -- again, that we knew the swabbing technique had very low efficiency and sensitivity and recovery, so that really wasn't -- and folks had stopped doing that for many years now, I believe.

The 10 percent destructive testing was the old USP method. There were other methods out there that we listed that could be used to validate, which were the references to ANSI and AAMI, the biological indicators, the bioburden based methods and so forth. Those methods were preferable, I think.

Help me explain that, I guess, if you can.

MR. WINTERS: The removal of the requirement for testing 10 percent, for example, of sterilized tissue -- what I can answer there is with regards to the reference to sterilized tissue is it's related to what I mentioned in my talk about the statistical relevance of testing 10 percent of something when you are trying to use it to verify a process, which is supposed to provide 1 in a million probability of a non-sterile unit.

So statistically, it was a complete waste of

tissue. There was no scientific rationale for the 10 percent value. So when we changed it to reflect that the radiation dose or the sterilization cycle must represent a sterility assurance level, then at that point there was no longer any need -- there wasn't much to begin with -- but at least in a validated process, there would be no need or no usefulness for the 10 percent destructive testing.

Scott, does that cover it for you?

Okay. Any follow-up questions to that while I'm standing here?

DR. KUEHNERT: All right. Stay right here.

MR. WINTERS: Okay.

I know what this is about. I talked to him about that.

DR. KUEHNERT: Okay. Then --

MR. WINTERS: Yeah, I can address that one.

DR. KUEHNERT: Okay. All right, if you want to.

MR. WINTERS: Okay. The -- this says you said on your next to last slide always screen for viruses, but all the methods that I mentioned were only

applicable to bacteria and fungi.

So what methods do I recommend for viral screening? That comment I made regarding that you should always screen for viruses is in reference to the donor screening process, that I don't think that should ever be removed in lieu of sterilization, for example. That should always be an important step.

But as far as screening for viruses, for example, post-processing or anything like that, it's very difficult to do. There's no one test. You probably all know this.

There's no one microbiological assay you can perform to detect a large number of viruses. Each virus has its own very specific growth requirements and host cells, so therefore it's virtually impossible.

So I just want to make that clear. I was referring to the donor screening methodology for viruses and that we should always continue to do that.

DR. KUEHNERT: Sort of a broader question.

MR. WINTERS: It says regarding validation of sterilization, please identify, if possible, the key assumptions made during the sterilization validation of

solid tissues. How might validation studies be improved?

That is a broad question. But I think I can narrow down those assumptions to a handful.

So key assumptions that must be made in order to do a sterilization validation of tissue: One assumption that must be made -- hopefully, it’s backed by some degree of data -- is that the bioburden on the tissues is known. By bioburden, I mean the number and the types is known and fairly consistent.

So a knowledge of the number and type, and the consistency of that number and type of organism on a tissue, is critical to any kind of validation, particularly in sterilization where you’re not doing any post-sterilization testing.

That’s probably the most critical component, is a knowledge of the number, and types and consistency is part of what I’d say there. BF obviously is critical in that.

Those are the ones that come to mind right offhand as far as the critical assumptions. Those assumptions should all be based on data. But there

comes a point where you got enough data, you have to assume it’s going to be consistent; then you no longer test at that same level of frequency.

DR. KUEHNERT: That was sort of a broad question. I was wondering if anyone else on the panel has any other comments on that issue.

DR. SRINIVASAN: Just more of a question, anyone from the panel or the audience.

I think one of the issues that we struggle with here is when we’re talking -- I think you’re making good points, Martell, about sterility assurance levels. We don’t say culture 10 percent of your scalpels when they come out of an autoclave. We say if a scalpel goes through an autoclave, it’s sterile. If a colonoscope goes through the standard processing method, it’s high level disinfected. It’s ready to use.

Someone might come and say well, why should we do any of this screening? I mean, why do we need to continue viral screenings? If we have sterility, if we feel confident in sterility, then isn’t the method enough?

Should we do away with all testing? Is there no need anymore for even viral screening? Because that again, it presents a different standard.

We say that for equipment, if it's sterile, it's truly sterile. But for tissues, if it's sterile, they still have to be free of viruses because it might not be sterile enough.

So I just raise that as a question for people to comment on on the panel.

The specific question being is would there be confusion as sort of a double standard for what sterile really means.

MR. BRUBAKER: I am not the scientist in the group here. But the term sterile and the way FDA has approached it, and the way we've approached it and others, is it doesn't reference viruses and activation of viruses. That's separate. That's different.

So I think the processing methods, though, that the presentations that have been given in our meeting and others, AOSSM, by tissue banks, it shows the reduction and possible elimination of levels of contamination by various pathogens, including viruses,

not just microbes.

I know at the AOSSM meeting, where there were a lot of the end users, of course, they were there. They had a misperception of what sterility meant. They thought it included viruses.

It doesn't necessarily do that, although the log reductions that banks have proven and shown in their process include viral reduction. But inactivation, different thing, and some of them can prove that as well. I believe there are some claims out there that make that claim.

I guess I'll try to answer a question or two here. I'm sorry.

Mary? Yes?

MS. MALARKEY: I was just going to mention that tomorrow morning's session, we're going to try to tackle that very issue as to what is sterile. I'll put the quotes around it because I do believe there may be some confusion.

In terms of viruses, as Scott was saying, it's been a longstanding expectation that if one wants to make a claim for a process around viral

clearance -- that would be removal or inactivation -- one does that using separate, generally laboratory small-scale studies.

We have a lot of experience with that in biotech and the plasma derivative industry. I have seen it in this industry as well. So we'll talk a little bit about that tomorrow.

MR. BRUBAKER: Thank you.

I have a question here regarding draft guidance ideas for AATB. What kind of bioburden monitoring is being considered? Where in the process should it be monitored?

This is a very new and recent project by the standards committee. We'll need to have board approval to get the studies done and to have consultants work on this with us, but that's to be determined.

The slide I had on it was basically what we've only worked on very recently as a general idea to do this. It does mention on that slide ongoing bioburden monitoring programs. So again, that at what steps, we haven't evaluated that yet.

Assurance of adequacy about sensitivity and

the specificity of the culturing methods and, of course, the BF testing and those recovery studies that need to be employed as well, at least at the very end of the process.

Then environmental monitoring is all part of that evaluation of potential contamination that could occur, too. Then using all that information together for release.

This one, I'm not sure. It says how is -- regarding AATB standards, how is compliance with the standards determined? That is via inspection once every three years. Unannounced inspections have actually occurred as well, related to processing.

How is compliance with standards reflected in the product label? Again, that can -- how is compliance with standards -- an AATB accredited bank can only use that claim that they are accredited by us and they follow our standards. That can appear on the label.

I'm not sure if exactly that's what that means -- that question means.

MR. WINTERS: I think the question is can

they put on the label or what can they put on the label. Can they put AATB accredited or --

MR. BRUBAKER: Not on the label. In the package insert and so forth, it's allowed.

One of the things I didn't put in my presentation, because I didn't think it would fit, was our standards for labeling on the graft itself, which go further than FDA requirements, and also the package insert requirements.

They're very extensive. I could show that if my Mac would work up here, but it's an extensive list. There's over 20-some items in the package insert that need to be on there, warnings and so forth, and single use, that kind of thing.

You cannot advertise that you follow our standards if you're not accredited. I wanted to say that.

Now, this one I think I probably need help from some folks who actually work at tissue banks. But with many synthetic products, NIST comes up with standards and guidelines. I think it's mechanical properties, et cetera.

Do tissue donations have integrity standards? The symbols here, I've never seen before. There's two of them. But there's one that's hardness and ductility. I'm not sure if I'm reading that correctly.

But different grafts have different properties, of course, soft tissue, hard bone, valves. Our HCT/Ps are not -- the FDA doesn't require pre-market approval, which would probably address these processes, these different specifications.

So I guess I know tensile strength studies have been done by banks for tendons to figure out if they can go up to a certain age. For hard bone, I know some other studies have been done for how strong is that tricortical wedge up to a certain age. But for some specific guidelines that have to be met for all grafts, that hasn't been done. I don't think that's in the future.

I hope I've answered that one correctly.

Heart valves. We tried to treat them gingerly and not to alter their structure and functionality. So we try to limit even the processing that's actually done on them.

DR. DOPPELT: May I ask you a question?

MR. BRUBAKER: Sure.

DR. DOPPELT: I just want to go back to the very first question. You said so the 10 percent destructive testing is -- that sort of went by the wayside. Number 14, the standard, you said all cells and/or tissues to be released for human transplantation shall have representative microbiological cultures obtained, but you didn’t define what’s representative, how many, what percentage. So it sounds good, but what does that mean?

MR. BRUBAKER: I know what you’re talking about. I don’t know the answer to that one personally.

DR. DOPPELT: It’s nice that the standard is -- representative is fine in theory, but in practice somebody has to make a determination that this is or is not representative.

MR. BRUBAKER: Well, I think part of the answer to that was really answered by Martell when he talked about the assurance that you can get from your validated process and do you really have to destroy tissue, or co-processed tissue, to prove that that

process worked, when in reality you may not have to do that.

MR. FRONK: From a cardiovascular processing standpoint, we do not terminally sterilize. It's disinfection only. A hundred percent of the tissue, each and every piece of tissue, is tested. So that is the representative piece. We also do companion testing of non-implantable pieces of tissue as well.

MS. SCHAFER: I think the issue of representative samples -- part of the crux of the matter, we've done a lot of BF testing at our tissue bank. As a consequence, the question of the representative sample has really risen to the top of the matter.

I question the standard of requirement to use a piece of skin and want to move into trying rinse aids. While we were doing bioburden testing, we found there was a lot of utility with rinse aids.

Martell gave the whole industry some really wonderful guidance on recovery efficiencies. It kind of opened the door to another way of looking at this.

So if there's good technical collaboration

with a laboratorian and good technical collaboration with media consultants, some of these things can be pushed through because a lot of them hinge on many different matters.

For instance, we learned in the BF testing that not only was our media inadequate and we did have bacteriostasis, but when we went to resolve it, there were a couple of pathways we could go. We used the USP.

I could say on Phase 1, we resolved the bacteriostasis. But that’s really just Phase 1 because now I’m working with people who make media, and they want to really kind of use the techniques that Dr. Srinivasan alluded to with the CDC to really specifically look at what neutralizes each component of your processing. So we’ve learned a lot and we’re building on that.

Additionally, on our bioburden testing, we found out the limitations of filters when we ran into all the lipids in the tissue and started reformulating the question of what was really a sample of incoming bioburden. So we really had to learn the hard way, I

guess, because maybe we're all on that part of the curve.

So I would like to see a lot of dialogue on what's a representative sample. I'm not convinced that a swab with a 16 percent sensitivity is adequate to detect the one culture of Group A strep that may be a disseminated systemic infection. That concerns me greatly.

I think I have probably more questions, but part of what this challenge is, is to talk about the concerns, and those are real concerns.

Once we removed our bacteriostasis, we increased our discards of skin by tenfold. There's a lot of difficulties with that because now are we -- we have to go back and look at the methods with the antibiotic treatments. Can we beef them up?

So it never ends, but I guess that's where our experience is on that. I think with more collaboration with the experts in the field and with the people who really have technical expertise -- because we don't know all of these techniques. I've picked up a few good ones already

today. Thank you.

MR. RONHOLDT: In terms of what AlloSource has done for the representative samples, since that's one of the questions for this panel, we actually have started to move away from that.

We found that it's very difficult to validate, that there's some tissues, that it's very difficult to run through typical fluid extractions. They just aren't good methods to -- there's just other better methods that you could use that would not destroy the tissue.

Unfortunately, some of these methods you need to use a companion piece. What we've done is we've actually looked at some of the osteoarticular and some of the DBM that we could go through and validate.

There are methods to test for homogeneity and stuff like that that we've done. Where there's something that we could benchmark or something that we could say, hey, this is what's been done previously, we tried to follow that as close as we could.

Some of the other questions that hopefully we'll get to is using tissue is a challenge. It is

very inconsistent. It’s donor to donor, variability of different densities. You have superficial versus cancellous. Where are the bugs? How do you get the bugs off?

It is a challenge to try to release these tissues to make sure that you have negative cultures. The physicians say they expect the cultures -- some of the skin or some of the products that we put out are not sterile. But they expect that when they get them they are free of microbial organisms.

I think the representativeness is a good question. In terms of what we’re doing is we’re trying to find other ways to look at the cultures on the back end and try to benchmark other industries and stuff like that.

DR. KUEHNERT: Okay. Thanks for those perspectives.

I just wanted to -- there were a couple of questions that were sort of in the other category. I’m not sure where to put them, so I’ll just bring it to the group and see what they think.

One concerns nucleic acid based testing

methods and are they helpful in the evaluation of residual bioburden in allografts post-sterilization.

I think what this is referring to is actually bacterial PCR methods. I'm just wondering what the experience has been amongst the panel with those methods.

No, no. We have a volunteer.

MR. WINTERS: That's an interesting question because something like that has the potential, perhaps, to be a little more sensitive, but then you also come back once again to the sample size question.

Even if you're looking at a nucleic acid type of assay post-sterilization, you're still back with well, so I did the nucleic acid testing on 10 samples. So I've proven 10 to the minus 1, but my sterilization says 10 to the minus 6. So where is the statistical representation there in the samples?

So I would go back to it may have some benefit perhaps in pre- or post-processing or pre-sterilization cultures. But in post-sterilization cultures, I'd get back on my soapbox and just say don't do it.

DR. KUEHNERT: This question involves the -- did you want to --

MR. RONHOLDT: I just wanted to --

DR. KUEHNERT: Yeah, go ahead.

MR. RONHOLDT: With respect to PCR, we did look into that. The difficulty is now you're getting down to the sensitivity where you can detect a dead bug.

So you're going to come out and your PCR is going to say, hey, okay, there's clostridium here. But you don't know if it's dead. I mean you -- or maybe not even clostridium; staph, something very easily killed by your process.

Then at the end of the day, you have a very sensitive method that is going to impede you into releasing stuff and you really don't have a good appreciation of what that really means.

The other thing that we've discussed and found is that PCR is very specific. The 16S DNA strain is consistent through bacteria, but you need to get a little bit more sensitive to that.

So you need a molecular tag for clostridium,

for staph, for strep, for E. coli, on and on and on and on. So at the end of the day, you're going to have this huge test and molecular tag. There's still other bugs that you're going to miss with it.

So there are some challenges that -- it'd be a sweet assay, but it's very challenging.

DR. KUEHNERT: This other question is sort of the flip side of this which is -- it concerns viral and parasitic testing.

I think that what it's getting at is it asks what donor tissues are tested for these pathogens. I'm assuming that serum is really what's tested for -- let's just limit it to viruses for now.

Are there any banks or other recovery agencies that are testing individual tissue or samples other than serum for infectious pathogens?

Okay. So that looks like a no.

Okay. The other questions -- I don't have any other ones. But I gave an avalanche of them to Arjun. So let's see what's in his stack and what we might want to address.

DR. SRINIVASAN: I redistributed some of

them. The hard ones, I gave to Scott. I'll do the other ones.

It's a question, and a very good one, about given the fact that there's a wide variation in the donor population, how would we get representative sample trending.

The point that the questioner makes, as I think the answer is, is that you would need to do some degree of mathematical modeling. You would need -- it's called here a multiple variant effect analysis. I don't know what that is. It sounds really good, though.

But, yeah, you do need to do mathematical modeling because you do need to understand if you're trying to define the universe of contamination, you need to know the universe of donors.

So looking at all of the characteristics of the donors so that you have a handle on that and absolutely, it would need to be mathematical modeling to ensure that the population that we're looking at is, in fact, the population that are tissue donors. I think that through mathematical modeling of that

population, it would be a good way to do that.

Another question is conducting -- about our investigations, how sensitive are we to maintaining the secrecy of a company's processing trade secrets?

That is a very important concern for us. We're very, very sensitive to that. We understand that these are proprietary issues. They are trade secrets. We have had careful discussions with our lawyers.

As you know, we are a government agency. We are subject to the Freedom of Information Act. So people can request information from us and we have to provide the information that they request.

But our lawyers have said that if trade secrets are provided to us in a voluntary manner, that there is a law under the Freedom of Information Act request that allows withholding of that information.

So we can make an argument to withhold trade secret information that's shared with us voluntarily, even if it's requested through the Freedom of Information Act statute. Trade secrets that are voluntarily shared with us don't have to be released.

So we go to great lengths to protect both

trade secrets and also the identity of the company. We do not divulge the identity of companies because we know that our ability to conduct these investigations is dependent on people wanting to work with us.

If we are not sensitive to those issues, then it will greatly impede our ability to work with tissue banks. So we work very hard to do that.

There’s a question about viral testing, if we test tissues for viruses. We don’t do that. I think some of the challenges with specific PCR testing with tissues have been discussed.

That’s what our laboratorians have also felt, that it’s not something that’s easy to do. It’s really hard to know how to interpret the results. So we don’t do viral testing of tissues and investigations.

There’s a question about molecular testing, specifically about the Group A strep. But I’ll generalize it to a general question about molecular testing, which is how do you know that when you have a strain from tissue recipient and a tissue from a tissue, just because the molecular patterns are identical, does that mean that the organisms are

identical? Does that mean that it came from the tissue?

So the genetic relatedness only tells you that the organisms are related. So the second question of whether or not that tissue was the source of the organism in the recipient is a valid one.

That’s why when we do these investigations, we feel like there has to be two pieces of information. There has to be the microbiologic information which establishes genetic relatedness. There has to be an epidemiologic relationship as well. We have to demonstrate that, in fact, it’s that tissue from that donor that’s in that recipient and then the organism is also in the recipient.

The advantage that we’ve had in our investigations is some of our investigations have been with relatively unusual pathogens: some of the clostridial species, very rare causes of post-operative infections; chryseobacterium meningosepticum, extremely rare cause of post-operative infection; Group A strep, not that rare but fairly rare.

So the fact that they’re rare bugs, and that

we get them from the tissues, and there is a molecular link, provides us that -- what we say to be very strong evidence that the tissue is responsible.

If we're talking about staph aureus and strains of staph aureus for which there is very limited genetic diversity, absolutely, we may not have a way of knowing. We may recover the same strain from the tissue and from the recipient, but that's an organism that's very common as a cause of surgical site infections.

We're learning that there's a very limited genetic diversity in some strains of staph aureus that are very common causes of infection. So the molecular typing in that situation may be fairly meaningless.

So how we interpret the molecular typing depends on the pathogen and also depends on the other information that we have. It's additive information that can't be used on its own.

I think those are the bulk -- I passed off some of the other questions that I've gotten. I'll let some of the other panelists come and discuss them.

DR. KUEHNERT: Okay. So whoever wants to

come up next with the remaining questions.

MR. WINTERS: Okay. A question here. Is it possible to combine bioburden testing with tracking of pathogen markets? For example, PGE2, this can be done on media extracts, on ELISA kits, and as it’s done in tissue banking.

I think one of the issues there again would be if those pathogen markers would likely be present whether the organism was alive or dead. I don’t have an extensive knowledge of those types of tests, but that would be my guess that that would be the case.

So I would think if a bioburden test is done properly, you’re going to get both the pathogens and the non-pathogenic microorganisms. So a more standard bioburden test, where you’re just looking for the numbers and types, would generally work very well. I don’t see that it would need to be improved upon for any other purpose.

Then when performing bioburden counts on tissue products, how critical is showing the tissue samples to release bacterial contamination within tissue?

I think that’s referring to -- and the follow-up question related, does maceration of the sample release intracellular bacterial contamination or just release of anchored bacteria to the tissue surface?

That’s a very good question. That’s one that we kick around occasionally. Some of us microbiology nerds get together and talk occasionally over dinner or whatever. So the questions that we ask ourselves -- and no one has the answer yet – is, well, is the surface extraction acceptable for bioburden testing, for example.

On the one hand we say, well, if you greatly underestimate your bioburden count and you’re doing a radiation study, then you’ll fail the sterility test portion because if your bioburden’s underestimated, you’re looking at the wrong part of the table; you’ll get a lower dose than you should get and you’ll fail.

Then we also ask well, if it’s not related to a sterility test or any type of validation, then we say how common is it to have microorganisms hiding inside a tendon, for example.

The general discussion, my understanding is, generally speaking, you should not have a high level, or perhaps any level, of microorganisms inside a tendon as opposed to on the surface.

So that’s a question we kick around as well. My guess would be that if you’re getting higher counts -- and maybe someone else can comment on this.

Arjun, I think you mentioned this in your talk.

If you’re getting higher counts on a macerated tissue, you’re probably just doing a better job getting the bacteria out of crevices or mated surfaces in the tissue, or the bone or things like that, rather than inside of a tendon or something like that. That’s my guess there.

Okay. That’s it.

DR. KUEHNERT: What else do we have?

I think Scott may have the last batch. Is that right?

MR. BRUBAKER: Yeah, I’m not sure. I just saw this.

When antibiotic rinses are conducted, is that

logged? During processing, yes.

Have there ever been instances of tissue processed with antibiotics causing allergic reactions?

I would have to ask the clinician users the answer to that question. But allergic reactions may be -- it could happen with the antibiotics. That's part of the labeling that goes on a graft or actually in the package insert, that there could be residuals.

I mentioned the chemical processing. So there could be chemical residuals. There are warnings about that in the packaging. Those should be reported to the tissue bank if it occurs.

I know with our TTSN project and other initiatives that we're working on, we're trying to increase and educate the end users how to best recognize the potential reaction, whether it's an infection or an other reaction; failures as well.

So we're developing guidance on that ourselves at AATB. A work group has started on it.

We want to increase surveillance in reporting, basically, and recognition.

So as far as how -- has that happened, I'm

not an inspector. I don't run our accreditation program. I do know that our inspectors always look at the complaints files, adverse event reporting files. That's part of the usual process wherever they go.

We haven't tracked and trended and logged those ourselves. But they do look to see how they're handled and how they're closed, and has the medical director closed the ones that are medically related, especially for infection. Actually, all of them the medical director does close.

This one, I'm -- of course, this could be controversial, I guess. But shouldn't AATB be responsible for disclosing these infections so that surgeons can evaluate tissue banks?

I don't think that that's necessary. It could actually deter reporting to us. There shouldn't be a punitive announcement, I think, for infections that occur. Plus you do find out anyway.

CDC doesn't post the names in their reports of the banks. There's probably other reasons that I'm not thinking of right now for being that punitive.

Recalls occur. Those are available on the

FDA Web site. There’s a delay in those getting posted, but those inform you of who the bank is and what the general reason was for recall.

So I just don’t see it would be a value to make that information available, say, on our Web site. That’s my feeling at least.

DR. SRINIVASAN: I would sort of reiterate, I think this is a huge problem. We’ve had some discussions many, many times before and also here.

The sad reality is that these types of events lead to lawsuits. The problem becomes that if we make reporting punitive, reporting will go down. That’s the opposite of what I think we’re all striving for.

The only way we improve the safety of tissues is if people tell us when they have a possible transmitted infection. We investigate it. We try to figure out why that happened.

I mean look at what we’ve learned. We’ve learned about the importance of neutralization. We’ve learned things about Group A streptococcus. We learn things from each of these investigations.

If we make this really a punitive thing,

where as soon as you report, you get up there and you're going to get sued and no one's going to buy your tissues anymore, then we will get no reports. We can't do any investigations. We can't learn. We can't move the field forward.

So it's a tricky balance. I mean certainly, action needs to be taken to remediate when these problems occur, but at the same time, we can't make that system punitive.

I really feel strongly about that because if we do, we're never going to get reports. We can't move the science forward if we don't get reports and investigate them and learn from them.

DR. KUEHNERT: Are there any comments?

I was just going to make the comment that I agree with that and that I think there is value for surveillance in that sort of a situation. But it's important to remember how, for instance, other fields handle this, such as infection control, where if you report data in the aggregate and you have some degree of anonymity and anonymized data, you can have much the same effect without going out and naming an

institution.

So I think that there's ways to disseminate information, as we have in a number of ORs and other methods of dissemination, and still not have it be punitive.

MR. BRUBAKER: When you asked the question about parasites, there was another pathogen, I think. There was a question about whether the testing was done --

DR. KUEHNERT: Yes. So it was a question about whether donor tissues are tested for viral infections and what tissues are tested. I'm not sure if that means routinely or in special situations.

MR. BRUBAKER: Well, for viral infections, we did look through relevant medical records, do thorough screening looking for any indication of infection, bacterial or viral.

Is the tissue tested? We don't have presentations, I don't believe, by banks' specific methods and how they've gone about their validation. In other programs, that's been presented. They can show, like I said before, a reduction in viruses

through their process, even though it’s a byproduct of the process, the many washes and rinses and treatments and so forth. But individual tissues wouldn’t be tested. For parasites, they’re not as well. That would get back to donor screening.

I’m going to plug a presentation I gave where it actually worked at BPAC last April. If you go to their Web site and look up BPAC meeting in late April, I believe, I did give a five-minute presentation on why we believe tissues are basically a low risk for Chagas disease. Some of that program was about Chagas.

Of course, a lot of blood donors are being tested in the U.S. If you go to AABB’s Web site, you can see how they’re tracking that. There are some cases that are proven to be real positives.

So in relation to tissues, we get back to the kind of processing, the type of tissue it is. We have a standard -- for years now, I think at least five years or more for Chagas screening – for valves donors because we know that the cardiac tissue is a place where the parasite can reside in someone who’s infected. The cryopreservation process can preserve

the parasite in the amastigote stage and other stages. So we've found that's a risk tissue, a risk processing method. We decided to basically put a screening requirement out there which is a verbal history screening.

Some banks have gone to the point of if they get a positive or they identify a risk in the donor from the verbal screening, then they might do testing. That's been voluntary, but I know at least of two banks.

We also presented there that the majority of the tissue that's processed and distributed today is lyophilized. It's bone or lyophilized soft tissue as well in some cases but mostly bone.

That goes through processes than can kill parasites because we just probably have to validate that, that that happens. Lyophilization itself, a parasite -- from my understanding from speaking with Dr. Herwald at CDC is that that would kill parasites. They can't live through a lyophilization process.

So a majority of the tissue today that's been released would be treated in at least one fashion and

that would kill that particular parasite or parasites in general, I should say.

So again, I think it’s a tiered-risk based approach to the type of tissue, type of process.

DR. KUEHNERT: Yeah, I think that’s a good point, is that there is a risk spectrum. A lot of times when we start thinking about a new pathogen, it often gets applied to all tissues, and that may not be relevant for all tissues.

But until we know, we sort of have to make the worst case assumption. So there really is a need for studies to look for, say, the viability of T. cruzi in tissue or, for instance, West Nile virus.

A recent investigation of organ transplantation -- I think there’s a lot of data from organ transplantation we can learn from -- it was shown that a organ donor was PCR negative and yet antibody IGM positive, and transmitted to organ recipients. So the speculation there is that there was West Nile virus present in the tissue but had cleared the blood, or at least cleared it to the point where it was too low to be detected by current methods.

Now, the question is what relevance that has for tissue. That's something that's an open question until there are more studies as far as what the tissue distribution is of West Nile virus over time. Just one example of some of the work we have to do.

Any other questions that were remaining?

Well, I'd like to thank the panel for a really nice discussion.

Before we close, I wanted to give the organizers at FDA and Drs. Solomon or Witten a chance to close the session today.

DR. SOLOMON: Actually, I just want to thank everyone for their participation today, the speakers and also the audience and the questions.

(Whereupon the meeting was adjourned.)