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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH,

FOOD AND DRUG ADMINISTRATION

AND

INTERNATIONAL SOCIETY FOR ANALYTICAL CYTOLOGY

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PUBLIC WORKSHOP ON SAFETY ISSUES PERTAINING TO

THE CLINICAL APPLICATION OF FLOW CYTOMETRY TO

HUMAN-DERIVED CELLS

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FRIDAY, APRIL 20, 2001

PART TWO…

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DR. KEANE-MOORE: Thank you, Adrian. That was a really excellent talk, and I believe that will also sort of become the cornerstone of the reference materials of this transcript. I think we're going to have to put you on payroll after that.

And I would also like to say that Dave Coder and I pulled out the website locations for most of the documents that you mentioned and they are part of your handout today.

Our next speaker is Dr. Thomas Wagner from the Greenville Hospital Systems and Lillia Holmes, who is his flow person. And they will be telling us about their system. Thank you.

DR. WAGNER: First of all, I'd like to thank Dr. Keane-Moore for inviting us to speak to you today. And this will be sort of a two-part

presentation. You're lucky. I'll do the short part. I'll talk about why we sort and Mrs. Holmes will talk about how we sort. Prior to talking about why we sort, let me give you the conceptual background behind our protocol.

Modern tumor immunology has indicated very clearly that tumor cells are antigenic. Indeed, we also know that tumors start in all of us all the time. A frightening thought, but try -- and usually they are cleared by the immune system.

So tumors persist partially because they select for secondary mutations which block the ability of tumor cells to present antigens appropriately.

If this is indeed true, which much evidence suggests it is, then an ideal approach to treating tumors might be to take advantage of our best antigen-presenting cell, the dendritic cell, and somehow try to use the power of the dendritic cell to present appropriately those antigenic characteristics of the tumor should elicit an immune response and clear the tumor from the patient's body.

How might we do this? Well, you might do this by fusion. Now that sounds simple enough, but it's not simple in this case. We're all aware of hybridoma cells, which have been around for a long time, and we know how to make hybrid cells.

We take one cell population and put one selectable marker in it, and take another cell population, put a second selectable marker in it and culture the cells for a long time and select from the two markers together.

That's fine, but it won't work in this situation, because dendritic cells are terminally differentially, non-dividing cells.

Furthermore, when you try to make fusion cells by this method, let's think what we do. In the process of culture, which we all know as

biologists, we're going to select for a particular subpopulation of the cell population we start with.

Tumors are by definition highly-diverse cells. We want to express that entire diversity in the final fusion cell product. And, therefore, we wish to make what we can an instant dendritoma. An instant dendritoma is a dendritic cell that is immediately isolated.

And we do this. The colors that I've been using, green and red, are not simply by chance in my slides. Not just to be cute, but indeed this is how we do it.

What we do is we take dendritic cells prepared from patients and stain them with a vital cell tracker dye, which is green in florescence.

We take the tumor cell from the patients. The tumor cells are then irradiated so they can no longer divide, and they're stained with a red florescent tracker dye, vital tracker dye.

We then fuse these cells under standard procedures with polyethylene glycol and a very small percentage of the cells fuse.

But those cells that fuse at that moment are a complete representation of the diversity of the tumor cell that exists in the tumor.

And we then create a cell which you can see. And these are not -- these are real pictures by the way. This is actually the fusion process going on.

We create a cell which is neither green nor red, but is somewhat orangish/yellow and we can then sort, use the cell sorter -- use the cell sorter that we've been talking about all day today, specifically to pick out those particular fused cells immediately after fusion, with no culture in between.

And this is the method we're using to try to generate a vaccine to then introduce back in to the patient to immunize them or to show

their immune system the antigenic characteristics of the tumor that their immune system has never before seen, because the tumor cannot present it.

Now, this is what we get. And this, again, are an example of how effectively we can sort these cells. And this is an instant sort. So that at the top we have the tumor cells, the dendritic cells and the sorted product, which is a highly-purified population of the instantly fused cells.

We want to look and see whether indeed these cells have the characteristics that we want. We're particularly interested in them having the characteristics of a dendritic cell, the ability to present antigen appropriately and indeed they do, as you can see. They express HLA A,B and C and HLADR, just as about as well as dendritic cells do.

They express CD80 and 86, which are very important accessory proteins in antigens presentations, just as dendritic cells do.

This cell is indeed a cell which is both a tumor cell and a dendritic cell at the same time. All the characteristics of the dendritic cell for antigen presentation and all the characteristics of the tumor cell that the patients immune system has never before seen.

Now we aren't just fusing to be cute. I didn't say that to be cute either. But there are other people who are fusing tumor cells with dendritic cells and putting the gamut into the patient.

And we've done very careful experimentation to show you something. The first -- let's see if this pointer works.

All of these here are really controls. You can look at them, and they're important, but this is basically controls. The importance of these three.

If we just take tumor cells or tumor lysate and incubate them with dendritic cells -- and this is an in vitro CTL experiment where we can get

some quantitation, because you can't get quantitation in the patient -- we get a certain effect -- generate a certain CTL activity.

If we now take tumor cells and fuse them to dendritic cells, but don't sort them, we get an increase. But now if we sort, we get a greater increase in the sorted population over the mixture than we get from sorting over just tumor lysate. That's very, very significant and a significant difference.

Furthermore, very recent work in our laboratory by Dr. White, who is here, suggests that the activated by non-fused dendritic cells actually may have a negative implication in CTL generation. That they alone may impeded CTL generation.

So this is why we sort and now Ms. Holmes would like to tell you how we sort. Thank you.

MS. HOLMES: I think everyone here is pretty familiar with how we sort. What I'd like to talk about is how we actually do prepare the instrument for clinical sorting.

First, I'd like to say it's a pleasure to be here. I think it's really fun for me to get to put faces with names that I see on the cytometry mailing list all the time.

And I'm very impressed by, as well as respectful of the expertise that is here today. So if you will kind of sit back with me for a few minutes, because I'm coming at you with a little different perspective than I think everyone else so far, in that I'm the operator. I'm the girl that puts into practice all of the things that you're talking about here today and try to make them a reality for our patients.

So my whole focus of what I do every single day is how can I handle the things in the lab so that my final product being injected into the patient is safe, which is our first priority.

And what I have found, actually, is if you accomplish this goal, if you make sure that everything is safe for your patient, you wind up accomplishing the goal of worrying about operator safety at the same time, because the same things are required that you're protecting your patient as well as yourself and it works out pretty well.

So you want to handle everything in every possible step along the way to insure the final product sterility and purity.

And you want to make sure that that final product is unaltered, accurate sort. I think that these are things that we've been hearing all day long.

So I'm going to give you the basic rundown of what I do to prepare the instrument for sorting. The first thing we had to consider when thinking about how to keep this instrument clean was the room requirements. This is actually the door or the room. So I have a variety of signs on the doors, trying to keep people out of there.

But the cytometer that we're using is solely dedicated for the purpose of the two clinical trials that we have going on, and we were lucky in that this is a very new instrument. And so nothing had ever been run through this cytometer. No mouse cells, no other animal-type cells have been run through his instrument. Only cells for this project.

And I only run things though this cytometer that have been proven to be sterile. We do do -- I don't think I've heard anyone mention this, but we do some -- what we call in-process testing for sterility, before we even get to sorting, so that we can show that the cells that we're putting through are sterile and of themselves. So that helps us out there.

The room also has filtered air and we have some UV lights as well in the ceilings of the room. At least half the lights have been removed and

UV lights have been put in. And you can turn those on before and after your sort to help insure sterility.

And you want to keep everyone out of there. And an issue for us was housekeeping. Being in a hospital situation, housekeeping goes everywhere. It takes trash out of everywhere and, initially, we didn't have a lock on this door. We now have a lock on this door. But that was something that was a consideration for us in the beginning.

Now you've heard this too. UV, or keeping it separate from the room, these are not absolutes. There is no absolute in this case. But you want to use all of these things together to try and achieve your goal.

So before I begin, I make sure I have everything possible that you need to sort. How many times have you been sorting and you have a nozzle clog and you go oh, I don't have this here. And you need something sterile to help clean that.

You want to make sure you have everything possible that you can imagine before you start sorting ready to go.

Just some ideas for you. Some things that I use. I sterilize some cotton swabs, some chem wipes. I make sure I have sterile gloves and mask available. We're using a metal injection port, so I sterilize that.

The sample tubing is sterilized. It's also changed for each patient. The nozzle tip. I also sterilize a little pair of scissors in case I need to trim any tubing along the way.

Any solutions that you're going to use, maybe to wash off your injection port, or if you have a nozzle clog and you need some kind solution, these bottles are sterilized themselves. They contain sterile solutions. I have some 1XPBS, some ethanol, some sterile water and some bleach.

Anything you think you can possibly use you want to have ready to go before you begin.

Something that we have done to sort of make our process easier is the use of multiple sheath tanks. For myself it was quite cumbersome to take one sheath tank and lug it back and forth between the flow hood and trying to use ethanol and PBS, or water, or whatever I was needing to use.

And so we decided to purchase multiple tanks. And so that way, once they're clean, then you can just clean the connections each time that you're connecting it, but you can just plug it right in. You don't have to go back and forth. And that kind of saves you some time when it comes down to actually cleaning the instrument.

We talked with Becton Dickinson at great length about how we should handle cleaning these tanks and they said yes, you can autoclave them, but -- and those of you who have with these things a lot longer than I have may have your own methods or your own opinions about this, but the feeling was you'd have to take off the connections and anything that might not survive the autoclave, and then after you autoclave the tank, you've got to reconnect all the stuff, which would open up a possibility of contamination while you're reconnecting everything. So that would be a difficulty there.

So we are not autoclaving our tanks, but I promise you they're getting a huge, healthy dose of ethanol. And you just might as well go ahead and plan on signing up for buying huge amounts of ethanol because that's what it takes to clean these tanks down.

I usually give them a good dose of a hundred percent to begin with, just to make sure, followed by a 70 percent rinse.

Then you want to make sure you get rid of all of that ethanol. And usually what I will is pour out as much ethanol as I can, seal it back up and

swash it around really good and then go to the flow hood to remove remaining ethanol, because you want to reduce any kind of contamination.

After -- so you make sure you rinse those tanks after your ethanol with you -- whatever your sterile sheath fluid is going to be. In our case, it's 1XPBS.

So after they're cleaned, I keep them lined up back here. And when you're connecting and disconnecting these things, I always -- I use my little sterile bottle of ethanol and make sure that I clean down every connection when you're opening and closing them.

Like I said, all the transfer of the sheath fluid is done within the bilaminar flow hood, which can be kind of tricky, but it's very doable.

And how we actually sterilize the fluidics -- this may look very minimal in comparison to what you just heard. I think it was a six-hour total thing, and then an additional three hours after the sort, but we run 70 percent through the system for a minimum of ten minutes at a high-flow volume.

A lot of times this kind of expands up to about 30 minutes for me, just because I'm in the lab preparing something getting ready for the sort.

After the ethanol, then we flush with the sterile sheath fluid for an additional ten minutes at high-flow volume.

I think this is a most glamorous picture of me ever. I think I'll submit it to Harper's Bazaar after the meeting today. But this addresses the two things I was talking about at the beginning.

One, if you achieve patient safety, you achieve operator safety. We're talking about a stream in air system here. Have you ever opened the door to your flow cytometer while you're sorting whatever, mouse cells, and breathe down the stream and you can see the whole stream moves.

So it's really important, one, don't open the door and breathe on it, but that makes the argument for be sure you wear a surgical mask, or some people were talking about wearing HEPA masks. Whatever makes you happy. You really -- this needs to be a consideration.

I have a lot of hair, so I always have on the beautiful hair net. I also wear sterile surgical gloves at all times. I do not wear just your run of the mill latex gloves. These are sterile surgical gloves. I have a lab coat and shoe covers.

At this time point, after sterilization of the fluidics, you want to, again, use that huge amount of ethanol you have and thoroughly clean the inside of the instrument everywhere you're going to have sample uptake and collection.

Now, unfortunately, we cannot cram the vantage into the autoclave. I was wishing that this morning after the talk about autoclaving, but we have to do the best we can with using the ethanol in this manner.

You want to clean the sample arm, you want to clean the injection port. You want to clean the knobs that you use for laser alignment because those may have been touched.

Anything that you can possibly see, you want to give it a heavy dose of ethanol. Don't be afraid. It works great.

Something -- I mentioned that we're using this metal injection port. And this was actually at the request of the FDA, as we began writing our IND.

And a lot of you may be using this if you're using a little pre-filter stuck on the end of the port. That's not something that we do. We filter our sample when we're putting it into the tube.

But a lot of times this tubing itself comes down into the sample. This has been -- and this tubing is changed for every patient. And it's also autoclaved prior to being installed on the instrument.

This has been something we've been discussing with Becton Dickinson and maybe might be a point of consideration after the meeting today. And these are the field service guys at BD, talking about the sheer act of trying to connect this tube or this metal port to the sample tubing might open you up for a point of contamination.

But if you simply used the tubing down through to the sample, you might have a better chance, if you don't need that filter on the bottom there, or something like that.

That's something -- at this point, this is what we're doing. Something to think about for the future. Like I said, when you're cleaning you want to clean everything. Anything you can see, you want to clean it.

When you're putting -- one thing I want to mention about -- and those of you who are used to changing your tubing probably already know this, but something that BD was very emphatic about cautioning us about, when you're putting that sample tubing onto that sort head, there's that little, teeny, tiny pin on top of the sort head where the tube connects right there.

You want to be really careful as you're putting it on not to push down that pin into the sort head by accident, because that will change the flow into the nozzle and could cause you huge problems.

So this is something that I try to be meticulous about because I'm doing it so much. So it's something to think about.

After you put that tubing on, because you've kind of had your hands in there, even though you've wiped down everything and you have on sterile gloves, I usually give everything another once over.

This includes when -- I'm talking about wiping down everything. The insides of the camera doors, the objectives, the obscuration bars, anything you can see in here, it gets cleaned. It's kind of tedious, but it works very well.

Something else I want to point out, which those of you who have a Vantage will notice this. I don't know what your personal preference is, but I have taken off the little red safety guards on my deflection plates. Hopefully, your operators will know don't stick your hands in there.

I found that having those protectors on there decreased my ability to keep those plates clean. If you have a problem with your side streams during set up or during your sort, and you get salt on there and you need to clean them off, it really inhibits the sterility process in having those little red caps on there, so I keep them off now.

After the sort, in thinking about the next patient that's coming along, you want to make sure your system is clean. So we run ten percent bleach through the system, again, at a minimum of ten minutes, a high-flow volume, followed by a sterile distilled water for another ten minutes.

And, again, you want to kind of go back down over your surfaces because you've had this patient's cells in that area. Any possible way that you think you've contaminated things.

Now again, you're going to change out your tubing and autoclave your metal injection port again, but better safe than sorry.

Kind of a lot of the points that have been made today -- like I've said, I've gone through the very basic technique of how we actually sterilize the instrument, but your methods are going to be specific to whatever your product is.

These are some ideas I think everyone, no matter what you're working on, is going to be doing sterility assays. We're also doing some PCR assays for sterility in addition to what's required by this regulation, by the traditional culture methods.

You can reanalyze your sample of your final product, check your markers, you can check your purity. We are fortunate in the fact that we're not using antibodies, so I can also look at my cells on a florescent microscope because we're using membrane stains so I can see them really well and check my purity that way.

And the basic method you heard about this morning, checking cell viability with Trypan Blue. These are just some ideas. There are thousands of ways that you can go about doing this.

Some things I want to point out to you in that we eliminated some of the limitations before we even started.

And those of you working with things like HIV or other things may not be as fortunate as we are. One, that all of our patients are screened very heavily for HIV and hepa, the different types of hepatitis and they are all negative.

So we don't run any one who has those infectious diseases and they also have no other pathological infection at that time, other than their cancer. So we sort of reduce -- try to reduce that risk before we even start. So that helps us out a lot.

In kind of -- the second thing which I mentioned already was that we're not using antibodies. So we're not as worried about our reagents in that aspect.

So in review, you want to consider your room requirements, get all your materials ready to go, consider using multiple sheath tanks. Talk to your operators and see how they feel about lugging those tanks around.

Sterilize your fluidics. You want to use appropriate operator-protective devices for those two purposes, to protect your patient as well as your operator.

Sterilize the sample uptake and collection services. Clean the system after sorting and confirm your product.

Now I'd like to kind of conclude by saying that this is done for each patient. And what I would like to tell you is, especially in comparison to like doing a six-hour cleaning method, and we're talking about doing a 20-minute thing, we've done eight injections into patients and we've had zero contaminate problems. Everything has come up perfectly clean.

And I want to tell you too, that we spend more time doing all of these assays than we actually do in product manufacture. So endotoxins, sterility, proving that the reagents we used during manufacture for fusion, any antibiotics used in culture media, all of those assays that we do to meet the requirements, actually, take us more time than the manufacturer.

But the point is, if you utilize even just the simple, basic things that we're doing, they're very effective and you will get a sterile, unaltered product at the end.

That's all I have. If you have any questions, I'll be happy to answer them.

AUDIENCE MEMBER: (Inaudible, speaking from an unmiked location.)

MS. HOLMES: The sorting is variable, but we are sorting anywhere from a million to several million outs. Our sorts are very short. The

last two patients we did were say an hour, an hour and a half. It was pretty quick.

MR. LAMB: Larry Lamb, again. Your patients are not -- are your patients compromised, immunoblated -- what --

MS. HOLMES: We don't -- we go through a great deal of testing actually.

MR. LAMB: No, no. The patient himself. Does the patient have chemotherapy on board, suppressed immune system?

MS. HOLMES: No, no.

MR. LAMB: Okay. Second thing that I was interested in is this a subcutaneous injection?

MS. HOLMES: Yes.

MR. LAMB: I think that that clears up some of the differences in some of the previous talks where patients have been fully immunoblated and allogenic cells being used, and also being infused as part of hematopoietic system.

MS. HOLMES: That's a great point.

MR. LAMB: You can get away with a lot more in that circumstance.

MS. HOLMES: That's a great point, and we actually started out doing intravenous injections and switched to subcutaneous, but the patient at that point is not having any chemotherapy of that kind.

DR. WAGNER: Indeed, it's the reverse. We screen our patients for immunocompetence because that's required for this therapy.

DR. KEANE-MOORE: I just want to thank Lillia for a very, very excellent talk from the trenches, really. The front lines. It gives a whole new perspective to concretely see what it is that we've asked people to do.

Our next talk is going to be by Dr. Albert Donnenberg, who's from the University of Pittsburgh Medical Center.

DR. DONNENBERG: Let's work offline. Well, this story begins a little more than two years ago, when in trying to secure a Shared Instrumentation Grant for the University of Pittsburgh, we recognized the need for biocontainment, particularly to protect the operator from potentially biohazardous cells that were being sorted.

As we formulated this grant, we have as a primary -- we had that as a primary objective, and as a secondary objective, to protect product from the laboratory environment, with an eye to the fact that an instrument like this, or along these lines, could conceivably be used for manufacturing of cellular products as well.

However, the purpose of this initial development was in an open -- was to develop a machine that could be used in an open laboratory environment, to sort potentially biohazardous cellular products.

After discussing this very briefly with the engineers at Cytomation, who assured me, of course, this could be done, we applied for the grant successfully and we started with initial ideas, that we would have a dual-containment approach, based on primary containment, using the already existing Cytomation aerosol evacuation system, and then a secondary level of containment, a fail safe, if you will, level of containment, and at the time we were thinking along the lines of a biosafety cabinet. And, as you'll see, that idea has evolved.

The third important component of this is that in the event of an aerosol, we would have an automated decontamination procedure that would prevent the operator from exposure to aerosolized material.

So the features then, according to the design specifications, primary and secondary levels of containment, continuous sort monitoring and this is done with the Sort Master, as Matt Ottenberg has described, correction of droplet break off during sorting, protection of disruptions due to nozzle clogs, which would be indicative of an aerosol condition.

Remote control of the sample. A sample station would be necessary so that we could access all of the bottoms, the analyzed standby and all of the de-clog options when this containment was closed. HEPA-filtered air in and out, audible and visible alarms and real time monitoring of air pressure temperature, humidity, et cetera. And as I mentioned the automated formaldehyde decontamination.

This is a view of our MoFlo. And as Dr. van den Engh has designed it in it's modular fashion, the sample station, the illumination table and the aerosol evacuation system, these are components that come in contact with sample, can all be isolated physically from the rest of the instrument. And this just shows that in schematic.

So we have the primary containment zone, which is the aerosol evacuation system, and the secondary containment zone, which is those three components that I mentioned.

This is -- if I can get it to work - this is a little animation. No, we went right past it. That would have been a little animation of -- it's not behaving. Excuse me.

It would have been an animation showing you a rotation of the cabinet design so that we could point out some features, but we can see it from these snapshots. Some of these you saw in Matt Ottenberg's talk.

There is a sash that maintains a face velocity, like a chemical fume hood when the sash is -the sash can either be open in a position where you can access the AES or flows.

Not shown there's a hatchback door that will lockout entirely when this unit is in operation. And you could barely see through the window where there would be -- would there will be a hot plate for vaporizing formaldehyde.

There are access panels that come off for servicing and there are quartz windows which allow for the laser light to enter and for the reflective light to be measured by the PMT's.

There's another view where you can see the illumination table and the sample station. Yet, another view giving you some idea of the scale.

This gives you an idea of the size. This is on the optical bench, such as the one that we have in our laboratory.

So I'm going to concentrate -- now that you've seen pictures of what the prototype looks like, I'm going to concentrate on how we picture that it will be used. Of course, actually, a large part of the design specification was deciding just how this would function in operation.

So we have a set-up mode. And that's the default mode when we power up the cytometer. And this allows for calibration, for sorting of feeds of beads and other non-hazardous materials and for the introduction of hazardous samples into the sample station.

So the status of the instrument, the cabinet blower. The cabinet is that large container. The main door is open, the sash is unlocked, which gives you a face velocity into the cabinet, .5 meters per second, which is typical of a chemical.

The sample station controls our accuracy. You can push the buttons. The aerosol evacuation system is active, and the visible alarm, this yellow thing that you are in set-up mode. You can sort in this mode.

For normal operation, however, this is when we're sorting potentially hazardous materials, the cabinet blower's on low, the main door, the hatchback door that you didn't see is locked so the air that's coming into the unit is HEPA filtered.

The sash is closed.

There is remote control of the sample station, probably by a GUI, although it could be by a mechanical remote control. The aerosol evacuation system is active. Sort master is active.

And this is important, because the sort master is not only keeping our drop delay on time, but it's also monitoring for deviations in droplet formation or for disappearance of the stream. Sorting is enabled and the visible alarm says things are green.

Recoverable failure. This mode is entered if the sort master detects persistent fluidics failure. For example, a change in drop position beyond predetermined parameters, or if the sort master detects that the stream has disappeared entirely.

So these two different conditions have slightly different affects.

In the event of an unstable drop position, the cabinet blower remains low. And if the stream disappears, we are assuming that we have an aerosol, so the cabinet blower goes on high, the main door it locked, of course, HEPA air is in, the sash is closed.

The sample station goes into the standby mode. So that means that we're no longer directing sample through the sorter and the de-clog options are available by remote control.

So the operator has all of the usual options that he or she would have in de-clogging, short of reaching into the cabinet and touching the nozzle.

The aerosol evacuation system, of course, is active and the sort master is off until a drop a delay has been controlled, visible alarm is on and red and importantly, the operator can revert to normal operating mode if the proper drop off -- droplet break off is established.

So the operator would have as much time as patience permits to try and get rid of the clog, to get the stream stabilized and to go back into the normal operating mode.

In the event that that can be done, then there's a mode called unrecoverable failure. And this would happen if the operator -- with operator input after having failed to reestablish normal operating mode, it would happen if there was a blower failure, we're relying on the blower.

And we would also boot into the unrecoverable failure mode if the system crashing or if power was lost during the sort.

So this is important because we have to take into account the possibility that the computer controlling all of this could go down.

So the status in unrecoverable mode, cabinet blower is on high, we're locked out, sample is in standby mode, visible alarm, and the only way to get out of the unrecoverable failure mode is through the decontamination mode.

In this case, the damper shuts, which isolates the cabinet from the environment. The cabinet blower is off, the main door is locked, the sash is locked. The sample is in the standby mode, visible alarm and we go through a decontamination sequence which consists of powering up the formaldehyde

hotplate after an interval power to the recirculating activated charcoal filter that Pat mentioned.

Then after formaldehyde was removed, damper open, blower on high and return to the set-up mode.

So that is how we're envisioning now in our specifications that this sort of a secondary containment cabinet can be used. I'll stop there with credit to Edwin Kennah, who is our biosafety officer at the University of Pittsburgh who participated in developing these facts, and that and Kris Buchanan and Ben who are here. Ben is here.

And to Ger van den Engh who was instrumental not just in the design of the instrument, but also sitting down at the first design meeting and helping go through these specs and making sure that we had something that was workable. I'll stop there. Any questions? Gerry.

DR. MARTI: What's the abbreviation IT?

DR. DONNENBERG: Illumination table. It's that central area. This is Cytomation speak.

DR. MARTI: I knew I'd heard it before.

DR. DONNENBERG: Any questions? Thank you.

DR. KEANE-MOORE: Our last speaker before the break is Jim Houston from St. Jude's Children's Hospital.

MR. HOUSTON: (Inaudible, speaking from an unmiked location.) Two major concerns that I use for cleaning. Two things. Patient to patient, which is ultimate and the most important, and which really causes me more problems as far as cleaning the instrument, than the system to patient.

Several points to consider when we're doing this. Sterility -- I put in quotations there, "clean environment," which means you want to keep the room clean at all times.

On instrument usage, what are we going to use it for? On the sterile clean environment, you use sterile procedures all the times. I use 70 percent ethanol usage on the lines just like Lillia had prescribed earlier.

I use ten percent bleach on just the surfaces. I do not use ten percent bleach in my system. I have found personally, on the research side that residual ten percent bleach, even after copious amounts of flushing still leaves enough that investigators that I had used it on before were complaining that cells after three to four months were not performing as well as those that I did with no bleach.

So there's still -- to me, there's still some problems with that on there, and we'll go over how we get by that.

And the use of a sterile hood. By all means, most everything I do, when I put the components together it goes on in a sterile hood. For sterile procedures, I use personal gear, just like it's been show here. Today I use gloves. Sterile -- not completely all the time sterile.

We have to remember that once you put on sterile gloves, as soon as you touch your keyboards you're no longer sterile. Anytime you touch anything else, you're no longer sterile gloves.

So I find it, to me, personally, trying to don sterile gloves out of a container, there's a difficulty in room, how you lay it out, how you get them on. It takes too much time.

It's easier for me to pull them out of the box, put them on, wipe off real quick with ethanol and go about my work. Obviously, a mask, hair covering, disposal lab coat and shoe coverings in our facility.

One other thing I want to mention about this personal gear here is if you have an operator who has a bad sinus infection, even this will not help you.

There's been several instances of people saying they only have one operator that does a good job. You need two operators that do good jobs. Not all the time you want that one operator in that room. If there's problems healthwise with that person, you do not want him in that room.

I use 70 percent ethanol just for general cleaning of the components and of the area, just like Lillia had describe earlier. Ten percent bleach, not to be used in areas that come in contact with sample.

Like I said, there's still some problems with residual contact with sample and all the bleach that's not there. I definitely use bleach on the work areas and the sorter waste disposal areas.

And sterile hood cleaning of components that are used and are installed. I definitely make sure all this is done in the sterile hood right outside my flow room.

Instrument usage, only for human material. Our sorter has never seen a mouse cell, never will and never will. Okay. We don't want any of that near the lab. It doesn't even come in the facility. It's actually not even allowed in the buildings where we're at.

Samples must be clean. Samples that are used on our sorter are in lab materials only. We will make some exceptions to any material that has left our facility, has gone back out, and then wants to come back in. Those samples have to be verified by QA as being tested clean. This is to make sure that our facility stays clean all the time.

Static instrument, the shut down is important. It's like which comes first, the chicken or the egg? If your system is not shut down properly and cleaned and left dirty, when you get ready to start it up, you got to clean out the dirt. So this system is cleaned both before and after use.

Replaceable components. I do a lot of replacement, which we'll see in just a minute. Sample lines, sheath lines, o-rings, tubing connectors. All these are replaceable components.

Non-replaceable components on the BD Vantage SE, the sort head, if you want to pay the price, you can replace that component if you'd like to. BD would be glad.

The sample insertion rod is not -- it can also be a replaceable item. It's not a very expensive item, but the wear and tear on the sort head, getting it out and the operator having to realign that particular rod in that system, more than likely, after about three tries you'll probably damage the sort head and will need a new one.

The sort nozzle that I use, when I'm done sorting, it comes off. It's cleaned with ethanol, stored in a 50 mil tube that also has ethanol in it. The sheath solenoid we'll get to in just a second.

Starting point. This is where I start. Inside this bag I have every piece of replaceable tubing, connectors, o-rings, valves. Everything is inside this bag that goes on that system.

I put these little kits together so I don't have to try to locate everything when I need it. So this is where I start it.

There's some modifications that I've done on my system. This is the original system over here. The problems I've had with contamination on these systems is that this has water lines. These two black lines here are your coolant lines, which come into a BD cooling device which slips over this tube and seals itself on this o-ring.

Underneath here, for those who don't know -- they actually had these sorters -- don't realize there are a set of o-rings up underneath this material right here. When you slide this bar back and forth, those o-rings slide

back and forth as well. That's what keeps your water from getting -- closes your water off to here and also always your pressure to your sample.

These have been a source of probable contamination. This is not a very easy thing to take off and clean and put back together.

What I have done is modified this a little bit, and notice I've taken off the black tubes that provide water. These are now gone. Also, the connectors on top are now gone, and there's -- no longer connected to here at all for the line for the sample pressure.

This is straight through with a one-way valve inserted in between -- cannot get any backflow of the pressure down this tube, which can happen.

Samples -- the sample insertion to the sort head, I will take all this tubing from right here, all these tubes are replaced. They are not cleaned, they are replaced. These all come off, they're disposed of and replaced with tubing from that bag I just showed you.

This is a little bit closer view. You take the tip off and you look, there's a small o-ring inserted right here. This o-ring is also taken off and thrown out. These are very simple devices to take off and put back on. They are very cheap. That whole bag

-- probably the total cost of that bag -- I haven't exactly figured, that was probably less than a hundred dollars to replace all these tubings and connections which I'm replacing here.

So expense-wise, for the materials it's fairly low. Time is another matter.

This is a shot of the cellanoid area behind the cage here on the Vantage SE. There are two cellanoids here that I am concerned about. One is it controls the sheath fluid from your sheath tank to your nozzle head, and the

other controls your waste. When you hit the purge button on your system, it opens up a waste valve to your waste tank. This is the next one that I'm concerned about.

A little bit closer view. I have actually moved one of these valves from the position two to the position one right here. It makes it a little easier for me to get the tubings on and off. The other one is this nozzle flush, which is actually a waste button -- the waste cellanoid right here.

There's the cellanoid taken out. There's also quick disconnect lines put on these so I can actually take them on and off the machine at will. You take this cellanoid apart and it looks like this. You've got lots of components in there.

You can take these apart. Currently, I take these apart and I actually clean these with ethanol and it's stored in ethanol until it's reused again, except for, obviously, the electronic component cannot be stored in ethanol. This is teflon inside here. Very easy to clean with ethanol.

So all this is basically clean and kept clean until it's reused again. We're currently trying to actually find the complete replacement and replace this as a component from the manufacturer.

We switched from the BD cooling system to a Cytech cooler. Right here, this whole system will come apart. You can clean the entire apparatus as it sits. You can just take it apart, screws all around, all these o-rings, take them out, cool them, clean them, put them back in. All samples that we have -- we put on the sorter are kept at four degrees.

This is my sheath tank. I autoclave my sheath tank. You can see the residual autoclave tape all over my sheath tank right here.

One comment, in respect for Lillia and what she had said. I do autoclave my sheath tank. I no longer have the tube in there which gives me

a sheath level. That sheath level is prone to problems. I don't think that BD would like you to autoclave it. I take that out.

You can put the polypropylene quick disconnects on this system and autoclave it, but you have to beware that the expansion rate between the two are different when they cool, so you have to make sure you re-tighten up all your connectors before you put them on, before you put fluid across them.

Right now I don't put anything -- the only thing that's autoclaved on this tank is the tank itself. There are no connectors on it. And one of the reasons why, what was pointed out earlier is, you validate your sterilization techniques.

If all of your closing -- all of your openings are closed when you sterilize it, you're not probably going to get the inside sterilized at all. It's just not going to heat up and cool down as much as sterilizer is.

There are -- all the openings are covered with aluminum foil and then taped down with autoclave tape. That's the way it goes in.

Now, on this tank I used a filter from -- ordered from Fisher. It's a power filter. It comes in as sterile in its own individual wrapper. You take it out of the wrapper. This is all done in the hood. This whole tank and everything is set in the hood and that's why I take care of putting everything on.

This is taken into the hood, taken out of the wrapper and this bottom connector is taken off, Teflon is wrapped around this, and then this is screw onto the tank right here. So it's a good tight fitting.

The tank I run -- my tank sits in front of me. Also it sits on a scale. I tare the scale before I put it on, and then I can tell by the weight of the tank after it's been filled with about several liters of PBS, I can tell exactly what my fluid level is.

A quick overview. Use sterile handling techniques. I use gloves when I do material on my sorter. I use gloves, I use hat, gowns, everything I can think of to keep me from contaminating it.

Over the past 20 years of doing flow cytometry and sorting, I still believe that most -- probably 99 percent of the contamination that's handed to a sample is from the operator himself, and not from the environment or the sorter itself.

So it's very important that your people practice the sterile handling techniques and be well gowned and covered up.

Replace all components that are replaceable. Obviously, we would like to throw our whole pathway out and stick a new on in, but at this present time on our instruments, we're not able to do that.

We do, basically, clean what we can, replace what we can. I clean with ethanol and air dry remaining components.

I was told many years ago when I did research work and had problems with contamination, I was told by infectious disease people -- and somebody else here may have a different opinion on this -- that it's not per se the ethanol itself that actually kills the organism, it's the drying effect that the ethanol does once it's dried out that actually does most of the killing itself on the organisms. So when I put ethanol in the system, and it is nitrogen filtered and nitrogen dried.

I flush the system after I put the filter on with about half a liter of a sterile Dulbecco's PBS in order to flush the filter out in the system.

And above all, I just keep it clean.

That's all I have. Any questions? No questions. Excellent. Is it break time?

DR. KEANE-MOORE: Let's break for about 20 minutes and come back.

(Whereupon, the meeting went off the record at 3:26 p.m. and went back on the record at 3:54 p.m.)

DR. MARTI: Thank you for coming back into the room. We would like to get the panel session started. And, for the record, starting at that end of the panel, I would like each of you to state your name and your affiliation for the record and for the transcription, starting with --

MR. CHRISTIAN: Todd Christian, BD Biosciences.

DR. MANDY: Frank Mandy, Health Canada.

MR. LAMB: Larry Lamb, University of South Carolina, Department of Pediatrics, Pediatric Hem-Onc.

MS. SCHMID: Ingrid Schmid, UCLA.

MR. PERFETTO: Steve Perfetto, NIH, the Vaccine Research Center.

MR. CODER: Dave Coder, University of Washington. Well, I guess, more appropriately, ISAC Biosafety.

MR. OTTENBERG: Matt Ottenberg, Cytomation, Inc.

MR. HOWES: Grant Howes, Beckman Coulter.

MS. SHAPIRO: Margie Shapiro, Division of Monoclonal Antibodies at CBER.

DR. KEANE-MOORE: Michele Keane-Moore, Division of Cell and Gene Therapy.

MS. CLARKE: Jane Clarke, National Institute of Aging.

DR. MARTI: Thank you. Now before turning the panel over to you and the audience to the panel, I would like Kevin Holmes to make a few comments about the experience in his laboratory, and that will then be

followed by a comment by Marjorie Shapiro. Kevin, please? You can just come up here.

MR. HOLMES: Nothing too lengthy here. I just wanted to comment on some of the efforts. We've worked together with BD in their biohazard containment unit for the FACS Vantage that you saw. He mentioned it earlier. There was a couple of presentations about it earlier.

There's been some delays in getting the biohazard containment unit out into the field. We're now beta-testing three biohazard containment units on two FACS Vantages and a FACS Star Plus.

The system, as Todd described it, consists of both an aerosol evaluation system, where you have a vacuum pump that is attached to the sorting chamber, as well a droplet containment system.

And for of you those familiar with the FACS Caliber droplet containment system, it's very similar to that in that you take the tube off, any backflow out of this sample tube -- the same tube is prevented by an outer droplet containment tube sucking it up.

That's been the real issue that BD initially struggled with, was that sample area containment and that's where they had the problem with. That, to my satisfaction, has been fixed.

In other words, you don't have any issues of your sample when you take the tube off now dripping out onto the sample area, which is a major -- which, I think, if you read the papers on -- some of which were handed out here, that's where most of the contamination occurs is in the sample area, as opposed to closed chamber. So that's a very important step forward, as far as the BD aerosol containment system.

I guess some other issues, it's a very robust vacuum pump system that they have on this system. It's a separate actual pump, as opposed to

going in-house vacuum. That keeps it separate entirely from any house vacuum system, and they have a very large HEPA filter system to isolate any airborne contaminants from being evacuated into the air.

I can't state the statistics. I don't know if Todd knows. There has been a lot of effort put into how much CFM's of evacuation from the sort chamber that this system will handle, such that in the event of a clog, et cetera, you can calculate how long, essentially, after shutting off everything you should wait until you open the sample door. And I think it amounts to two to three minutes depending upon the CFM draw.

And, I guess, in terms of practical experience, one of the questions that we're working with BD right now on is the HEPA filter system and exactly how many hours of use that this HEPA filter system can be utilized, and how often you have to exchange them.

There was an initial figure of only 80 hours, and I know I just talked to Todd and he's working on trying to determine if that's a hard number. 80 hours is not very long, if you think about sorting either hours a day or so. You'd have to exchange these filters quite often. So we're working to see if that's a real number or not.

I guess the last comment on the system is that from the evacuation chamber in the sort sample station, the tubing goes down into the stainless steel tank before it gets to the HEPA filter and that acts

-- similar to, you know, an Erlenmeyer flask set up, where you could theoretically bubble your aerosols through bleach or something.

And the comment there I would have is that with the amount of CFM's that these vacuum pumps draw, the bleach evaporates by the time you finish your sort.

So, I was talking to Steve earlier. Maybe we could go do some betadine set up, as opposed to bleach. So, anyway, that's basically our experience on the BD biohazard system.

DR. MARTI: (Inaudible, speaking from an unmiked location.)

MR. HOLMES: Cubic feet per minute.

DR. MARTI: If there aren't any questions, then let's go on.

MS. SHAPIRO: I'm not here to talk about flow, but I'm from the Division of Monoclonal Antibodies. So I'm going to talk about the reagents that Dr. Gee so wonderfully introduced in his talk.

If you submit an IND or IDE to CBER for a gene or cell therapy protocol, that would go to the Division of Cell and Gene Therapies. But if it uses a monoclonal antibody, they would come to us and request a consult review.

So the types of things that we look for in the antibodies, I suppose the bad news is that we would want the antibodies to be purified, following the same guidelines that we would look if the antibody was the therapeutic itself.

Dr. Gee referred to two guidance documents. One was the 1997 points to consider document for the manufacturing and testing of monoclonal antibody parts for human use. And I have about 20 copies of it here for people who are interested.

That would be the document that we would use more than the other one that was put out as monoclonals for use to make immunoaffinity column for purifying other things.

However, when this document came out in 1997, it was well-received by the industry, in that we did have a couple of innovative ideas, so things aren't always as difficult as perhaps they used to be.

One of the improvements was if your clinical protocol is for a serious of life-threatening use, there's minimal testing that we would require a phase one -- for a phase one trial.

However, the product reviewers in the Division of Monoclonal Antibodies don't decide if your protocol is serious or life threatening. We would, obviously, consult with the clinical reviewer on that.

In their view, it may not always be the same as your view as to what is serious and threatening. For example, if some protocol comes in to treat and underlying condition of a serious of life-threatening disease, then that wouldn't be considered -- that underlying condition would not meet that criteria.

The other good news would be if you want to make a lot of different monoclonal antibodies, some to purity T-cells or some for B cells, or stem cells or whatever, if they all come from the same cell -- if they're all from SB20 cells, and they're all murine, not a mix of murine or humanize, and they're all of different subclasses, the different subclasses, and you purify them the same way, your virus of validation studies, your virus clearance studies that would be required, you only need to do that once.

We have a generic or modular system now where -- because all antibodies -- if you harvest them the same way, if you put them -- purify them in the same way over a protein A column, anion exchange or whatever, then the viral clearance that you get with one antibody should pertain to the others.

Now maybe many of you aren't making your own monoclonal antibodies, but you would still be responsible for knowing how the

antibodies that you might use in your protocol are made, if they meet those requirements.

We not want to see any monoclonal antibodies that are produced for research use only. The azide is a major concern.

So if you don't make them yourself, one thing to do would possibly be to find an industry sponsor that has already made monoclonal antibodies specific for your antigen and perhaps strike a deal with them.

And even if at that point in your own lab or some contract manufacturer, you got them to label it with a fluorochrome for you, at least the purification of the antibody itself has gone through the rigorous safety testing for the adventitious virus and we've asked them all the questions that we typically worry about.

And at that level, that wouldn't be a concern of yours. But what you would still have to do for the end product is show sterility, the bacteria, fungi, microplasma and things like that.

So it's a little bit difficult, because many of you out there probably aren't thinking of making the monoclonal itself, but it may come down to that because it wouldn't be allowed to just go and buy something that's for reagent grade. I think that's enough for now. I don't know if anybody has any questions.

DR. MARTI: (Inaudible, speaking from an unmiked location.)

MR. CODER: Great. As I mentioned -- it seems like many, many hours ago -- that the end point of this whole exercise was eventually to -- is this microphone on? I'm just not getting any feedback here.

The whole point of this exercise today is to get feedback and solicit comments and raise new questions from everybody in the field who is

doing this kind of research, such that this group of people assembled here at the table and in the chairs in front of me eventually is going to put together a document that proposes the best way to approach keeping your instrument clean and making sure that the product coming out the other end is the one that you think it is and meets all the criteria.

So the panel itself is -- I think, in some ways good that it's grown to a larger size because what that means to me is that we can probably partition it into subareas to deal with a fairly broad range of fairly technical topics that we're going to have to investigate, and put together in order to formulate what these protocols are going to be.

So just some of the areas that seemed obvious. Well, let's see. Bob Sausville is no longer here, but there is a whole area of sterilization -- Marjorie just brought up one of the issues as far as reagents go.

From the biological standpoint we've dealt with some aspects of other reagents, of having pyrogen free sheaths, for example, is going to be one of the requirements. Not have animal proteins floating around in the various preparation mixes, a variety of things like that. All of those are going to have to be addressed, as well as in the specific instrument sterilization protocols.

Since we have three different manufacturers report, depending on how you want to count them -- I guess I should put as a note to transcriptionist for the panel members, Ger van den Engh is also a member of that panel, but he had to leave early. So I'm passing on that footnote.

I just lost the train of thought that I was talking. Ah, yes. The variety of instruments and the fourth instrument, or the person who wasn't here, constitutes completely different designs, and each of which will have their own probable best ways to sterilize them and keep them sterile during the process.

So I think it sort of suggests that it will be incumbent in some ways to find the individual manufacturers, once we've identified what the criteria are that we need as far as keeping things -- well, starting with sterile system, keeping it clean, frequency of monitoring all of those other issues, for the individual manufacturers then to propose what is the most effective way, in their opinion, with presumably some sort of demonstration, that that's the best way to proceed.

The other thought that came to mind, seeing this increasingly large, growing body of existing regulations, and you look at one and you find, oh, there's a whole bunch more of them.

But I think in some ways that's good, in that we don't have to reinvent the wheel. There's probably a lot of pain and suffering that's gone on to formulate some of these existing regulations, and there's no reason in the world why we should not see what those are and try to use them as a quick way to arrive at a solution.

So that then brings up an issue of trying to tap the person who has the most expertise dealing with the regulations and see -- there are a number of people who work in this very, very place. Things that we can sort of divide up later one. But that's one obvious place.

I think we've identified a number of individuals who can tell us about sterilization methods, and procedures, and so on, the regulations, certainly.

There's another issue that has come up that we really have sorted batted around without must resolution, and that was from the protection issue of the operator in a general environment, where we have identified at least some cases of where cells may well be sorted from an individual who has a known disease, or several identifiable diseases.

And so given the fact that you'll be forming aerosols of some potential pathogen, what is the acceptable limits for that minimal infective dosages? What's the best way to treat that?

So we need to find someone who has expertise as far as infectious disease goes and, specifically, modes of transfer.

And it seems like someone at the CDC -- we tried to get someone here to sort of represent them, and we couldn't shoe horn them all in at this point, but we should be able to identify someone from that area. Gerry? Grab a microphone.

DR. MARTI: I think that we have a resource available to us. I'm not trying to put down our colleagues at the CDC, but something that has emerged in these discussions is that where the various institutions have been serious about undertaking this endeavor, you find out that their infectious disease -- they have an infectious disease consultant on their team.

And Jeff Miller, I don't know if he's still here, his laboratory is working with the sorting of cells infected with malaria. And the question that he raised is do we actually have any evidence of operators being infected by aerosols?

And I'm not -- I'm not aware of any data. That doesn't mean that it doesn't happen. There was a very famous case of an aerosol here at the NIH a few years ago, HIV. Something like a 200X concentrate in a centrifuge.

And it may have even been involved -- there may have actually even been a puncture involved with that person.

But I am not aware of -- I'm aware of people in hematology and chemistry who handle --literally stand in blood up to their knees or their elbows all day long. It's not uncommon for those individuals to become sera-positive for hepatitis B or hepatitis C. It's unfortunate, but it happens.

But in terms of aerosols, or even any other serological conversions, we probably don't have procedures in place in flow labs to even detect a serological conversion would be my guess. But that would be my first comment.

MR. CODER: Well, specific to that comment, I've had a number of people in my laboratory working with various microbacteria. And one of the things that in the absence of specific guidance or anything else, I suggested to them before they embark in this work that they might want to have PPD before, such that if they are in a condition where they're exposed to an active case of tuberculosis and they show that they're sera-positive, so when did that happen?

And so that might be -- I guess, I'm sort of -- that's a question to go out. Does anyone know are there specific requirements for people having particular kinds of testing prior to working with infectious agents like that, and should that become part of the protocol that we have of the operators. Yes. Jane?

MS. CLARKE: I know at the NIA there is a -- actually, two levels of -- one, is specifically for office workers and the other is for people who have the potential for exposure. And that would be anybody actually in the lab. Those people are required to have a PPD test and take a follow up.

And, currently, they're just using -- (Inaudible, speaking from an unmiked location.)

MR. CODER: Well, what about other things as well as say, is it recommended that people working with blood products have hepatitis B vaccination, for example?

MS. CLARKE: Well, legally, according to the OSHA regs, you are required to offer hepatitis B vaccine within ten days of the start of

employment for any employee who has the potential. They're not required to take it.

It's also generally recommended if they decline it, that the sign a declamation form. But I'm happy to say almost everybody has already had it when they come through the NIA.

MR. CODER: Yes. Ingrid.

MS. SCHMID: We at UCLA have been working with HIV since 1983, and it was laboratory practice that anybody who started in the laboratory was drawn and was HIV tested, the results in private. But anybody would have a serum sample available so in case there was exposure, you could take that sample into the -- and you could find out if that --

It was not -- you could decline it. It was just recommended. It was offered to anybody to go through that testing.

DR. MARTI: (Inaudible, speaking from an unmiked location.) I think that probably the most important thing would be that we start thinking about the flow operator much the same way that we think about a hospital employee coming in contact with patient samples. Blood or otherwise. That probably would not be unrealistic.

MR. LAMB: David, I believe the College of American Pathologists also has regs for all their laboratory personnel as well. And just using good, sense if you -- to follow those regulations, even if you're in a non-clinical setting and don't require inspections, or that sort of thing would go a long way towards monitoring the incidence of these problems and helping people out who might be in contact with these agents. That was the College of American Pathologists.

MR. CODER: Yes. Frank.

DR. MANDY: Frank Mandy. In Health Canada, those who work with infectious diseases, it's condition of employment to receive protection against hepatitis B and also it's compulsory to give a blood sample where the serum is frozen for the very reason that Ingrid pointed out. If there are complications, then we can establish if they were exposed to that particular pathogen prior to employment.

MR. CODER: I guess I'll say just to sort of extent this out, there is a hepatitis C vaccine that's pretty much ready to go at this point, or not yet? No. Where did that come from?

DR. MANDY: Wishful thinking.

MR. LAMB: There was one other thing. Something that struck me in this entire day today of conversation that I think are broad brush things that we need to consider when we're talking about machines and sterilization, that sort of things, is we're not all playing on the same level of field.

We heard from Dr. Wagner's group, from my adoptive State of South Carolina, who is preparing cells for vaccine into immunocompetent individuals and doing subcutaneous injections.

And we heard from my colleague, Dr. Frits van Rhee, who is preparing CMV-specific lymphocytes for haplo-identical transplant patients who are heavily immunosuppressed and could be susceptible to just about any type of containment. And I think there's a wide variety in between.

And I think you can ask several questions. Do we need a uniform protocol for the entire spectrum? And second is, one of the things that I swore I was going to bring up today, was how we take care of the instrument when it breaks, the service that comes from the company within.

In some situations, when you're working with ablated patients and you're sorting a graft, you have to have an instrument that's going to be there. As a matter of fact, it's probably better to have two.

Or you have somebody getting on an airplane who's going to be there in an hour, which is kind of hard to do as well.

But in situations where you're sorting cells for vaccine, if your instrument goes down for a couple of days, you can just postpone the procedure and then move forward post haste. But a 12-hour shutdown's not going to kill you.

And there's a lot -- again, a lot in between. We're working at different levels and I think the requirements and the records should reflect that.

MR. CODER: I'm glad you brought up that point, because one of the things I had sort of thought about initially , as far as the -- well, some of the statements I had brought up earlier from the GTP, quotations about trying not to make excessively burdensome regulations, I think it probably does have to be something to reflect what the actual needs are.

And we don't want to make things that that so onerous, and so difficult, and so expensive, that people aren't going to do it.

And, also, thinking farther down the line, if these are really going to be clinical procedures that are successful, eventually, some insurance company is going to pay for it.

And if the things are so incredibly expensive to do, nobody's ever going to pay for it and, therefore, it's not going to happen.

So we have to have something that's going to be reasonable, as far as getting the job done safely and effectively but, again, I completely agree with you that sorting in somebody that has no -- or in grafting back into

someone with no immune system is a very different context than somebody that has some degree of protection against pathogens.

And I don't know, there's likely -- somebody has already answered this question, somewhere I would think. And so where do we find that information, would be one of the questions.

DR. MARTI: I think what Larry has is really two major questions here. The variation in patient population, or heterogeneity of patient population into which a biological product goes, that's a problem that's faced at the FDA all the time. I mean some investigators want to treat everybody with every known cancer. And some will say, well, just solid tumors. And some will just say hematological malignancies. And now Larry's breaking them down into immunocompetent versus incompetent.

I think at this point in the history of this field, I would not give too much concern to the actual clinical protocol. I would still concentrate on integrity of the product that's going to be given to the patient.

And from an FDA standpoint, we would say that those products should be the same. There shouldn't be any difference in those two products, if it's going into the different patient -- I mean as a rule of thumb.

Now this other issue of service, availability of service, I'm thinking of Carlton Stewart's laboratory at Roswell Park Cancer Institute in Buffalo.

He has an engineer on site all day long, five days a week, and he doesn't do any clinical sorting for clinical use. His is a laboratory that does 100 or 150 samples a week for analysis. But he feels it necessary in that setting to have his own engineer.

I don't think it's unreasonable to think about that at this point in time. However, I realize it's unrealistic to think that you would have to add

the equivalent of one FTE for an engineer for every site where you were doing sorting.

But I think if there is an area where there is a conglomeration or a congestion of these instruments, then that might not -- that might be a way to work -- but I don't think that should be something for a regulator agency to concern itself with, or perhaps even this committee.

But it's certainly worth discussing and hope that some agreement can be made between the manufacturer and individual labs. That would be my thought on the subject.

MR. OTTENBERG: If I may, one other consideration is the level of redundancy, and conceivably in the worst case we said you could have another instrument. Something tells me somewhere between that and single instrument with an on-site service guy is reality.

I was wondering, is there similar instrumentation that we can use as a baseline to try and define some of these things and what level of redundancy is practical?

MR. HOUSTON: Dave, can I speak over here?

MR. CODER: Yes.

MR. HOUSTON: I'd like to speak on the problems of technical failures on the instrumentation while you're sorting please.

I'm Jim Houston from St. Jude's Children Research Hospital. On this particular topic, I think one of the key involvements on this is not particularly per se the company and their response time to get you going, because in reality, you can't wait an awful lot of time when you have cells on ice that need to be used, and sorted, and put back either in a freezer or in a patient. You can't wait 12 hours. You can't wait five or six hours.

Redundant systems are grade and fine. A whole total redundant system sitting there is an awful expense. It takes up space. A lot of institutions have a hard enough time justifying one, nevertheless two.

One of the key components to making sure this is taken of, is having an operator that is well versed in that machine, not in just running a flow cytometer, as far as putting samples on and off and doing analysis, but that a person probably needs to be trained as well on some the electronic components of the system as well.

I personally have been in flow cytometry for 20 years, back in the days when we did a lot more mechanical stuff that what we do now.

I have the ability to do a lot more just because I've been in it a long time, to do a lot more with lasers, jumping pins, jumping wires, using paper clips, whatever it takes to make a flow cytometer run, mostly because I've been doing it a long time.

We have a lot operators out there now that are getting into this field that have only been doing it for a year or two, and to expect them to able to pull a card out and jump over a wire, or troubleshoot a laser by pulling the head off is nothing you want them to do, particularly if they have not been trained.

So part of the issue, I think, on clinical sorting is this operator issue. You definitely need a good, qualified operator who has been trained in more than just basic flow cytometry. They need to be trained with a little bit of mechanical aptitude to pull a card out and replace it.

We at St. Jude, we keep a redundant system that's in use in the research tower, that's an exact duplicate of what we have in the clinical realm, to the tee.

They have a little bit extra that we don't particularly need, but we have already stated -- and the problem -- if we have a problem with one of our boards, we will shut that research instrument down and take a board out of their machine, clean it up, put in ours and move on.

But in that case though, we have a machine that's sitting over that's being used in the research field. So I think that the board does definitely address probably the possibility of something about operator competency, as far as being able to operate

a clinical machine in this realm.

MR. CODER: Yes. That sort of brings up a couple of issues, especially in the diversity in the types of instruments that are out there, and the particular problems that each of them is going to have.

And so having some sort of requirements as far as the operator, as part of the specification for what is required in laboratories is probably a real-world phenomenon. Exactly how far that goes, is something I guess that we'll all be determining. Yes. Frank.

DR. MANDY: Well, I think you could summarize this whole issue as a question of resources, that if you're going to go into this field, you have to take into account the cost of doing business, and I think you can leave it at that.

Because you can go further if you have P3 or class 3 facility. Then you have duplicate all the instrumentation, the service instrumentation that you're going to have, because you can't have someone fly in, and then wait until everything is autoclaved, and then give him a big pile of plastic instead of instruments back to him. So I think it becomes just to resource management and a cost issue of doing business.

The second comment I have is that this afternoon we heard of a variety of ways of recovering cells, anywhere from a fairly open system to double protected systems.

And what I would suggest is that the speakers we have this afternoon, to go after them and see if they've published their method, because nothing like evidence-based conclusions.

MR. CODER: That's exactly the case. I didn't -- is Adrian Gee still here or did he have to -- he had to leave. Okay. Because he had a fairly extensive list of things and also -- well, for starting to get into the issue not only of sterilization, but also evaluation of product quality. Because he had made the one observation that they went from a 60 psi to 30 psi. He didn't say which cell type and he also didn't say what criteria were they using to evaluate loss of function at higher pressure. But it seems that they've worked with it fairly extensively.

MR. LAMB: There's more think I wanted to say and then I promise I'll just shut up. But I think that one of the things that has been very helpful to me in making these decisions, to take myself out of the role of lab chief, or investigator, or whatever, and put myself someone in the role -- after being in pediatric transplantation since 1991 of being a parent of somebody who's receiving a graft.

Now that misfortune hasn't happened to me, but if you get into that mindset and you think what all you would like to see done if you were putting a member of your family in this situation, I think that your thoughts clarify a lot more.

Ingrid, I remember you had sent me a summary, I think of the protocol. At least it came from your lab.

And Larry's point that he just brought up, of the patient scenario, of thinking like the patient was one thing I recall in that particular protocol. Was I correct?

MS. SCHMID: I'm not sure. From the patient's standpoint?

MR. CODER: Yes. And I was wondering if that had been worked out in any further detail?

MS. SCHMID: Not to that point. There are at UCLA trials currently going on where patients are receiving products in immunology that are processed in laboratories. And there are suites there where these cells are processed.

And the concern is that these cells are handled -- you know, like there were issues because they're building a GMP suite, and they have, for instance, students working on these products.

Is it permissible that you have personnel of that kind -- would you wish if you would be receiving a product like that, somebody that is temporarily in a situation, hasn't been -- has been trained, but hasn't been trained well, somebody who's not invested in that so much handling that product and getting it back into the patient?

MR. CODER: Yes, you do have something to say about that.

MS. CLARKE: I come from a clinical background at the (Inaudible, speaking from an unmiked location.) And just have been in research --

MR. CODER: I don't think that microphone is working.

MS. CLARKE: I come from a clinical background at the University of Pittsburgh Medical Center for a number of years, but I've just recently started working in the research room as a safety officer.

And I know exactly what Ingrid's talking about when she says there are students who are untrained.

It's just been my opinion as a safety officer that people in research backgrounds do not have the same kind of guidance and awareness of all the rules and regulations that people in a hospital. The CAP regulations, the JCHAO inspections, and a lot of those things because they've never had to do them and in many cases, they're not aware that they're even being done someplace.

So I'm seeing like a very surprising attitude towards safety, and sterility and proper techniques, and who's working with the animals and the instruments.

MR. CODER: Well, I guess one of the issues anybody doing flow cytometer, say in the clinical lab, in the hematology departments, I think there is a requirement that the people running those tests are at least certified medical technologists.

So it sounds like we're rapidly working to that point, that it won't require that particular level of training and then a super level of training, including the things that Jim had brought up, of knowing the guts of the instrument perhaps, or perhaps not, depending on how this shakes out.

But, at least, basic training in how to handle human cells with aseptic conditions.

And having been an editor once, or sort of always an editor, as far as terminology goes, we're tossing around a number of terms, and sterility and so on. Sterility does not refer to the final product, for my inference as an editor. That means nothing is growing in there.

But we're talking about maintaining aseptic conditions, and not adding other things to it. If you have a sterile product, I'm sorry, that's not a

good product. You want an aseptic product with only the thing that you say is in there coming out the other end of it. Yes, Gerry?

DR. MARTI: I wanted to make a comment about that. Somebody used the expression a little bit earlier of being able to make evidence-based decisions.

And I think that with regards to sterility testing today, we probably saw two extremes. We saw kind of the 20 minute version versus the two-hour. I don't know which one's right. Maybe they're both wrong. Maybe they're both right. But I think that perhaps some type of rinse testing.

David was eluding to Dr. Gee's data. I would have loved to have seen the data on why the psi had to be dropped from 60 to 30.

I've never seen Carlton Stewart's data, but he said he was absolutely shocked when he saw that he could sort cells up to 100 psi and they didn't explode, because that was the general teaching for the past 20 or 30 years that the minute that cell got into that stream, it would just go all over the place.

But, on the other hand, he probably has some functional data.

So that's something the FDA will always ask. Where's the data? What are the results? And if there aren't any data, then it will probably say go back to the drawing board and find the data before we proceed, or before you proceed.

So I think that one way to find out what your sterilization procedure is is to use one of these tests, and please don't ask me to describe the test, but I'm going to use the buzz word of bioburden, which I think is not too onerous and, hopefully, not too expensive.

If you can sterilize in 20 minutes rather than two hours, I think that might be very beneficial information to have, and the same thing also if speed is really an item, then this psi and droplet frequency is going to be an

issue, and you may have to test that, and I'm willing to bet that isn't the same for all cells.

That peripheral blood lymphocytes, you may be able to do anything with them, but I'll bet these pancreatic islet cells I probably would -- I have no idea, but I bet you have to be pretty careful with them.

MR. PERFETTO: Gerry, with regard to dendritic cells, I know that because of the surface area where they decompress, they cannot be sorted at high-pressure speeds.

So, typically, those are run on lower speeds, and there's a lot of data really out on that. But they're probably other cell types. But, certainly, the culture cells are a little more resilient and can withstand more pressures.

But I would like to go back real quickly to the safety of the operator. We're at the VRC right now where we're interested in protection more than sterilization and therapeutic needs at this point.

And we found that -- actually, we've not migrated to a whole bodysuit. One that I know that's being produced by the surgical -- for use in surgical teams with a helmet and a HEPA filter unit that's attached to that which draws air down through and out the bottom. It's a complete suit, rather than worrying about all the other devices you have to wear, goggles and everything like this.

It's actually a very good devise by Dupree, which I think is something that if we're going to look at guidelines, we've got to start looking at those kinds of things and maybe look at some stricter ways to protect people.

The other thing that I think we should try to look at is regulations to monitor whether or not you are contained. How good is containment? And try to have a very fast, effective way to measure that.

There's some papers here that I know -- sorting beads. We used to sort beads on overhead film, and then wash the overhead film into tubes, spin those down and run those through a cytometer to look and see if, in fact, we had containment.

I mean there may other simpler procedures, and I think we had to have adopted some kind of standard procedure that even industry can use to say that we have containment.

But I think before the operator gets in there and starts doing these kinds of things, we're going to have to look at those kinds of approaches as well.

MR. CODER: The point came up earlier, we were talking about various requirements and also the ease of design beginning with criteria, rather than trying to go the opposite direction, of let's put Band-Aids on it and hope it works.

I think one thing that we can -- and the industry people here are probably deliriously happy if we come up with some starting criteria that things that we need to meet, such that we can then go back to engineering and say these are the things we have to at least be able to do to simplify the procedure.

MR. HOWES: I think this is the issue. The fact of the matter is whilst one is aiming for asepsis, let's say if you're dealing with reinfusion of a product, the issues of the manufacturers -- and I'm just one of them from Beckman Coulter, have to do with our buyer has a containment which isn't necessarily the same as protecting the sample that you're dealing with for reinfusion.

So one has to define the difference between the two, I think. So protecting operators, protecting sample.

And I think there do have to be guidelines -- not guidelines. There do have to be goal posts set on what users, what consumers expect of the manufacturers, and the frequency with which you expect that from manufacturers because, of course, everything requires resource from a development standpoint as well.

MR. CODER: My feeling is having glanced at some of the GMP requirements and also -- well, another place, if you really want to go out and look at very stringent systems, you can go and look at the cleanliness requirements for the microelectronics field.

Particle counts are much more highly-regulated there than for medical things. And so that's really an extreme approach.

But for the minimum that we require for biological things, my feeling is there's a lot of this. It's probably already out there in terms of how many colony-forming units per unit, whatever, is going to be a tolerable biological burden within this area. If you're doing swab tests, how many colonies can you recover from that, et cetera.

So, I think, I a lot of this stuff is already out there and that will be -- whoever takes on the search of the existing regulations project can report back to us. Where do we go from there? I think that will be a plus. Yes. Frank?

MR. MANDY: A couple of observations. One message I got today from at least two speakers, that if you protect the sample, chances are you are protecting the operator at the same time.

And the second point is that since we have the manufacturers here, if we lean on them and suggest that they build these instruments so that, in fact, the sample integrity is controlled, and controlled well, then the operator does not have to wear a space suit.

And, in fact, we know that from other practices. We don't dress up in asbestos suits when we autoclave. We restrict the autoclaving to a box.

And why can't we do the same thing for sorting?

And that's why I think acceptability criteria need to be looked at as well. I mean, is it acceptable to put something the size of a MoFlo or an Epics Ultra in a hood, is that okay, or do you need the whole thing in a containment room, a category 3 or even more stringently, category 4 containment environment? What's acceptable to the users? So you raise a very important point.

We've solved the problem. Just put in a containment 3 laboratory. There you go. No issue. But, of course, that's not the issue. There was a question.

MR. CODER: It's going to be somewhat expensive. Yes, Jim, and then Kevin.

MR. HOUSTON: On this issue of -- I guess, of either operator exposure or sample exposure, we heard a lot about different things here today, but the thing we haven't about is really validation of these systems.

We are creating systems that – why are we creating them in the first place? Do we know for sure or are we being contaminated as operators by the aerosols to a degree that we are in danger? Are we creating all these containment systems? This is knee jerk reaction, before we have the actual written proof through validation of our own systems.

Point in case, for the difference in extreme of cleaning the machine between myself and Adrian Gee. If he wants to do it for that long, let him do it. If I can validate I can do it in five minutes, then that's the way we're going to do it.

MR. CODER: I was going to ask you and, in fact, have you done any of those experiments, to see what the -- how many --

MR. HOUSTON: We just talked about that a little while ago with our QC person here. One of the problems we have with the GMP facility that the system sits in is there is no way that we're going to put in live containment bacteria or virus into that system, into that room. You not only contaminate the room, the you contaminate the system, the room, and the whole facility by doing that.

MR. CODER: That's not necessary to do. There's enough stuff floating around there to begin with, and those are things that you're looking for under normal conditions.

To go back again, the two things that we had as handouts, the cytometry reprints, the biosafety guidelines for sorting of unfixed cells, well that was spoken of as a guideline, and not necessarily from that you should infer that there's any minimal standard of cleanliness that's involved in that.

That just says a method for aerosol production within a system. It says nothing about what the level of containment is going to be at all. It just makes a very sensitive way of measuring that contamination.

But in any system, and many of the things that are hardest to get rid of our things like gram- positive spores, for example. You know, very resistant to a large variety of things.

You can go and take swabs off the -- what you think is a relatively clean area and you're going to get stuff growing on it. And those are the things that we're talking about, the adventitious agent.

And also, depending on your heating, ventilation, air conditioning system, aspergillus spores floating around, those are also

particularly nasty from patient standpoint, depending on the degree of immunosuppression and so on.

And so those are the sorts of things that you have to be able to demonstrate that are absent at a particular level, as far as the successful results of your cleaning and defining an endpoint.

And those things are pretty well nailed down in a variety of other documents, and we'll find those and use that as a starting point.

MR. HOLMES: Just to sort of bring together a couple of ideas. The thought was brought up that the research environment is a lot different than clinical environment, in terms of the biosafety, and I agree entirely with that idea.

And I think what we have to remember is that the majority of these instrumentations were built as research instruments, not as clinical instruments to begin with.

So what we're doing is trying to play catch up here with instruments that have now become or have the potential to become very important clinical instruments.

And so what it may ultimately require is a redesign or brand new designs to incorporate some of the ideas we're talking about.

But probably in the meantime, that's not going to be readily available. But what we're trying to do here is just make do with what we have, and in all instances, it's not going to be an ideal situation.

DR. MARTI: I agree with you, Kevin. In fact, as one of the things that became clear to me today was that perhaps there's two major types of instrumentation.

There is the air jet stream that we're historically used to and now it looks like we may be moving toward something that's much more -- well

to use the word, a desktop, high-speed sorter, that part of it might be able to be sterilized. And we're starting to hear about disposable things too.

But in the interim, I think that the community have definitely demonstrated that decisions can be made, even on the traditional air and jet stream, what is disposable, what can be replaced, and then what has to be sterilized.

And I think after this meeting, or this workshop today, a little more attention will be given to validating that sterilization.

Still, the question about the product, I think that perhaps the best thing, from my point of view today, is seeing the wide acceptance, or at least the ability of the community to approach both GMP's and GTP's.

And although I can assure you that it was not planned this way, it impresses me how the facility aspect emerged today, that if we began to start just paying attention to the facility I think that will be an incredibly big step along these lines, and eventually, even in the GTP's, there's a tremendous consideration for a donor, the actual donor, safety, donor screening.

And as this -- if this method becomes more widely used, the community will learn what those guidelines and recommendations are and they will use them because they're common sense. Thanks.

MR. CODER: I guess from everyone's sort of fixed stare at this point, we may have exhausted certainly not the questions, but -- Jim, you have --

MR. HOUSTON: Can I say a few more comments? I know we're running out of time and everybody wants to home.

MR. CODER: Please do. You've got five more minutes before we sum it up and have to get out here.

MR. HOUSTON: Right. A lot of people already left. Two -- three things I wanted to enumerate on real quick. One was some nomenclature, like you had just talked about, about using sterile versus aseptic.

The things which you've seem today was analysis rates versus sort rates, that that's still something that gets crossed linked.

And when people do slides or presentations or talk on the web pages, or whatever, that there's definitely a difference between analysis rates and sort rates, that we should make sure that we use the right terminology for that.

The other thing I saw today also was the use of speed as a liability. Speed has nothing to do with viability. It's the pressure involved in the tip that has the problem to do with viability, not the stepped that we do it at.

So we need to make sure that -- it's a little bit tricky there in terminology, but it's something we need to pay attention to.

MR. CODER: I think the thing you're -- to summarize the comment, and particularly, the last -- it's what's the proper cause and effect.

And I completely agree with you that we have to know what the causes and effects are such that we know what to look at, as far as getting the solution to the problem.

MR. HOUSTON: Right. There are more conditions to having good viability when you're sorting. If you start out with a lot of dead cells, you're going to have dead cells when you sort. So there's a lot more to that than just the sorter itself.

The last thing I'd like to comment to about the committee, is I assume this is a committee that's probably going to get a spearhead, as far as writing regulations and some guidelines and all for doing this type of clinical work. Is that the essence?

MR. CODER: We'll probably not be writing regulations, per se. That falls in somebody else's bailiwick.

MR. HOUSTON: The guidelines then?

MR. CODER: Yes. We were going -- recommended voluntary guidelines.

MR. HOUSTON: Okay. One of my -- I've been trying to do this for many, many years. And it's gotten more and more -- it seems like more and more difficult as I got into the clinical realm.

When you go from research, doing basically research, doing basically research, jump in there and sort type research, you've got to fill the paperwork out first for clinical stuff, it makes a whole lot of difference.

And one of the fears I have is making this so difficult that it can't be done, or so expensive, most people can't do it, or so tedious to do that you'll need somebody with 20 years of experience to do it.

MR. CODER: That was one of the implicit goals -- well, I sort of said explicitly a few times that we don't to make this so difficult to do, that nobody's going to it. And we have to be reasonable and try to do it in as streamlined fashion as possible.

Because I could imagine as far as some of the paperwork stuff, if we get some enterprising people who can put together -- just talking off the top of my head or something -- a very nicely, well worked out Excel spreadsheet, which summarizes all of the forms that you have to do in order to verify what you're doing, you're doing it the right way, that would make a big step in simplifying the whole process.

MS. SCHMID: I want to make one comment. From my experience in writing the guidelines about biosafety of sorting for the operator, it is also very important that this is done.

You don't want to make it too hard on people, but it's very important that people have guidelines because otherwise other people will not do it because there are no guidelines, and then people will say I don't know what to do, because nobody knows what I should do. So that's my opinion on that.

DR. MARTI: It's about seven minutes until 5:00. I think we should promise to all be done by 5:00. Any further comments from the floor? We didn't really have a specific comment period. Fatima?

MS. ABASSI-LATIF: I was just curious, since flow is in for a long time now, should there be prospectus study on operators? If there are any incidents or any database on people who are actually sorting. If they are infected with anything they are sorting or any effects of sorting on them.

DR. MARTI: Well, my response to that would be that your question has the makings, the sounds of at least a survey.

And I think unanimously the committee would suggest if you maybe draw up a draft form of such a questionnaire -- and I don't mean to be making little of that.

I think that if somebody could design a reasonable prospective study about operators, their concerns and actual issues that have arisen, I think that would be useful to the committee.

MS. SHAPIRO: I just wanted to make a quick comment based on the perspective of someone who belongs to a product division that we now have a lot of mature products. And your field is still very new in this respect.

And I'm not suggesting that anything I'm going to say you have to start doing today. Like when we do inspections of our manufacturing plants, there are always things we look at.

And one of the things, for example, is training of the personnel. So you would never see a student in a manufacturing plant, for example.

And when we saw the examples from the lab here at NIH, there were pictures -- you saw somebody's hands in a hood with gloves, but you saw skin and you saw a watch.

In a manufacturing plant, you would put on -- you would take off your clothes and put on a suit and you would be covered -- there wouldn't be any skin showing. I think you were the one who said most of the contamination comes from the operator.

So I think as your field evolves, and I know you want guidelines now. Most of your trials are phase 1 and phase 2 and, obviously, we do have different standards.

But sooner or later, you're going to get phase 3 trial and somebody's going to want to license it. And whoever comes in with the first license is going to have the hardest time.

They're going to -- they're definitely going to open the doors for everybody else, but they will get the most scrutiny and the most questions, and everybody after that will know what they have to do.

But it's not too early for you to start thinking now about -- and maybe inspections of your type of labs will be different from the manufacturing plant, and that may be an evolving field that our full-time inspectors have to think about as well, that you might have different requirements than a plant that makes a monoclonal antibody.

But I think you need to start thinking about if we want to get this procedure licensed, what kind of requirements will we have to meet, in terms of personnel training, and gowning, and validation, absolutely.

If you can validate that your ten-minute cleaning protocol works, then -- and I think you just need to start thinking about these things.

MR. CODER: Thank you. Marjorie. Frank?

MR. MANDY: Just to reiterate the validation. I think that those who attempted to write guidelines in the past had a really hard time because they had nothing to go in terms of published data.

And now I think there are enough people in the sorting business that you must use evidence. And if we know that there's a difference between running a sorting instrument without a laminar flow in front of you, then we don't need it. And if there's evidence that you need it, then we insist that you use it.

And this becomes way before regulatory parts. This is just for the guidelines. You've got to have evidence-based data, and we should go out back to the people who spoke today and see if any of them published any of their stuff.

MR. CODER: That's a good point. Did you have a question?

MR. WHITESIDES: Yes. John Whitesides from Duke University. I want to know -- I'm an operator in a -- operator and manager of a CORF research facility, and we have many, many clinical people that want to use our machine for doing clinical sorts.

You talked about asepsis and those things, and I think once in a while it was mentioned on decontamination procedures.

And if you're decontaminating a machine from patient to patient, depending on what infectivity level patient A had versus patient B, is there a different level, or is there even a level where you can decontaminate across species lines?

DR. MARTI: My immediate -- I would use the same rigor for all infection in humans, but I would not cross species. I would not use mouse and primate.

And I'm assuming that those would primarily be in analysis setting and not -- well they could be sorting also.

MR. WHITESIDES: No, it would be like in a stem cell sort between mouse and human. So would you need a dedicated machine for all human sorting?

DR. MARTI: Yes.

MR. WHITESIDES: And a dedicated -- well, it wouldn't matter if your machine's dedicated for mouse if you're going the other way, but you can't go backwards.

And are there any procedures to that machine where it could be converted from one to the other?

DR. MARTI: Well, I suspect that there probably would be a way to validate that, but I would -- just using the example of primate versus human, and just using DNA as your way of validating that, the hybridization of those two is within 90 percent -- 98 percent or greater.

So I don't know how you would -- they claim that that they can prove the difference between a human, a chimpanzee and an ape by less than one percent hybridization, but I don't know if I'd want to be applying that to a flow cytometer that was then going to be used clinically.

MR. CODER: Jim, you get the comment here before we finish up.

MR. HOUSTON: On this issue of cross use, I think that's probably one of the guidelines. It would definitely have to be a guideline as to no, thou shalt not cross lines.

DR. MANDY: Based on what?

MR. CODER: Stay within your own species, please.

MR. HOUSTON: Yes. Because if you're operating these systems within a GMP facility, I think that the GMP regs would almost prohibit you from bringing those species into the lab area altogether. They will have to pass all kinds of testing to get in there.

MR. CODER: It does remind me of one line, I forget the movie of Jean Garofalo describing her difficulty in finding dates, and she said well, I just have minor standards. I'm willing to stay within my own species.

DR. MARTI: You know, Marjorie makes the point that we do allow so-called multi-campaigns in pharmaceutical facilities. If you could definitely show that -- if you have evidence, again, and I like Frank's comment about being evidence-based, we would certainly review that.

But I think as a rule of thumb at this point, and I think it's emerged somewhat in this meeting, that the units are being restricted to humans.

Well, listen, I promised -- I'm already one minute past 5:00 and we need to stop. Did either of the other co-chairs want to make a comment?

MR. CODER: Yes. I had promised Larry Lamb, and he is gone now, so at least we can read this into the record that, in fact, as far as follow up for this meeting goes, the most proximal meeting where the same issue will batted around again, will be at the Ice Age meting in Quebec City, June 14th of this year, going on for a couple of days thereafter.

Michele will be speaking at that meeting on this topic, summarizing this particular meeting.

And then as far as further follow up throughout the year, there is the clinical cytometry meeting that will be in November and then the ISAC Congress will be in -- I guess, it's late April of 2002.

And I would hope that at least before then we have a reasonable draft document put together. But I'm sort of looking at the subsequent meetings as other forum where we can discuss the topic more if it needs to be, and then also to get some feedback for some preliminarily things that we're doing based on our recommended protocols.

And with that, that's all the comments I have to say.

DR. MARTI: On that note, I'm going to declare this meeting over. Thank you.

(Whereupon, the meeting was concluded at 5:03 p.m.)