UNITED STATES OF AMERICA

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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

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The workshop was held in Wilson Hall, Building 1, at the National Institutes of Health, Bethesda, Maryland, at 8:45 a.m., Joyce Frey, Ph.D., Deputy Director Division Cell and Gene Therapy Presiding.

PRESENT:

TODD P. CHRISTIAN
L. JANE CLARKE
DAVID CODER
L. SCOTT CRAM, Ph.D.
ALBERT D. DONNENBERG
JOYCE L. FREY, Ph.D.
ADRIAN GEE, Ph.D.
LILLIA HOLMES, MS
JIM HOUSTON
GRANT HOWES

ALSO PRESENT:

MICHELE KEANE-MOORE, Ph.D.
LAWRENCE LAMB, JR., Ph.D.
FRANK MANDY
CHRISTINE NELSON
MATT OTTENBERG
STEPHEN P. PERFETTO, MS, MT
ELIZABETH READ, M.D. (CC/DTM)
ROBERT SAUSVILLE
INGRID SCHMID
MARJORIE SHAPIRO, Ph.D.
GER van den ENGH
FRITS van RHEE, M.D., Ph.D.
THOMAS WAGNER, Ph.D.

ALSO PRESENT:

RANDY T. FISCHER
KEVIN L. HOLMES, Ph.D.
WILLIAM JANSSEN, Ph.D.
HOWARDS MOSTOWSKI
JOHN F. WHITESIDES

I-N-D-E-X

Page

I-N-D-E-X (Continued)

P-R-O-C-E-E-D-I-N-G-S

8:47 a.m.

DR. MARTI: Good morning. My name is Gerry Marti, for those of you who don't know me. I'm one of the co-organizers of this meeting and very shortly you'll meet the rest of the committee that's organized this meeting.

As you can tell from the agenda, we have quite a few speakers, topics, packed into this one-day workshop. So I probably will have the appearance of being some type of Nazi master before the day is over in order to get through this.

Just to remind you that the meeting is being taped and is to be transcribed in 15 working days after this meeting, and will be available on the FDA website. So that might, hopefully, be of use for us.

I would like to next introduce Joyce Frey from the FDA. Dr. Frey got her Ph.D. in immunology from Kansas State University where she did her work in the cellular therapy of skin tumors.

And as a post-doctoral fellowship, she worked at the NCI, studying receptor recognition in NK cells. In 1993, she came to CBER as a product reviewer with a specialty in cell therapies.

In 1999, she was appointed deputy director of the Division of Gene and Cell Therapies and she is going to give the introductory welcome today. Joyce.

MS. FREY: Good morning. For my background, I know with cell therapies we're learning more and more as time goes on. And what we're seeing in our

division in regulating cell therapies is that over time, people are figuring out which cells are the critical cells that they need in order to produce a therapeutic product.

And as a result, one of the moves people have gone to is for cell sorting. That's one of the reasons that this meeting is really very critical to the FDA, because we quickly recognized that there was a lot of issues related to cell sorters and insuring the safety, purity and potency of the final cellular product.

And I think the organizers of this meeting have done a great job in identifying speakers to address many different areas of safety concern related to cell sorters.

I would encourage you all to be active in the discussion of this meeting. We are here to learn and to try and apply appropriate regulations so as not to impede this area, because I think this will become an area that will become increasingly more valuable to producing cellular therapeutic products.

So I welcome you and I hope you have a good day. I will apologize right up front. I'm not going to be able to stay for the whole day. For some reason, my division director decided he had to go give a talk about in California, so I have to go run our division meeting.

But I'm hoping to step back in here this afternoon when the talks get into the more details of safety. So, hopefully, I'll be able to make it back, but if not, I hope you have a good day and I look forward to reading the transcript from this meeting and your ideas and suggestions.

And be rest assured that this is not going to end with this day.

The organizers have assured me that this is going to be an ongoing process of

collection of information and input from industry and academia. Thank you very much and welcome.

DR. MARTI: Thank you, Joyce. It's now my honor and privilege to introduce one of the co-sponsors and co-organizers of this meeting, Dr. David Coder, who's director of the cell analysis facility at the University of Washington School of Medicine.

David has been involved in just about every aspect and phase of flow cytometry in the last 15 years. I know that he is quite interested in various methodologies for analyzing all aspects of cell -- at the intercellular level and perhaps even the subcellular level, and he's also had a lot of expertise in writing and producing software and analysis of flow cytometric data.

His major interest and background for this meeting is that he is chair of the biosafety committee of ISAC, the International Society for Analytical Cytometry and with their interest in where cell sorting was going to go, in terms of the potential clinical use and his previous experience in that committee, agreed to chair and organize this meeting. David.

MR. CODER: Thanks, Gerry. Well, now that she has stepped out -- I missed the opportunity to thank Joyce for a very good introduction to this and hitting some of the major topics that we're going to be addressing and sort of the sense of things as well.

Again, to emphasize, one of the major things is that -- the reason we're having this meeting is to get comment from all of you. That we're going to have an opportunity at various times throughout the meeting, and then particularly at the end of it, trying to tie everything together such that we can get your comments, because the major

piece of work that happens after this meeting that we have to as a working group sit down and produce a document, which is the -- well, as we'll go through, which is going to be the guts of this meting.

So as far as the origins of the workshop goes, Dr. Frey at the start mentioned their interest in trying to isolate different kinds of cell flow cytometry, that is cell sorting, has been a method that's been used for a variety of applications, and then also for stem cell isolation over the last ten years or so, and also in a clinical context.

But with the increasing numbers of applications to do these kinds of research, it seemed that there was really lack of guidance for the proposed therapies that involve sorting of human cells. And so -- well, lack of guidance within FDA specifically for these new kinds of proposals.

So from the story that I was told, FDA contacted ISAC as being one of the professional academic societies involved with this, and then as a source of information, Ingrid Schmid, who is also here, used to be the -- preceded me as the chair of the biosafety committee and had produced a number of documents dealing with biosafety more from the aspect of protecting the operator and protection of the environment of aerosols.

But the emphasis here is really sort of the opposite direction, of how are we going to insure that the cells coming out, in fact, are the ones that we expect them to be and are free of contaminants?

So for that lack of information from all sides, we proposed that we have a workshop to address this issue. And it's really not the first time that this has been sort of proposed. Larry Lamb with Ice Age was involved in trying to get a workshop for this over

the last year or so, and it seems that there is just enough interest now and a groundswell of new proposals coming in to justify putting this meeting together.

So we can approach this some ways from the standard database product in that what do you want to do as far as reports at the end of it go. And it's really going to be a fairly straightforward thing, at least on the face of it.

We want to produce a set of voluntary safety protocols for clinical flow cytometric sorting of human-derived cells at the base of it.

And there is a working group that is listed on your handout, composed of a number of individuals, most of whom we will hear from today. And this working group is going to produce this document, we hope, by the end of the year, which for things of this sort will be a fast pace and we hope we can do it, because work will be held up in the absence of that.

So in thinking of ways to address the problem and looking at things that are currently in the works, there is a document recently put out, the "Good Tissue Practice," GTP, and this contains some of the concepts that we can start to get our hands on for defining the criteria for sorting cells safely.

I put just this one web address at the FDA, as sort of the introductory document to this and then I think also, from that same page, there would be the 50 or 60-page detailed document.

And the thing to really emphasize here, these are proposals. This is part three of a larger set of rules that FDA is producing to regulate cellular and tissue-based products.

And so the DTP -- well, we're all familiar, at least in part with GMP. I suppose cell physiologists might think was there an intermediate GTP. Not that I know of.

But in any event -- okay. You're all still asleep. In any event, some of the issues brought up in the GTP as a proposal will probably have quite an affect on how we proceed as far as safety issues go.

And there are a few things that I can just quote from that that will probably be helpful in framing our thinking on it. And this one is a -- well, it's a comprehensive risk-based regulatory framework designed to help insure the safety and quality of products, including new technologies, without imposing unnecessary regulatory requirements.

And Dr. Frey had sort of mentioned this, that we want to have new technologies out there without getting in the way of things.

But also, the other point to bring up here is a risk-based framework. It's a concept that the entire thing is not going to be absolutely free of risk. There has to be some degree of acceptable level of risk that can still produce safe products.

And this is something that's implicit in almost everything we do, either you know that obviously, or it's always sitting in the back of your head that most of us came here on an airplane or some sort of vehicle that has a definable degree of risk, probably greater than many other things that we're dealing with here.

But the airplane didn't fall out of the sky. We all arrived, and that was an acceptable level of risk.

And so to define that in the context of sorting cells, we have to arrive at some sort of acceptable risk level.

And so as the other issue -- you know, without unnecessary regulatory requirements -- again, elsewhere in this document, one of the goals is to permit significant innovation while keeping the regulatory burden to a minimum.

And so the concept of this -- it's recognized this is going to be an ongoing and developmental process. And to have a fixed set of rules that would impede ongoing development of instrumentation of processes that are eventually going to improve the quality of healthcare is not something that we want to do. So we want to make sure that we have some way of making sure that things are safe without getting in the way of ongoing development.

So as some of the criteria then that we might think of as far as what's going to involve safe and effective sorting, it really comes down to a fairly short list of things, and they're fairly obvious, at least at this point. Putting those in the document will be somewhat more difficult.

But very simply, are the cells sorted -- sorted the ones that you think they are. And so this brings up issues of purity, of recovery and the like and things that most people doing cell sorting are very familiar with.

The other aspects are the degree of contaminants that might affect outcome. And so here the contaminants is a fairly wide issue.

Biological contaminants and the obvious things that you think of, of bacteria or fungal, or whatever microbial things that might be present in the environment that may, in fact, be part of the cells that you're sorting.

And a very important issue is when you're dealing with clinical things is that you're sure that patient A's samples only contain patient A and not the subsequent or previous samples as well.

And then lastly, as far as the quality of the cells collected, are they able to function in the way that you expect them to.

Now some of this, of course, is going to depend on the individual protocols involved, but there is some fairly well-established methods just regarding cell viability at a very crude level.

All of us that do this though recognize that simple viability alone, based on a wide variety of different criteria may not necessarily be enough. Because you can have cells that say -- appear to be viable just on the basis of dye exclusion or something like that.

But the ability to respond to say various antigens secrete cytokines as one indication of function may not necessarily be there.

So this is an area that, again, gets to be a little bit close to the individual protocols, but it's probably a useful concept to include with this as well.

And so areas that are likely to be included in these guidelines -- obviously, one of them is going to be instrumentation and the facility.

So the instrumentation itself, anything that's going to come in contact with the cells is going to be an important part to know exactly how that's being treated,

individual parts defined. If you make changes in it, can you justify the changes and justify that they're going to work the way that you expect them to.

Then also. the facility where the instrument is located, you expect that to involve a variety of things, including heating, ventilation, air conditioning systems, particular counts, microbial counts, et cetera, all of the things that you would expect as for sources for potential contamination in the process.

And the secondarily, maintained aseptic conditions in the instrument and in the cells as they're collected is going to be a major issue.

So the instrument itself, as far as contact with things in the environment, previous cells, the reagents that are involved, how you're collecting the cells, what sort of conditions you're collecting them in and then what do you do with them immediately after are going to be aspects to consider in keeping the product aseptic.

To distinguish between that and sterile, sterile is going to be -- it's real easy to make everything sterile. You want nice, happy, viable cells, but free of other things, hence, aseptic.

And then also some of the cell manipulations and the conditions while you're sorting, are you keeping them, and is it necessary to keep them at say four degrees, collect them in particular kinds of media to insure that their functional state is preserved.

And then lastly, the area that biosafety and sorting that's mostly dealt with is protection of the personnel involved.

Again, this has been approached mostly from the standpoint of are you producing any aerosols that may contain pathogenic cells?

Now in some cases, this would be sort of an issue to deal with -- you expect that many of the samples are going to be coming from patients that do not have pathogens that can be spread by aerosols. This is an issue that I think that is probably outside of this. But if that kind of sorting is permitted say for an autologous transplant with somebody who does have a defined pathogenic condition that can be spread by an aerosol, that maybe something that has to be considered within this context.

And the reason I'm hedging on a lot of the stuff is nothing is fixed right now. I'm tossing out ideas.

And so just to try to limit the scope of things -- things that are definitely not included, since we're dealing with a variety of therapeutic protocols and they're going to have their own context, the safety and effectiveness of any particular protocol is something that's not going to be dealt with in any of these procedures.

And in a similar vein the sample preparation, that is removing cells from the patient and whatever tissue, and then the post-sorting manipulations of those cells, if it's going to be ex vivo expansion and so on. Those are outside of the realm of what we're speaking of as far as cell sorting goes and the safety of that particular technique.

So in order to get the solution, as Dr. Frey had mentioned, it's going to come from a variety of people, principally those doing the work in the laboratory and the clinic -- and fortunately, the afternoon speakers will give us a fairly good overview of some of the approaches that are currently being taken.

And then to follow up with that, the various scientific and professional societies involved with ISAC, ISHAGE, the Clinical Cytometry Society all have an interest in this and they are represented as well.

And, obviously, the manufacturers of the equipment will have an input in terms of how are they producing the instrument, what needs to be done, and I expect there will be a very good amount of feedback between what is possible, what is effective and what's cost effective as well.

And then, finally the FDA, CDC and also -- well, at least with Frank Mandy representing Health Canada, we have a variety of governmental agencies involved in this as well.

And so the important thing is since we're dealing with a wide variety of groups, it's important that each of these groups which have their own specialties are able to understand what the other groups are doing.

And with my involvement in this over the last three months or so, it's pointed out that you can't know everything in this field. It's vast.

And you can get more sort of information overload in dealing with various aspects of this talk than you can imagine.

So our goal here is get representatives of each of these fields, such that all of this can be at the end of it sort of reading from the same sheet of music.

So that brings us to today's agenda. And so as far as the technology of cell sorters goes, we will have a presentation by Scott Cram on that. This idea of sort

adventitious agents, how do you get rid of them, how do you clean up and sterilize the instrument -- Bob Sausville from the FDA will be presenting that as a topic.

Some of the regulatory issues, specifically GMP, Christine Nelson, also from FDA, will be speaking on that.

We have all of the commercial manufacturers represented here. We'll hear a number of presentations from then and then lastly, as I mentioned, we have the whole afternoon of users to sort of present with works in progress and what we're doing now.

And then, finally, we will convene this working group that's going to produce a document at the end of it and, hopefully, get a lot of discussion from all of you.

And so, again, to emphasize that, the major purpose of this particular meeting is to get input and comment from all of you such that this working group can sit down and produce a document that we can all agree on will be a workable protocol to do self sorting in a safe and effective fashion.

So with that we can sort of move on to the rest of the morning sessions. Thank you.

So we're actually running a bit ahead, as far as time goes. If there are any immediate questions that can be brought up -- I'd sort of try to like to keep this within the time scale, as Gerry had mentioned, but also not to get in the way of presenting -- preventing discussion as it would come up. Yes.

MR. FISHER: (Inaudible. Speaking from an unmiked location.)

MR. CODER: Let's see. We are having this transcribed. We're sort of set up -- we have a microphone over there and I thought there was -- yes, there is one far over

on the other side. To be kind to the transcriptionist, if we can all use the microphones when we have questions, it will make life much easier at the end.

MR. FISHER: Dave, my --

MR. CODER: If you could -- sorry to keep interrupting. Name and affiliation, such that we know who's speaking.

MR. FISHER: My name's Randy Fisher. I'm from the National Institute of Arthritis and Muscular Skeletal Diseases here at NIH. My question was is the end result of the working group and everything that we're going to be working on here to eventually get something included in the Code of Federal Regulations, like GLP and GMP are included.

MR. CODER: That Gerry can answer much better than I can. The point of this, as previously said, are voluntarily guidelines. And the degree to which this is codified in law, I will leave that to the people who are going to be producing that document.

DR. MARTI: I think I can speak to that. I think that the full process in the next two years will be the production of -- the preparation of voluntary guidelines. And the lack of the speed at which the rest of the world moves, I wouldn't worry about that.

MR. CODER: Okay. Anyone else? Well, with that then I will introduce the next speaker, who is Bob Sausville. And -- no, you can't run away. He is now with the Division of Case Management. Was with the Division of Manufacturing Product Quality and he's going to give a quick overview, as I clarified a bit earlier, on some of the aspects involved in cleaning and sterilization.

So what do we have to do in order to make these things free of the bugs that we don't want to have.

A minor change in our schedule. A reversal. I'm the person who's been writing this document. It's gone through at least 14 revisions within the past couple of weeks. You'd think if anybody could get it right I could.

So, to apologize to Scott -- well, we know that Bob is going to be speaking very soon hereafter. But, yes, as I mentioned in sort of lining up the people to introduce various aspects of this meeting, an important thing was to be able to summarize all of the important aspects of it, and I was very happy that Scott Cram agreed to come here and talk about how cell sorters work, such that all of us will know exactly what it does, some of the problems involved.

And Scott is a particularly good person for this, since he was former director or the National Flow Cytometry Resource at Los Alamos National Labs.

So we have sort of the person representing the origin of where this beast came from in the first place to tell us how it works, and to make us aware of some of the aspects of it that may cause problems and how to avoid them. Scott.

MR. CRAM: Thanks, David. Good morning. I guess the first question of the day is how many of you are knowledgeable about flow cytometers and cell sorting? Raise your hands. Okay. So I'm going to talk to the two back there that didn't raise their hands. I do want to keep this informal. So one, if you can't hear me, let me know. And two, if you have questions as I go along, please ask them.

I'm going to give a high-level overview and move it along pretty fast because a lot of you I know are have been involved in flow cytometry almost as long as I have.

I guess the other question is how many of you are familiar with flow microflowtometry? Gary, you can raise your hand.

I'll tell you why I ask that question. The original terminology back in the late '60's was flow microflowometry, but so much of the applications focused on cells that the title became flow cytometry. In fact, I think the earlier title is more descriptive of the technology today because we're analyzing and sorting a lot more than cells.

My apologies for the headers and the footers that say unclassified. If you work at a national security laboratory, they now require us to put that on there. So if anybody steals them, they know that they're not getting anything in secrecy.

I thought what I'd do is talk a little bit about the technology, make sure we're all on the same page there. The second case of examples of DNA measurements, I only listed that as an example of how things have matured over the years.

Obviously, you're all familiar with making whole cell measurements. I think most of you are familiar with chromosome analysis and sorting. And these are just examples from the DNA world.

There's now a lot of emphasis on doing snip analysis with flow cytometers, doing DNA fragment size analysis, something we've worked on very hard in Los Alamos. One application being bacterial discrimination.

And then at the end I thought I would give you some of our work that pertains to the topic of today's meeting, having to do with analysis versus sorting rates, sort purity, cross sample contamination and how we have dealt with bacterial contamination in both our commercial and our experimental cytometers.

So what is flow cytometry? It's an apparatus for counting or measuring cells. As I said earlier, the name really isn't terribly descriptive of what we do today.

There's such a wide variety of particles that are being analyzed in not only the specialized, but the commercial flow cytometers as well.

Some of these slides I took from Jim Jett. He prefers the title florescence particle analysis. Take your pick. Why do we say that?

Well, here's a list that Jim put together, and keeps expanding, that illustrates the variety of particles based on their size that have been and are being analyzed in a flow cytometer.

And they extend everywhere from large plankton, the algae, obviously, the mammalian cells, chromosomes, bacteria, virus particles. All the way down to phcyoerythin and other single molecules.

In fact, with specialized instruments, one can detect single florescent molecules in a specialized flow cytometer.

There's really two reasons we do flow cytometry in my mind. One has to do with rare events, and the other one, that you're all familiar with, I'm sure, is doing the individual particle analysis.

And the analogy that we've used is in the case of why do we single particle analysis has to do with the analogy of applesauce.

If you're eating applesauce, you taste the average of the ripeness, the sweetness, the tartness and the average color, which is fine for applesauce.

But if you're a connoisseur of individual apples, you want to taste the individual apple, likewise in flow cytometry, you want to measure the individual cell, not the average of the whole population of cells.

And the other reason is to look for rare events. Many of us have often used the analogy of looking for the needle in a haystack. If you want to look for the needle in the haystack, you want to look at, ideally, if you can do it very, very fast, individual straws and have a descriptor that allows you to detect that needle.

So you'd want a descriptor, a measurement, something that was metallic, something that was reflective, and you could add other descriptors as well, so that you could pick up that needle as you went through very fast.

But you also need to validate, which means sorting, and recovery of the object for additional applications. And that's one of the reasons we're here today.

So flow cytometry then focuses on single particle analysis and an analogy is that is that it's moving microscopy. If you think of the microscope that many of us used in undergraduate microbiology class, we've replaced the slide with a fluent stream, we now put the cells in suspension, we've replaced our eye with a photomultiplier tube, and we're doing quantitative measurements, rather than descriptive measurements.

Analysis rates can be very high, easily 10,000 particles per second or higher. So remember we have zero spacial resolution, and people are starting to cross that border now that we don't do spacial resolution as the image analysis folks do.

So, obviously, then the first step is to prepare the particles for analysis. It involves staining the components or the particles to be analyzed.

And here are Easter egg cells. We're going to flow those down through the flow cytometer. We introduce the fluid flow concentric with our sample so that we align the cells like beads on a string.

That way we can analyze individual cells one at a time, very rapidly.

So at the base of this device we put one or more lasers so we can measure the florescence of our Easter egg cells.

So the cells are flowing through those laser beams at a high rate, several meters per second. They're spending a very short amount of time in the laser beam, depending upon the system, micro seconds or less, and we're measuring both fluorescence at multiple wave lengths and light scatter at two different angles, both forward and 90 degree light scatter.

It's useful, I think, to remember some of the characteristics. The probe volume that we actually measure can be very, very small. From picoliters -- from nanoliters down to picoliters.

What that allows one to do, of course, is to measure cells in the presence of a fluorochrome while not having -- while we don't have to separate the cells from the fluorochrome that might be in solution.

Because you're interrogating such a small volume, you can reduce that volume down to almost limitless to the size of the cell, or the size of a few molecules.

So that the separation of bound florescence from free is not something you had to do in a flow cytometer.

It's sensitivity we can gain -- as I said before, it can be as low as a single molecule, in the case of florescent molecules. Resolution can be -- can be -- this is a dangerous thing to put in print -- as low as one percent.

For example, in terms of DNA measurements, resolving male and female lymphocytes, based on their DNA content, which is more than one percent. That's the type of thing I'm thinking about when I talk about the very high resolution of a flow cytometer. It depends on a lot of different things.

So, again, the measurements are light scatter, particle size and surface characteristics. The florescence detection is done in multiple wavelength band, measuring total intensity and/or maximum intensity.

We sometimes measure fluorescence polarization and, more recently, lifetime of the fluorochrome, which tells us something about the environment in which the fluorochrome resides.

So with appropriate labelling agents, you get information about DNA, RNA, protein, surface molecules and the environment of a cell or membrane constituent.

Let's start talking a little bit about sorting, since that's really the crux of the meeting today. Obviously, after the cells are detected and analyzed, there's a period of time -- David, do we have a pointer? I thought I saw one earlier.

So after a period of time, the cells enter the two laser beams, as illustrated here, where we analyze them -- detect them and analyze them. They then flow down, generally. That stream of liquid is broken into droplets by the action of piezo electric crystal.

The cells are captured in small droplets, and then those droplets are electrostatically charged and deflected through a high potential. As the artists illustrated here, it can be sorted into multiple containers.

Some sorters, such as the one Tom Jovin had originally developed, were sorting six -- three right, three left. Generally, we sort in only two directions. So these individual droplets are charged and deflected, based on the cell's characteristics.

That's a picture I like of a flow cytometer. It's not a commercially -- commercially- available flow cytometer.

When I first got into the business, lasers were the size of this table. Power supply was twice the size of the podium. Laser beams were bounced all over the walls.

And that's sort of the case here, where the laser beam here is going across, hits a mirror comes back, hits at this point. Here's a fiber optic picking up the florescence. There's another bit of optics up here measuring the florescence at that point.

That stream is now broken into droplets, and you can see the droplets here in a separate photograph. It's really a picture taken in this area.

This is a high-tech charging collar, which is actually a paper clip.

But, nevertheless, it illustrates the beauty of the technology that you're all familiar with.

This is a very stable, reproducible, beautiful system that as you're familiar, when things go wrong, it doesn't look like this. When there's a plug or other obstruction due to bubbles, clumps of cells, then this stream of droplets can go array, creating aerosols. Sometimes you also get microdroplets in the formation of these very large droplets.

Remember, these droplets, when things are working right, are quite large. They're on the order of hundreds of microns. Maybe 100 to 300 microns. And only a few of those droplets contain the cells that we're actually sorting.

In the old days it was one in 40. With different systems now, the frequency of cells in the droplets has changed. So let me illustrate some of those numbers as shown here.

In a conventional flow cytometer, the operating pressures were reactively low, around 12 psi. Droplet frequency around 32 kilohertz. Analysis rates around 2,000 particles per second.

In the more recent developments of high speed, high pressure, high frequency, whatever you want to call it, sorters, we're now operating up around 40 psi.

With systems at Livermore and Los Alamos, we've operated over a hundred psi on occasion. Those turn out to be very challenging to work with, as Gary can tell you, because things become much more sensitive to vibration and other problems.

These operate at a higher frequency, so you get more droplets at a faster rate, and your analysis rate can go up quite substantially.

Realize, of course, that to get the higher analysis rate, and the same resolution, you have to have a much higher concentration of your sample. Think about that for a minute.

Your resolution, in part, depends on keeping those cells going through the focus part of the laser beam. If you simply expand the diameter of your sample stream to get a higher through put rate, you're not going to get as uniform illumination cell to cell.

So sure, we've worked at higher rates. And with chromosome sorting, there's a limit, as with cells, how high you can up the concentration of your particle, whether they're cells or chromosome.

So you're limiting rate here can be sample driven in that if you can only get your cell concentration up to 10 to the sixth, 10 to the seventh, somewhere beyond that you're going to get mush because the cells starts sticking together due to free DNA and other problems.

I couldn't resist putting on the sort rate. It really depends upon the fraction of sample to be sorted. I think most of you realize that having served on too many shared instrument grants -- and my apologies to the instrument manufacturers -- I see comments well, like we're going to sort chromosomes at 20,000 per second. It will only take two hours to sort enough chromosomes to be able to clone into a bacterial, or a YAC, or whatever vector.

What people are forgetting is that your sort rate depends upon the frequency that the chromosome of a cell is present in the sample.

So if you're sorting chromosomes, for example, from human you've got 24 different types -- depending on whether male or female -- you've got 24 different types of chromosomes. You're only going to sort one out of 24. Maybe two if you're sorting right/left.

So your analysis rate might indeed be at this speed, but your sort rate's going to depend upon what fraction you're actually sorting.

I thought I'd share with you some of our experience based primarily on chromosome sorting, in terms of sample contamination, sort purity, bacterial contamination.

As a predecessor to the human genome project, the Lawrence Livermore National Laboratory and Los Alamos National Laboratory were both involved in what was called the National Laboratory Library -- the National Gene Library Project. Thank you, Gary.

So we were sorting chromosomes, human chromosomes at very, very high purity, very large numbers, because the cloning efficiencies weren't that great in the old days. They've certainly improved substantially now.

But, in fact, in the case of Los Alamos, we were sorting chromosomes in some cases with two flow cytometers back to back, or face to face, actually, and we would sort around the clock for seven to ten days to get adequate -- we're talking about one to two micrograms of sorted chromosomes.

The advantage is you've only got one sample. The disadvantage is you've got to run different shifts of operators, and that's what drove, in part, high-speed sorting.

We have certain purity requirements, we had contamination requirements that are somewhat different than what a lot of you face, and I think some of the things we did might be useful in terms of thinking about new ways to look at some of these issues that a lot of you will be talking about later today and are certainly a lot more expert in than I am.

Let's talk first about sort purity. Obviously, it's a function of analysis rate, how fast are you going to push yourselves through there, and most importantly, the degree to which the population is resolved, and also, the amount of background florescent debris. Depending upon one's sample, these can be -- can vary enormously.

For bivariate two color flow karyotype analysis we could, at best, I would say, on a routine basis, with large numbers of sorts, get up in this purity range. And that's analyzed by florescence and in-situ hybridization.

So the procedure is to sort chromosomes at regular intervals, sample the sorted population, put them down on a slide. For sorting a single human chromosome from a somatic cell hybrid, then hybridize down and probe the specific or the mouse, or the Chinese hamster background, or for the human chromosome in assay purity.

We also did a lot of data analysis on the distributions, but actually, I think, FISH analysis was the most definitive.

One of the things that we were aware of, but not overly concerned about, was cross sample contamination. Obviously, you don't want to cross contaminate, whether you're sorting chromosomes or cells.

One of the things I always found curious and surprising was when we did our alignment of our system, we would use very small microspheres which imitate the size of a chromosome. So these are about 1.2 micron plastic microspheres.

So we'd run those through, optimize our alignment, as all of you do, do a minimal rinse, back flush, rinse off the tube, put your sample on and start sorting chromosomes. We'd sort 10,000 chromosomes.

Generally, in those 10,000 chromosomes we'd see less than one or two microspheres, which I always found kind of encouraging, that there wasn't must contamination based on this sort of analysis, with these small microspheres.

It's one way, not the only way, and maybe not the best way, to start to get a feeling for how much carry over there might be.

Let me put an asterisk after that comment. If I were running the machine, that number would probably be much higher. Our operator was fastidious in operation of his machine. He wouldn't let me close to his machine. Let's be honest.

And this fellow is the ideal instrument operator. He takes care of his machine and knows his machine, and loves his machine like you want all your operators to do. Regularly changing filters, changing tubing.

Look at your flow cytometer that does sorting. Are there any salt crystals on it somewhere? Not on Joe's. This guy really takes care of his instrument, and that's part of the reason we saw very little cross contamination, although none of these things are absolute.

We were concerned about bacterial contamination. I think everyone is. Our approach to that was we use a millipore sheath tank for our sheath. So we'd autoclave that. We'd prepare our sheath of solutions, all our reagents in a sterile fashion.

Generally, what we were doing was doing a 70 percent ethanol rinse, sometimes, depending upon the situation, we'd leave it in overnight, rinse it out in the morning. We'd almost never see any bacterial growth as acid, by taking five or ten milliliters out of the receptacle where we were sorting into.

Take five or ten mils, concentrate it down, put it out on a nonrestrictive agar plate and grow it for a week at different temperatures. We almost never saw any bacterial growth.

Again, the asterisk after that comment is I believe in a very dry environment. It's not high humidity of where some of you live and work.

We can do -- we can almost do tissue culture on the lab bench. It's that dry. It's 7,500 feet in Norther New Mexico, and that makes a big difference as well.

So these are some of the characteristics of flow sorting that we came across when we were doing a lot of chromosome sorting.

I'd be fascinated though to hear about the talks this afternoon, what some of you all face and your approaches to it. Another pretty picture of a flow analyzer, laser beam microscopic -- the intersection point.

So, in summary, remember that we're talking about analysis rates of 1,000 to 10,000 per second. Particle analysis time, microseconds to milliseconds.

In some systems we're analyzing up to 50 parameters per cell. The spacial resolution is essentially zero dimensional and, obviously, the sorting particles in our cells, chromosomes, can be physically separated for further study and/or applications, which really leads us to the rest of our meeting today. Thank you.

I'd be happy to answer a few questions if we have a little bit of time.

MR. MARTY: Scott, as a rule of thumb, when you're talking about analysis rate versus sort rate, if you know the percentage, but not enrichment of the cell or particle that you're looking for, you can then estimate the sort rate to be that fraction --

MR. SCOTT: Sure. If your sort rate is the same as the analysis rate -- and that's the mistake that new flow cytometrists sometimes make in their NIH-shared grant instrumentation, the shared instrument grants. Of quoting the analysis rate as their sort rate, and that's what someone told them. It's going to be a deadly error to apply for a grant application.

DR. MARTI: I don't think I explained it right. It's not just the analysis rate, it's also the number of (Inaudible. Speaker not near miked location.)

MR. CRAM: Good point.

COURT REPORTER: I'm sorry. I can't transcribe unless you use the microphones.

DR. van den ENGH: I'm Ger van den Engh, Institute for Systems Biology and the sort rate is not only dependent on the analysis rate, but also depends on the number of drops that you make per second.

MR. CRAM: Other questions, comments, clarifications.

MR. CODER: Yes, Scott. Dave Coder. You had done the one illustration of contamination of microparticles into the funnel-sorted sample. You brought up the one point also for high-speed sorting you have to have much higher concentrations of things. And it's been my sort of observation that the higher concentration you have between things, the greater the burden you're going to have as cross contamination goes. And is that something that you observed as well.

MR. CRAM: Absolutely. I don't think it hurts to stress again the importance of thinking about your sample concentration in these high-speed sorters, because as you drive your concentration up, we found we get into a lot of unexpected problems, not just clumping, but other spurious results that force us in some situations to back off from what we thought we could do in terms of cells sorting at very high rates, requiring very high concentrations.

MR. CODER: Thanks. We'll take one more question.

MR. JANSSEN: Bill Janssen, the Moffitt Cancer Center. This is actually on a slightly different tangent, but you notice as the speed goes up, the pressures go up. And doesn't -- wouldn't this have an affect on your sample integrity after a while. You were talking about pressures up to 100 psi. That's like sorting under 60 feet of water.

We didn't do much cell sorting at 100 psi. Those are mostly for chromosome sorting experiments. But certainly, we didn't find many cell types other than the standard rubberball scope CHO cells that could withstand not the pressure per se, but the decompression as the cells come out of the nozzle, go from 100 psi down to atmospheric,

and that's why I think most of your commercial cell sorters are operating in the neighborhood of 40. Is that right? 40 psi. What do you guys operate at? 60.

MR. CODER: Thanks, Scott. And now after the premature introduction, we will have Bob Sausville speaking, and just to bring up his first slide, he is with the Division of Case Management and Scott had illuded to some of the issues of how to keep the instrument sterilized and keeping things not growing that you don't want to grow, and Bob is going to tell us all how to keep it clean.

MR. SAUSVILLE: Thank you very much. I hope that I can actually lend some education to this process of how to keep things clean. As pointed out, I am with the Office of Compliance, and I cringe when I hear the cross contamination percentages.

I guess I'm looking at this from a little bit different perspective. I know that this is a new field, but I want to try to get everyone to start thinking about good manufacturing practices, even in your research, because down the road, hopefully, your research is going to be productive and you're going to go further and maybe eventually go to licensure of something.

I first want to thank Michele Keane-Moore for inviting me and Joyce for supporting her in that invitation. Dave, thanks for the introduction twice.

So I think we'll start with just a little bit of basic background here for me. I've got a few slides here. Michelle told me that I was to come and talk to you about sterilization, cleaning and sterilization and flow cytometry equipment, and I had so minutes to do it. I thanked her for all of the extra time she gave me.

Really, cleaning and sterilization are two topics that could conceivably be two weeks each, so I'll give you what I can in 20 minutes.

Another gentlemen asked about regulations. I don't think you need to worry about regulations. We've got plenty. I'll show you a few that I think might apply.

Just a quick general considerations for cleaning and some special considerations for cross contamination issues. Quick discussion on validation and then, again briefly, cleaning and sterilization and then a short example that we've used in the past for the gene therapy products and how we would apply validation procedures, I guess, for really early phase clinical material up through, hopefully, licensure.

But, again, this is just to get you people, hopefully, thinking about this early so that you'll know eventually where you're going with it.

As I said, we've got a few regulations. 1270 are published. There's proposed 1271's for good tissue practices, for producing products for humans. We have the 210, 211's, good manufacturing practices for drugs, also for biologics.

Special biologic product regulations in the 600's, and the 800's medical devices, and Christine Nelson's going to speak next on that. So I won't touch those. I'm not going to touch the 1270's either.

So we'll start with the -- the next four slides will be about the good manufacturing practices that could apply, if we were so disposed to need to use them.

To begin with, 211.63, equipment design, size and location, is to facilitate operations for intended use, equipment construction, and here we go, product contact

surfaces shall not be reactive, additive or absorptive, and equipment cleaning and maintenance, 1167, equipment must be cleaned, maintained and sanitized regularly.

And for product contact surfaces in a aseptic process, that sanitized would really be sterilized.

211.113. And, again, this is a very important component, control of microbiological contamination. Must have procedures designed to prevent microbiological contamination and it shall include the validation of any sterilization process.

Under subpart J, records and reports, 211.182, you must have equipment cleaning and use logs, and a written record of major equipment cleaning, maintenance and use shall be included in individual equipment logs.

So a flow cytometer with different samples would have to have record of the different samples that were put into it.

Go onto the 600's, the more biological regulations. Under equipment, 600.11B, apparatus and method of sterilizing equipment shall one, insure the destruction of contaminating microorganisms, be at least as effective as maintaining a temperature of 121 for 20 minutes, designed for thorough cleaning, inspection for cleanliness.

Product contact surface shall be clean and free of surface solids, leechables or other contaminants and equipment shall be sterile unless product sterility is insured by subsequent procedures, and that generally means that you filter the product at some point down the road, or it's terminally sterilized and, theoretically, at least, I don't think your products would fall into either of those categories.

The 121.5 for 20 minutes, again, that is sterilization needs to be that effective, but that still needs to be demonstrated. You can't just do 121.5 for 20 minutes and say that, we did what we were told. It's only to see the biological indicators show the death of those, lethality of your process.

And then the last slide on regulations, 600.12C, records of sterilization of equipment and supplies must have the mode, date, duration, temperature and other conditions for the equipment and supplies in the production process shall be made in and by an accurate, reliable way.

Now the general considerations, it's control the facility, but really it's control of the process, and again, as you can see from the regulations, our main control concern is the contamination and cross contamination and that, again, can be microbiological, it can be chemical, it could be from other cells, someone else's cells.

So we need to minimize the potential for that.

One way to do that is to segregate activities and processes and you also need standard operating procedures in place to prevent any mix ups.

Special considerations. These are a few of the potential routes of cross contaminations. Centrifuges are a typical problem area for us, because of the generation of aerosols and in those we would expect one sample to be processed at a time, and cleaning and sanitization of centrifuges between lots.

I'm thinking flow cytometers fall into this area. Pipettors are another area. We all have used them, but we need effective cleaning procedures for your pipettors, especially your automatic ones with filters attached.

Incubators, obviously, are a perfect place to grow everything, and I guess this would be the -- expansion of the cells that you've sorted and, obviously, flow cytometer would be a special consideration for a potential route of cross contamination.

Now, ideally, all product contact services should be replaced with re-cleaned and pre-sterilized components. Now I don't know really -- I'm not that familiar with flow cytometers. When I dealt with them, it was a long time ago and it was of minimal duration.

So we'll let the manufacturers tells us where we are on that part. If we cannot -- well, one of the concerns, even if you do that would be to maintain sterility during the replacement of the components, and at a minimum, in the class 100,000 area the best possible scenario would be handling at an aseptic manipulation under class 100 conditions.

Obviously, that replacing after each use is probably not too feasible, so we need to then consider cleaning, sterilization and have that process validated.

And this is a stock validation description. Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product or, in this case service, meeting its predetermined specifications and quality attributes.

So when we clean, we want to make sure that we get to a certain set point, and when we sterilize we want to also meet certain parameters.

And a validation approach for clinical manufacturing. This has been modified over the years to be a little bit more stringent because when you actually get to the

clinical trials, the facilities involved in the clinical manufacturing should be in compliance with applicable GMP's.

Do not expect full validation in the early stages, because you may not have three repetitive runs, or the worse case configuration. Any facility supplying clinical material to other institution -- this would be a contract manufacturing. I mean, I can see you folks being in a contract manufacturing mode, but that would imply that you meet CGMP's and you should meet what is expected for commercial facilities. And the phase three materials should be manufacturer's on full GMP's.

In the validation of cleaning, we need to be concerned about the items being removed, that includes the product, cleaning agent, microbial contaminants, endotoxins and sanitizing agents.

There are different methods of sampling from the equipment. We always like to see a visual inspection, if possible. Take swabs in open areas, or swabs in inaccessible areas.

One of the other main methods is a final rinse sample. Generally, a final rinse sample has been performed in conjunction with swabbing at some point to show that there is a correlation.

I mean, obviously, swabbing's going to show you whether something's clean. Probably better than a final rinse, unless you show that correlation.

And then there are analytical tests that can be run. I mean, obviously, product specific assays. I don't know how that's applicable, although if you're somehow labelling the cells, that label may be left behind. TOC, total organic carbon, ph conductivity.

We do have water standards if you wanted to meet WFI specifications as your standard and protein assays.

Now one or two rules to keep in mind for cleaning are Imbesi's law of conversion of filth. In order for something to become clean, something else must become dirty and Freeman's extension, you can get everything dirty without getting anything clean.

We'll move onto sterilization. I'm sorry for the short time on cleaning, but we don't have -- we can't get into that depth.

I'm going to talk mostly about moist heat sterilization, with a brief amount that I talk about sterilization will be about moist heat. It's a very efficient sterilization method. Two important requirements are water vapor and elevated temperature.

Another that we see quite a bit in the dry heat is dry heat sterilization. That's higher temperatures, lower water vapor, or no water vapor.

We quite frequently see that in depyrogenation ovens and tunnels.

There are other methods of sterilization. There are gaseous sterilants. I've been furiously writing down notes as we've been going through this this morning to try to touch on as much as I can.

I didn't go into the couple of different methods of sterilization. Obviously, gaseous sterilants, you still need to worry about the residuals that are left behind. I guess that's a redundant statement.

But if you were to use a vapor, hydrogen peroxide or chlorine dioxide to sterilize your equipment, you have to make sure that that's not left behind and that's a validated process.

I also did not go into SIP, or sterilize in place. Most of what we're talking about here is sterilize out of place. I mean, I can see that potentially that for the flow cytometer you might be able to do a sterilize in place in the proper configuration, but I didn't go into sterilize in place because, again, that's a whole another week course on what's involved.

So anyway, steam sterilization, the validation. The three very important components to this validation, heat distribution, heat penetration and then bacterial challenge to show that you've actually distributed and penetrate at the rate you're supposed to and held the temperature for the appropriate amount of time.

Most steam sterilization cycles are based on direct contact of the item of saturated steam. Removal of air is a common problem and trapped air prevents penetration of steam and depresses the temperature in the space of the autoclave, or in areas of the autoclave.

A preparation for sterilization is an important issue, different components, the components that make up the flow cytometer that would need to be sterilized, the product contact components would fit into either one of these headings.

Preparation includes how they're washed, rinsed and drained. I mean, that gets back to the cleaning validation, and then how they're wrapped and stored before they're loaded into the autoclave.

Components must be stored in containers or wrapped in materials which allow steam penetration, but it protects it from the environment once it's sterilized.

And again, it cannot contain excessive water from the rinsing because water may act as an insulator for the equipment or components that are covered in water that will insulate the surfaces that need to be sterilized.

Loading patterns in the autoclave can affect removal of the air from the chamber and the flow of steams throughout the chamber. Loading patterns need to be defined during validation and should be consistently used during production.

Heat penetration probes and microbial challenges are placed at the slowest heat areas of the sterilizer. That generally is the drain, or the vent, vent filter which supplies the incoming air after the sterilization.

Maximum and minimum loads should be defined and the reason for extrapolating data for these loads needs to be explained.

And minimal loads are important to validate also because a whole load obviously takes a certain amount of time to heat, but a minimal load can have its own problems.

And some of the other issues, obviously, handling of the components or the equipment after sterilization is a big issue. How long can it be stored prior to it being used and how do you maintain that sterility during the storage?

And some component or equipment cannot be sterilized more than once. So you need to keep records to make sure that you're -- you haven't ruined whatever it might be or are using something that was ruined because it was sterilized more than once.

Finally, we'll finish up with this validation approach, an example for clinical applications. An autoclave used to prepare sterile materials. I think that's appropriate in this case.

But early phase one and phase two, you need to demonstrate that you have a proper cycle achieved. You monitor the temperature, the pressure, and the time at temperature, use biological indicators for verification that you've reached sterilizing mode, but the loads are not well defined.

Early phase three temperature mapping should be done to determine the cold spots and hot spots. Biological indicators should be placed to verify at the problem points. Again, the cold points, the slowest to heat areas. And at this point, the loads should be somewhat better defined.

Then late phase three or onto licensure. Obviously, we validate a cycle determined at the monitored points. So that's at the cold points and you usually always monitor your cold points.

The loads are well defined and standardized and each load configuration has been mapped or worse case load has been validated.

So if you have a minimal load and it turns out to be the hardest to heat, the slowest to kill, you'll define that during the validation process or whatever configurations you have at your worst case load.

Another example, to just throw it in here, sanitizer effectiveness, phase one and phase two to be supported by literature. Phase three and beyond to be supported by

a validation. You actually show the sanitizer effectiveness. So really a sanitizer in the sense of surface sanitization.

I have a couple of references here. Christine has a whole list of references at the back of her thought, but the guidance registry for the submission of the documentation for sterilization process validation and applications for human and veterinary drug products were published in 1994.

Guidance on sterile products produced by aseptic processing was published in 1987. It is currently in draft for the updated version, but there's -- I can't give you a time when that will actually be published, because no one will give me a time.

Lastly, I'll just put phone numbers on the board here. I am now in the Division of Case Management. Before I was with the Division of Manufacturing and Product Quality. These folks really are the ones who review the applications or submissions that come in. They can be involved in the IND stage and beyond. Their number is 301-827-3031.

If you have specific questions that you want to ask me, feel free to call me. I want to keep involved with this area. So I don't want them getting all the phone calls, but maybe the majority.

But that's all I have. So I guess we have a little bit of time, if anybody has questions. Excellent. No questions.

MR. CODER: Just briefly. Dave Coder, again. Most of the emphasis that you were talking about and most of the things that we were thinking about as far as sterilization goes, literally relates to killing microbes that are there down to sporicides or

killing gram positive spores, probably the most refractible things, except as far as some of the things that we'll probably be looking at, contaminants from the standpoint of non-viable cells is one issue.

But if someone is dealing with say gene therapy, having non-viable, but potential chunks of foreign DNA sitting there from another patient is one issue, and then also potentially other antigens that, again, non-viable that -- something that would be not allowed in a final product.

Are there specific ways that those have been dealt with, as far as validation, the absence of those as contaminants?

MR. SAUSVILLE: Well, I think that that's been addressed with the cleaning, in the cleaning arena. And yet the product specific -- I mean, it doesn't really fit, but people have suggested PCR, but PCR's liable to pick up too much, or if you don't have the right segment, you won't get the part that you're looking for, or you won't see that it's there, but it still will be there.

So that's why I think I said that the best possible scenario was if you use -- each cell sorting experience was its own lot. I mean that's the way we would handle cell processing, and that's the way we've looked at it in cell processing, that each donor or individual cells are a lot. So segregation is important.

But as far as sterilization, there may not be a way to -- I mean it might be sterile but dirty. I mean we don't really want that.

MR. CODER: Thanks, Bob. Gerry Marti is going to introduce Christine Nelson, our next speaker. Our next speaker is Christine Nelson. Presently, she's director of

the International Affair Staff. We're drawing on her experience today for the last ten or 11 years.

She's been in the Division of Small Manufacturers assistance and her experience has primarily been in the area of device GMP's, and more recently, the quality system regulation, which is something that evolved out of GMP's. Christine.

MS. NELSON: Thank you. I'm pleased to have the opportunity today to talk to you about the quality system regulations.

We typically spend a day on this regulation when we do workshops, so I was challenged to condense this to 20 minutes.

What I'm going to do is to go over a couple of definitions and then I'm going to talk about the seven subsystems of the quality system. I'll give you the purpose of each subsystem and then I've listed some tools for achieving that purpose.

The tools happen to be the requirements of the quality system regulation. I'll talk about one tool per subsystem, but I wanted you to have a complete list of all the tools.

And then finally, I'll end with where you can find more information about the quality system.

So the definition of quality system is the organizational structure, responsibilities, procedures, processes and resources for implementing quality management.

This means that a manufacturer needs to establish a structure for the seven subsystems I'm going to talk about, assign responsibilities for the work that needs to be done, establish procedures for what needs to be done, so that what is done is done

consistently the same way, and supply adequate resources to carry out these procedures and processes.

Also, develop processes that are robust that -- as was mentioned, you want processes that consistently produce the same result each time.

Going back to resources, these include trained and qualified personnel and adequate equipment and facilities.

So how can this quality system cover so many different types of devices? It covers in vitro diagnostics, hip implants, cardiac pacemakers, a lot of different technologies.

Well, it was written to be flexible. The requirements are stated in general terms. We don't try to tell you how to do something, we tell you what you need to achieve.

With this flexibility comes some responsibility on the manufacturer's part. The manufacturer is responsible for determining -- given what that manufacturer is doing, how best to comply with the regulation.

Here are the seven subsystems of the quality system. You notice that management control is in the center, and that is because management influences the other six subsystems.

So how management sets up the quality system, what management's commitment is to having a good strong quality system really makes a difference in a company.

The first subsystem I'm going to cover is the management control subsystem. The purpose of this subsystem is to make sure there are adequate resources for the operations.

And resources include qualified people, training, buildings, equipment such as computers and software, and manufacturing equipment.

And then it's important that management monitor the quality system and make necessary adjustments where they're needed to assure that the quality system is functioning properly.

Monitoring can take place through an appointment management representative and through period reviews of the quality system.

I'll skip over those tools and focus on conducting management reviews. Management reviews, there's nothing specified in the regulation about how often they should occur. This is where you're given some flexibility.

But management must review the quality system and the corrective and preventative action activities periodically.

How often management reviews take place depends on the situation and the company. You probably should have management reviews more frequently with a new quality system, when there are problems with the quality system and/or manufacturing or testing, and when there are major changes taking place, such as expansion of the company, adding a new product line.

When the situation is stable, when you're not have a lot of problems with the quality system, you can do management reviews less often.

So that's why we didn't specify how often to conduct management reviews. It depends on the situation.

Design control is the second subsystem and the purpose here is to control the process of designing a device to assure that the device will meet the user's needs and specified requirements and regulatory requirements.

Now there are many tools for achieving this purpose, but I think one of the most important ones is right in the beginning of the design control process to identify the design inputs or the requirements for this product.

What do you want the product do to? How should the product perform for the user? Are there standards that you want to -- want the product to conform with and what FDA requirements do you need to conform to.

So these are some of the things that should be listed and identified as requirements for the device.

And then you're -- once your designers know what the requirements are, they can design to those requirements and then you can conduct verification and validation later on to see if you've actually achieve compliance with those requirements.

The next subsystem is the production and process control subsystem. This one has a very simple, straight forward purpose and that is to manufacture products that meet specifications.

Now as simple as the purpose is, the actual achievement is very complex. I think one of the most important tools for achieving this purpose is to control and monitor manufacturing processes.

Now control and monitoring can take a lot of different forms. It can be testing and inspection of output to make sure that the output meets specifications. But it should also include assuring that the process operating parameters are set where they should be.

So if temperature, pressure, flow rate are important operating parameters for the process, you want to monitor and control that process to make sure that it's operating at those established parameters, the ones that are validated.

The corrective and preventative action control subsystem is, I think next to management, key subsystem in the entire quality system.

The purpose here is to collect and analyze information that will help you identify non-conforming products and quality problems and then investigate them to identify their causes.

You need to take effective corrective and preventative action to correct quality problems and prevent their recurrence. So this is a subsystem that impacts on all of the other subsystems.

And collection and analysis of data really is the keystone of this subsystem. You probably have a lot of data available that can help you identify quality problems and non-conforming product.

You're probably more aware of the data that would help you identify a non-conforming product, but are you using it to identify quality problems and improve your operation?

Some of the data that you might want to look at is the results of monitoring manufacturing processes.

And inspection and testing of incoming products can also help you. It can help you identify problems with your suppliers and complaints that you get. That's another useful source, and an obvious one.

But I think this is really a very essential step in establishing a corrective and preventative action control system that is going to be viable.

The documents, records and change control subsystem is the next subsystem I want to talk about. And this has a variety of purposes.

It's there to assure that specifications and procedures that you're using are adequate, and that's through review and approval of them.

To assure that only current documents are being used. If you have a new procedure, you certainly don't want people using the old one.

The purpose -- another purpose is to assure that changes are reviewed, approved and incorporated into documents. Changes are made because of problems, generally. So when you make changes, you want to make sure that they are actually implemented.

And we've seen situations where companies did all the right things in making a change except implementation. Somehow that got forgotten.

And, finally, you need to assure that documents are maintained for the required length of time, and that's for the FDA investigator, and also for you to refer back to.

So if you go to investigate a problem with a product, having the records of its manufacture and a record of acceptance -- of components that went into that product will help you investigate and pinpoint the source of the problem.

One of the tools for achieving the purpose of this subsystem is to have a system for controlling your documentation. Establish a format and a system for numbering or otherwise identifying documents. You want to know what their revision level is, their implementation date, and what their status is.

For instance, you want to know whether a document is a draft or a final document that's been implemented.

You should have procedures established for reviewing documents and approving them, distributing them, and implementing them. And you should have procedures for retrieving out-of-date documents.

Now, as we go more into electronic documents, if you prohibit people from printing them out, this is a little bit easier, because they're working off the computer. But that's another seminar all by itself.

The material control subsystem is our sixth subsystem, and the purpose here is to insure that all products that are accepted, used and distributed meet your specifications. Here I need to define the word products. It includes, under this quality system regulation, components, manufacturing materials, in-process devices, finished devices and returned devices.

So an important tool for achieving this purpose, and one that may be unfamiliar to you, is the requirement to evaluate your suppliers, your contractors and your consultants.

So what you're looking for here is can your supplier supply you with components and manufacturing materials that meet your quality requirements and your specifications?

Can your contractors perform the kind of work that produces the quality that you need? And are your consultants the right ones to help you with the problems you have or the job that you want them to do?

It's kind of a novel idea to evaluate a consultant, isn't it? But we suggest that you ask them what their experience is in. If they've done work for FDA? What kind of topics they speak at at industry seminars? Try to find out what their background is and see if they really are the appropriate ones to help you with your problem.

The final subsystem is the facility and equipment control subsystem. The purpose here is to assure that devices are not adversely affected by the manufacturing environment, buildings or equipment.

You need to make sure your buildings, facilities and equipment are adequate for the operations that you're conducting.

If you need a class 100 clean room, don't try to make do with a class 1,000 clean room. You're really going to be sorry if you do. It's going to cause you a lot of trouble.

I think the most important tool here -- the first one is to design and build facilities that work. Keep in mind what you're going to be trying to achieve in these facilities and what they need to be like in order to enable you to achieve that with a minimum of work and rework.

So if you need controlled temperature and humidity for processing or storing, make sure you have adequate controls in place, and make sure you have enough room to get at the equipment to clean and maintain it properly.

Now where can you find more information? Obviously, I've just hit a few of the high points of this regulation. The regulation and the preamble are at this website. I think the preamble is important because it really tells you what FDA's thinking was regarding these requirements. It tells what FDA's response was to comments.

And when you're wondering about a particular requirement, wondering what it means really, it's often helpful to back and read the discussion of that requirement in the preamble.

I would recommend that you sit down and read the preamble from cover to cover unless you're suffering from insomnia, but refer to a discussion of a particular requirement when you have questions.

My division, the Division of Small Manufacturers International and Consumer Assistance, has a lot of information on the website. You can get at this medical device quality system's manual, and we also have a device advice page, which has a tremendous amount of information on it.

And if you can't find what you need on the web, you're always welcome to call us, fax us, or e-mail us. Thank you very much.

MR. CODER: Are there any questions? No. Well, thanks. So, I guess, in the interest of time, we'll sort of move on at this point. We have the mid-morning break coming up right now.

And just as a thank you to some of the financial help that came through ISAC from the manufacturers for setting up the coffee breaks and lunch, I'd like to thank Bechman Coulter, Becton Dickinson and Cytomation for providing the money such that we can all be well watered and fed during this.

And Gerry had pointed out to me also, we do have a couple of documents that have been published in cytometry on the biosafety guidelines for the sorting of unfixed cells that came out several years ago as a biological assay method, and then a more recent non-biological assay method of trying to get some idea of how many aerosol contaminants may be spewed out in the environment.

So we'll have the break now and if we could be back by about a quarter of 11:00, we'll continue on with the rest of the morning session. Thank you.

(Whereupon, the meeting went off the record at 10:25 a.m. and went back on the record at 10:48 p.m.)

MR. CODER: So if we could start the morning session, finish off the last half here. So as I mentioned in my introduction, we're having a variety of sources of expertise brought to this.

And so the balance of the morning we're going to hear about the manufacturer's instrumentation and what they're currently doing and some of the things that might be going on, such that you can know, one, who they are and then all of us can become familiar with what the requirements might be. And it's going to be an interactive process to come to the final document.

So as far as the start of this, we'll have Todd Christian, with Becton Dickinson begin with session with the manufacturers. He's market manager of instrumentation and also manager of custom products for Becton Dickinson, and he's going to give us the quick overview of sorting of Becton Dickinson flow cytometers.

MR. CHRISTIAN: Thanks, Dave. What I wanted to do is just give a really quick overview, because I think all of the important work will be happening this afternoon, where we could all start working together to understand what needs to be done to get us where we want to be and define where we want to be.

So what I wanted to do is just define and give an overview of our current mechanisms of sorting and what we have available currently, and it's really twofold.

We have a version of sorting, which is a mechanical sort on the FACS Calibur. Can everybody hear me okay? On the FACS Calibur instrument and then a lot of what we heard about already this morning is stream and air sorting on the FACS Vantage SE.

And, again, just briefly, I want to go through what are the differences and what do the -- how do the implications differ for what we're talking about today?

So with the FACS Calibur, it's a mechanical catcher tube mechanism, and I'll have some diagrams to illustrate that, to collect cells of interest. In concept, similar to any sorting. You want to identify a cell and bring it -- get it back.

The catcher tube is -- it's located at the upper portion of the flow cell and it is actually a mechanical movement. It moves in and out of the stream and captures those cells that have been identified as a cell of interest.

One of the implications is, you'll notice, it's a relatively low performance sort because it's a mechanical movement.

So you can sort desired cells at a rate of up to about 300 cells per second with greater than about 95 percent purity.

Another thing is it's -- in comparison to stream and air, it's -- I used it in quotes, "a closed system for sorting." It's enclosed from the sample injection through to the sample output into the tubes for more of an aerosol-free environment and better safety.

A key thing to note is sorting applications on the FACS Calibur are for research use only, and for those of you who may not have seen it, this is the FACS Calibur. Here's the sample introduction area.

If you're not aware of the sorting mechanism, it's actually located in this chamber here. And this is just a diagram of how it happens. The sample flow in the Calibur, obviously, is going up, which is reverse from the stream and air sort.

It goes through the flow cell, intersects with the lasers and here's really the point of interest. Once you identify the cell of interest, this is the mechanical arm. It goes into the stream and captures the cell and then deposits those cells in a series of conical tubes.

There are a couple of issues. There's three tubes, sorts one -- you know, fills up another tube, then fills up another tube. It's not sorting into them individually at the same time.

The system can be dealt with for aseptic sorting, not in terms of a replacement, like some of the regulations and ideas that were discussed earlier, but through an ethanol rinse. It can be used with sterile PBS.

And a lot of the uses are sorting for culture and functional studies. One key thing is recommended for just cell function is to sort into BSA-coated tubes and then you centrifuge those tubes for further use and processing.

Some of the nice things about this method of sorting, although it has its performance limitations, there's fixed laser alignment and stream velocity that you don't have to deal with special operators or any intense set up.

So then with that, moving into the FACS Vantage SE, also sorting for research use only at this point, using a different mechanism, which was described earlier this morning, stream and air.

So on here there's really two areas of greatest concern for what we're talking about today. One is the sample injection, or sample input area, and then the other is the sample output chamber.

With the sample output chamber you're actually creating droplets in air, and it's all enclosed in this chamber right here, so that's something that definitely needs to be considered for a variety of perspectives. From a sample perspective, as well as from an operator and operator safety perspective.

So what happens in this space, creates droplets and then goes into a series of output tubes all in this open space.

What are the implications? Again, as discussed earlier this morning, higher pressures for higher speeds, so you're dealing with sample issues. As a result of that the -- it's a contained sample path, really, from the sample injection up to the nozzle where we use high-pressure tubing to deal with the higher pressures to attain higher sorting speeds.

However, one of the biggest areas of concern from a safety perspective are in the -- is the open environment from that laser intersection -- the sort nozzle to the collection devices.

So what have we done to kind of address those needs? We do have an option, which is called the Aerosol Management System which helps to assist in managing those aerosols.

A couple of key points. I don't want to get into a lot of specifics of it but, basically, what we do is just it encloses that sort output chamber, creates a negative pressure environment within that chamber, as well as provides a droplet containment module on that sample injection, sample input area.

Some really, I think, key points is that this assists in aerosol management from a safety perspective. It doesn't eliminate any of the health risks and it always -- anything that we do on this instrument and just sorting in the environment has to be used in conjunction really with good laboratory practice, from a safety perspective.

So I think the key thing from my perspective, I will be here throughout the day and we also have a couple of other representatives from BD here.

And it's really to -- my objection is to work with all of you to really understand where we need to go and how we an get there together to meet the requirements, and defining the requirements.

And then another -- I think there's a few areas that we need to be considering. One is just laboratory protection, obviously, for the personnel at the lab. Another is sample protection, and what is the product of that sort.

Then what about performance? We may have to make trade offs as we get into making these -- meeting these requirements, what kind of performance trade offs are people willing to make?

And as part of that trade off, which I didn't put up here, is also a cost trade off. I think we have to look at all of these things together for how to do these things best in the lab.

So that's all I had. I just wanted to really give a very brief high-level overview of kind of our current mechanisms of sorting, what it means in the context of what we're discussing today, and then use this as a launching point to kind of keep things moving based on the rest of today's discussions and subsequent discussions. Any questions?

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

MR. CHRISTIAN: There's a -- I can tell you a little bit. I don't have a lot of the details. On the sample input area, it creates -- actually, I can get information and go through it with you later today if you want.

There's a vacuum on there, so it creates a vacuum pump and it's -- has an aspiration area, so any droplets are contained and aspirated out and away from any external exposure. Larry.

MR. LAMB: Larry Lamb, South Carolina Cancer Center, Pediatric Oncology. Question about your droplet containment center. We heard the talk this morning about validation. What procedures would you suggest or have you done to show the efficiency of this containment system?

MR. CHRISTIAN: There's actually -- there's a number of different procedures, I think, that can be done and that's one thing that we're in the process of doing right now is looking at that validation process from isolating any containment and where any potential might be, determining and identifying where that is.

There's some new florescent particles that can be used and run and actually then used with a -- I can't remember the name of them. They've got a funny name.

But they can be used to identify where aerosols may have been generated, and it's just using like a black light and looking at that. So we're going through that validation process right now, both on the sample input and output area and chambers.

DR. MARTI: Todd, I had a question about the comparison between the mechanical sampling and air and stream. It would seem to me that the mechanical sampler would not be very competitive for the kinds of sorting that one envisions in today's meeting.

On the other hand, I was thinking would such sorting at the level that that works, would that be useful for animal studies, in other words, where you didn't need such large amounts of cells and is anyone using the FACS sort in reconstituting either marrow, or immune systems, or perhaps other systems that I'm unaware of in mice?

MR. CHRISTIAN: I think that's definitely a potential use. I think the thing with the mechanical sort is just the limitation on the rate of that sort.

And if you don't need a lot of cells back and don't want to sit there for several hours, or if you don't have to get all of those cells back, then that's certainly a viable option. It's certainly lower in performance than stream and air.

So it really depends on what the individual needs are and getting those cells. I mean you then have to deal with more -- a more dilute sample of end product.

DR. MARTI: I guess the argument would be you would probably be doing five or ten mice, so it would still be better to use the air and stream.

MR. CHRISTIAN: Potentially. It really comes back to, I think, individual needs and those -- the sorting performance and speeds at which -- and then also the pressure -- implications of dealing with higher pressures for the stream and air, and how that affects your cells or particles. Great. Thank you very much.

MR. CODER: Thanks, Todd. So next up we'll have Matt Ottenberg of Cytomation. He is vice president of product development for Cytomation. And I need a computer to plug in.

MR. OTTENBERG: Thank you. I thought I would approach the various product developments that we've done at Cytomation around the MoFlo kind of

chronologically, because over the years as we've placed the MoFlo into various sites which we call clinical research sites, around the country the requirements have come to us and we've done various product developments along those lines.

Now, initially, most of those requirements really dealt with biohazard and biosafety and protecting the operator, and those have now evolved into -- I think the term was coined here earlier a sample integrity issue where we really want to make sure that we don't have cross contamination sample to sample.

So under the guise of MoFlo clinical research sorting, the MoFlo, as you know, stands for modular flow cytometer, as the brainchild of Ger van den Engh.

And one of the things the MoFlo system does, since it is open architecture, it allows us to try out new things and adapt fairly rapidly and design new systems and subsystems.

So quickly, I'll do that -- I'll do a brief technology overview of the MoFlo. We'll talk a little bit about the operator safety that we've developed to date. There's some new products. It comes under the heading of CytoShield, a marketing name for sure. And then finally, some new things we're doing in the sample integrity area.

First of all, in general, the MoFlo has very high sort rates. I won't argue the statistics as we did earlier. The 70,000 events I would argue is an analysis rate and sort rates are dependent on all the things we discussed before. Our specification always greater to 99 percent purity. Again, the system is open architecture and allows custom configurations to be adapted fairly readily.

And finally, I talk a little bit about the Sort Master system, which is a droplet control and monitoring system which we think can be used a good feedback mechanism to look at failure modes in a flow cytometer.

First of all, in operator safety, originally, we developed a basically passive containment. It's a -- basically, a protective door. This is the business end of the flow cytometer, the stream comes there. There's a sort receptacle behind the shield and this door swings to the left and that door has been tested as that guideline to the current phage test, as people call it, and passed all on its own.

We then developed -- there were several requirements to create a negative pressure in that area, and then evacuate that area and so we created the aerosol evacuation unit.

It's an in-line evacuation motor. It's got an ULPA filter system ahead of it. So here's the entire unit. There's a hose that goes over to that chamber I showed earlier, creates a negative pressure there.

There's a filter system. The motor can be taken apart, the filter removed and everything upstream of the filter then can be autoclaved.

It has a filter clog warning system built it. It's an audible alarm, real loud, to let you know if there are any faults either with the motor or the filter system.

This system has also been tested to the ISAC guidelines and we probably have, I would say, roughly 30 placements of this unit.

We then kind of went to the next level of biohazard containment. Typically, we've been working with our customers -- with the individual lab safety officers.

In the absence of general guidelines, we've kind of been listening to them and talking about their individual needs and eventually, we saw some overlap lab to lab.

This is the latest thing we've built. This is a beta unit you see here. The fielding of this unit is occurring as we speak. We call it the secondary containment system, where the previous, if you will, sort chamber, we call it the primary containment system. We now have wrapped another level of containment around the sort area.

To give you an idea of some of the principal features of this system, it's basically a fume hood and the fume hood -- I'll go back here a little bit -- has a continue sash here and the sash keeps a constant air flow.

Lasers are shot through into this -- through a quartz window into this chamber and you can see that same IT we talked about there, the primary system is visible there. The sample injection unit fits inside here and, actually, a cyclone cloning array -- a cloning motor -- an automatic deposition unit fits on the left side of this.

Again, the principal components here, with the -- the blower creates a negative pressure and is evacuated through a HEPA filter. We also have some electronics which look at the airflow sensor, again as a fault indicator.

And then also there's some warning lights that can be activated either automatically, or by remote control, or based on various sensor feedback.

The other thing here is this is the waste tank. We've made provisions on this so that the waste tank can be handled externally without having to go into the shield, into the containment unit.

And, finally, recently we had a requirement for a decontamination unit, a formaldehyde plate, basically, to do a formaldehyde bombing of that system.

And then this subsystem here is an air recirculator, basically, a charcoal recirculator to get all the formaldehyde out afterwards. It's a very lengthy process though. It takes about four to six hours.

Again, I've talked about some of these things. It's a fume hood. This is a back view of the fume hood. This is an air-cooled laser shown poking through one of the quartz glass windows.

This is the detector array, basically the photomutliplier tubes and the other items. We've separated the internals of the sort chamber from the detection electronic and some of the other photomultiplier tubes.

We talked about the formaldehyde decon system. This system also has some lock down capabilities. We've considered putting some interlocks into the system as well. If a contamination alarm does go off, it could lock down the doors to prevent access until such time as the system has been decontaminated. The system comes with some remote controls to drive all this stuff.

Recently, we added to this system. Again, since the fume hood was providing a negative pressure, the next logical question was asked, well, instead of providing a negative pressure to your primary containment, can you put a positive pressure in there to provide for sample integrity.

And we've done that now. That's what we call a sample protector. It's basically a fan unit with a blower, also another HEPA system which some clog warnings as well.

So this is the primary that will be kept at a positive pressure with the fume hood externally under negative pressure.

To talk some more about the sample path, I've kind of drawn a diagram there of the places where the sample actually comes into contact with tubing and nozzle, et cetera.

So this is what we call a Smart Sampler, a sample station. It's a system we designed which encloses the tube completely to prevent the mechanic -- one of the problems we had with the original unit were when you rely on the tube to provide a seal at high pressure, any slight crack or such can crack the tube.

So we developed the sample station originally to allow for the tube to be completely enclosed within a chamber and then pressurize that chamber. And this eliminates the faults you can get from cracked tubes.

The system then draws sample into the nozzle, obviously, where we also have sheep and vacuum lines, and then there's a sort receptacle area. So these are the major components of the sample and production system.

Now one of the things we strive to do in that is to have a continuous tube length. So this is the clamshell, if you will, of where the cylinder, which rises up and seals the sample tube right there -- there's an update tube that you can barely make out right there.

That uptake tube goes through a pinch valve assembly here and then goes off to the nozzle assembly. That has now been made into one continuous tubing length and that's now consumable. It's a piece you can buy. It's about a five to ten minute exchange.

Again, there's an exterior pinch valve. With the original in-line sample valves we had we saw lots of cross contamination and we developed a pinch valve that's been tested in our labs but not tested by many individuals on certain cell lines yet.

One of the things, since we're pressurizing this chamber, we necessarily want to keep outside contaminants from potentially getting into the tube.

And what we've done there is there's a HEPA filter that proceeds the pressurization of the cylinder and also similarly on the output side of the sample sedation, when that chamber opens that tube -- that chamber vents to outside air.

So we've also provided a HEPA filter for that venting to the outside air. And there are various other devices there too, including an expansion chamber and some other things that make that go quickly.

The nozzle itself is easily removable. Well, that's a relative term. It takes about -- a five minute exchange, I would say, is also maybe a bit optimistic. It's probably closer to ten or 15 minutes.

But the system is autoclavable. Here are the components of the nozzle assembly itself. You can go ahead and take that off and autoclave it. We don't -- I don't know how many people have actually done that, but we've tested, certainly that the system survives, or that the nozzle survives the autoclaving.

Finally, within the sort chamber itself, this is a blown up picture of the IT. So normally, there would have been that primary containment door there, but I wanted to show this because this is, if you will, the sample collection area.

And you can see it's an area that's mostly closed. There's a hole in this particular shot right there, but that whole area would be closed and it could then be swabbed down and cleaned with some of the cleaning protocols that were mentioned earlier.

This is the sort receptacle itself. It can be removed from the unit, and then all the individual pieces autoclaved as well. This is a piece of aluminum, anodized aluminum and that's polycarbonate.

Finally, one of the questions that's come up, what is the viability? Earlier on we suggested what are typical operating pressures for flow cytometers.

We, typically, run at 60 psi on a MoFlo, roughly 100 kilohertz drop frequency using the 70 micron nozzle.

There have been various publications over the years. I think some of the -- here are some references. There are more. You're welcome to come to our website. We have a new link, which is a reference link that shows all the reference publications that include MoFlo's and so there may be more information of interest to this audience.

Some work has been done, of course, on the viability of sperm cells, mainly in cattle and bovine sperm. But there's also been, obviously, some work done in humans as well.

So I would suggest that there's some evidence that the viability is there, even in high-pressure systems, but I think there's a lot more work to be done there as well.

Finally, I thought I'd give a reference list of some of the customers that are doing this type of clinical research sorting. Some of those folks are in the audience, I noticed, and that's really it. Can I answer any questions?

MR. CRAM: I'm impressed with what you're doing. Scott Cram with Los Alamos. With that sort of a system, are any of your customers either using it agents which are biosafety level four and if so, how are they using it and what context? Do they move the whole instrument into a BSL4?

MR. OTTENBERG: That's been the mode to date. Most people have moved it into a BL3. I'm not aware of anyone in a BL4, but there may be one exception to that in Europe.

A lot of the initial design concepts really came from the European community. They had made a lot of requests of us to do lots of these things and that was the genesis of a lot of these projects.

I'm not aware of the various kinds of organisms that they're working on.

MR. OTTENBERG: If anyone's planning to put in a BSL4 I'd be interested in hearing from them and how they plan to manage things.

MR. CRAM: Yes, I would too.

MR. OTTENBERG: Thank you.

MR. CODER: Dave Coder, again. You had mentioned one of the mechanisms for monitoring contamination, a potential lock out system. Is that something that you've implemented yet? And I was curious how you decided one, what the threshold

was going to be, and then how are you actually doing the monitoring -- what that threshold for contamination is?

MR. OTTENBERG: Well, there hasn't been monitoring done as far as identifying the contamination specifically. That could be done and, again, that could tie it into a feedback system that could then generate some automated sequences.

So to date we haven't done anything. It is worth mentioning. I talked a little bit about the Sort Master droplet control system. One of the things it allows us to do it takes, basically, an image of the droplets as they're being formed.

And we found that to be a very useful feedback mechanism to initiate some of these cleaning cycles or at least warning cycles.

Since the -- it's a fairly sensitive device. It's, basically, taking an image of the droplets and we can detect even the slightest deviation of the stream.

The typical failure mode on these instruments is, of course, to get a partial clog, which deflects that stream slightly and causes aerosols.

One of the things that we've done is that that Sort Master monitor camera is useful for doing that. If we see a large deviation from the stream, it then initiates a shut down sequence, which we've designed but not implemented yet, is to use the cloning motor and actually have the sort receptacles on the cloning motor.

So if the stream is deflected and some failure mode occurred, it actually pulls the sample of the way to prevent contamination of the sample.

And that was, I think, shown here recently at a trade show and, again, we have yet to field it, but I think it's quite useful.

MR. CODER: And one more also.

MR. OTTENBERG: Yes.

MR. CODER: You had mentioned the formaldehyde was one was of doing a complete volume sterilization and activated carbon to scavenge that. Is there any research known about what the residulations will be as far as the effects on physiology? Because the integrity of the product is going to be one of the major issues.

Because it's, well, as Bob just pointed out, it's easy to kill everything in there, but you want to make sure that what you get out after the fact is, in fact, viable he physiologically functional.

MR. OTTENBERG: With a question like that I'm going to defer to the fact that this is really a -- the group that helped us design this is an engineering group out of England, and they are professionals at fume hood and making fume hoods. That's their game.

And we we've -- a lot of the references for what we're doing, I think you can go to fume hood manufacturers and get similar types of data for what -- for the type of thing you have.

MR. CODER: Thanks, Matt. And so the last manufacturer talk will be done by Ger van den Engh. His name has been bandied about here several times during the day. And we'll -- well, without giving his entire history, Ger was at Los Alamos on developing the high speed sorter there.

He eventually came out to the University of Washington where I was and then just recently, is now at the Institute for Systems Biology.

But more importantly, he's been setting up doing his own custom-built cell sorters under the company name now of Cytopeia in Seattle.

And in contrast, all the high-tech presentations we've had before, he will have overheads.

MR. van den ENGH: Can everybody hear me? So cell sorters are extremely useful tools. I don't need to elaborate on them, but despite all our engineering efforts, they're still very unwieldy pieces of equipment.

They're temperamental, they have large lasers attached to them. There's water streaming at bath temperature. Illuminated, but strong light. That's not going to be sterile for very long. Whatever precaution you take, there are lots of components generating heat, upsetting air circulation.

There are electronic components with fans that may blow dust from strange environments and deposited in your work environment.

And, obviously, these are pieces of equipment that have no business being in a GMP facility if you can avoid it.

So my efforts for the last five or six years or so have been to redesign the cell sorter so that we can distribute it over separate rooms. We can take all the equipment that is generating a nuisance for the GMP worker and put it on one room, and then only take the business end of the machine, only those parts that are absolutely essential for the sorting process itself, for the cell purification process, and only place those components in the GMP cell processing laboratory.

And this is what I want to present to you today, how we have solved some of the engineer problems and some of the plans that are now on the drawing board.

Now to put this in the appropriate context, I have recently joined the Institute for Systems Biology, an institute that's been started by five renegade professors of the University of Washington who, under the leadership of Lee Hood, have started an academic, non-profit institution particularly with the purpose of trying to speed up the transfer to technology from the research lab to the users.

We are now in a society where biotech highly depends on technology and the usual way of licensing to a manufacturer, and see what they do with it, and then come back to you, ten, 15 years later. Obviously, it doesn't work anymore. We'll need to transfer whatever we're doing in the labs very fast to the labs of our colleagues, and for that we need some industrial quality production unit or manufacturing capability, and that's why we founded Cytopeia in Seattle, which you could describe as an instrumentation boutique.

We do not want to be a mass producer of standard instruments. We'd like to identify challenging applications of flow cytometry, such as GMP use and develop with people who really critically depend on this technology, a better way of doing it.

This is sort of a consortium. Cytomation has chipped in and is a partner in the Cytopeia establishment and a lot of the intellectual property that you are using still comes from the University of Washington.

Here are some of the things that we're doing. I am particularly interested in sorting in the GMP facilities. Have some experience that really focused me on how

difficult it is. In the early days of the gene project, we used to sterilize our equipment pretty much the way you heard before, with ethanol or with bleach.

We would grow bacteria, or a set of cultures, find no signs of life, but as soon as you make a live read, you find lots of clones that contain bacterial DNA. And, obviously, there's a huge difference between something being sterile and something being absolutely clean.

And cell sorters are a nightmare. There are lots of nooks and crannies in the tubing that are, basically, uncleanable. Thin tubing cannot be cleaned. There's too much surface area, there's too little flow to clean tubing efficiently.

The second experience that I've had was with Systemics, a company that did over 200 bone marrow transplantation. With high speed sorting I helped them build seven MoFlo-type cell sorters in that process.

They had very good results, good takes, good survivability. As far as I know, some of that information is still proprietary. But the effort that was involved, wrapping an ordinary MoFlo in plastic and running it in special rooms was really heroic.

And it's quite obvious that taking existing experiments that were never designed for GMP use in mind, and use them in a laboratory in a responsible way, is such a large effort that it's not going to work and most of us will not be able to do it. We need differently designed cell sorters in order to make it work for GMP.

So what is so special about GMP sorting. If we were to design an instrument from scratch, what are the things we would think about?

Of course, there's a safety issue. We want to do it so that whoever runs the instrument is protected and also, there should be absolute sterility and cleanliness. Whatever we put in should come out without any additions, either they alive or dead.

Then we need to really concentrate on ease of operation. We all know that the current instruments that we have are individuals. You heard the story this morning.

Joe can really keep the cell sorter in Los Alamos running, but not everybody can. Only the operator who is married to a particular machine knows which corner to kick when the machine is not running, which wire to jiggle, where the rubber band needs to be replaced.

That needs to get out of the picture before you have clinical sorting. You're dealing with a community of people who could not care less about the technology. They want the cells. The technology should be invisible to them. That means we have to automate it and we have to make the operation of the instrument totally transparent.

That means we have to have converging alignment procedures. We have to have procedures that tell you if you turn knob A until a certain condition is met, then do you knob B, then you know the machine is properly aligned and you do not need the critical eye of Joe who says well, maybe you should tap it a little more and then it's really optimal.

You have to have standardized procedures that tell you when the machine is running properly and when not.

Of course, that is a link to quality insurance and also you need, whenever you do something, experts that agree on what the criteria are. You need to be able to consult

a second and third opinion and, in a perfect world, those opinions would all agree with yours. They should be objective criteria that those opinions should be based on.

This involves probably remote monitoring. The world experts in this field are not that plentiful and it will be ideal if an instrument that was running about which some questions arose you could in due time access the manufacturer, ask them what they think about the experiment that's going on. They should have access to all the data and then be able to tell you this is right, or maybe you should stop, or maybe you should replace stuff.

We need increased reliability. We need to do much better than we do now. Again, that's basically an engineer problem. The biologists would not be interested in that.

And so reliability will involve redundancy. There are a lot of critical components in the cell sorters that maybe we could have in multiple elements so that if an element is failing or is suspect, we could put it to sleep. This is particularly easy to do with electronic server boards, and then maybe wake up a board that is in a standby mode and kick it into action, and deal with technical failures that does not total block or hinder the experiment.

And finally -- and in this mode, remote monitoring is very important. This diagnosis of the cause of the problem should be done by experts and, ideally, at the manufacturer or at some control center that is specialized in these things.

And finally, of course, we need an enormous amount of documentation, as you already heard this morning, that's really in clean procedures, spells out what the machine should do, how it should perform and how you should behave.

Now the instrument that I've designed lately is called the Influx. It's shown here as naked aluminum shell, black anodized aluminum. It doesn't photograph very well.

But this is a very small instrument on a two by two foot footprint. All the electronics that need to be operated by the operator are here under this plateau.

And then we have a cavity that is totally enclosed, which the cells are collected. There's a separate motor and slides that can bring the sample collection to thin it out automatically, and then all the steering optics and the sample are placed on top.

Here's a top view of the instrument. This is the area that works, where the cells are being sorted, where the live path are brought in and collected.

This is the detector ray. It is strapped onto the back of the instrument. You can have up to 11 or 12 detectors and in the smallest version, a small laser would fit on the same footprint and you have a self-contained cell sorter.

Now one of the nice properties of this instrument is that there's one plane to which all live path go to. So we can isolate this instrument, the business end of the instrument, from all the supporting hardware by a glass window or a plexiglass window that separates two rooms.

And to illustrate that I have this picture, where you see the Influx sorter encased in a plexiglass housing that one could characterize as a containment and right here is a nice, clean partition that could actually be the partition between two rooms.

This instrument is now being produced as a small laboratory instrument that you can place on your lab bench for high-speed sorting. It's quite a bit more complex

than the current generation. This was the official introduction at ISAC last year that we did in association with Cytomation.

And what I want to show here is that this is the actual influx sorter still in the covers, but all the support electronics is highly flexible. You can rearrange it, you can put it in any configuration that you want, so that it suits your needs.

So how are we going to adapt this instrument to GMP sorting? I've already told you this instrument lends itself to be operated across a partition between two rooms.

We are going to separate, or we are separating the cell separation module from the modules that require technical service.

Now this is maybe even more of an advantage than you now realize, because it means that the people are comfortable with the technical stuff, with the lasers, with the computers, with the electronics, can stay outside of your GMP environment.

They can wear their street clothes. You don't have to have this rare breed of a technician that you can train him in both the aspects of technology and cell processing.

There's two spheres. We have the cell tech lab where the cell technicians take care of the cells, and we have the technical environment where the service guy from Coherent, or from the computer manufacturer, can come in in his street clothes, doesn't to put on a bunny suit, can do his repairs and get out of the way. We also do not need to shut down the GMP facility whenever there's a problem with the laser.

Secondly, what we're doing is that all parts that are contacted to fluids must be replaceable. And we don't mean a quick exchange or replacement of part. But we

have designed a disposable nozzle with all tubing external to the instrument. No tubing should go through holes.

You should not have to splice things through little orifices, but all the tubing is external. The whole units, sheath, tubing, valves, nozzle is one piece that can be manufactured at a reasonably low cost and can be replaced within one or two minutes.

And then the instrument has the terministic alignment procedures. There are processes that say if you do this, this, and this these are the diagnostic criteria. If that's the case, the machine is optimally aligned. Alignment, drift detection -- there's ways of detecting whether the machine is going out of operating range long before the operator sees it.

We make sure that we have a quick turnaround time. If it is down, it can be quickly fixed and up and running again, and then finally, we have a primary and secondary containment area and we're installing remote monitoring operations for quality assurance procedures.

Very quickly I want to show you a few aspects. This would be the set of a GMP sorter. We have the lasers, computers, electronics, detectors in a machine room. There is a plexiglass shield separating the two rooms, and only the machine that has a primary containment unit is sitting inside the secondary containment box, and some of the electronics that are needed for diagnostic purposes are duplicated in the GMP facility.

But operators in both rooms can take hold of the instrument, align it and diagnose what's going on, and the two rooms can easily communicate because there is a plexiglass partition.

This is the disposable nozzle. A very simple unit. The parts that are in contact with the liquids are straight -- either plastic tubing or stainless steel tubes. There are no cavities, complicated areas. It's all one straight shot from the sheet tank to the nozzle tip.

In our case we add the acoustic vibrations that generate the drops in a separate vibrating body that horn shaped. This ring here is the vibrating element and the acoustic rays dribble down the plastic body and then are transmitted to the liquid here at the tip.

We do not have the problem with the nozzles that some of the acoustic baths go through the liquid where it might be disturbed by air bubbles or that require a construction of the nozzle that give undesirable quantity. Cavities -- this is a straight shoot, and the parts that need to be replaced can be made for pennies, except for the tip.

In this case, when you have a nozzle clog, you do not unclog the nozzle. You do not take off the tip, but you basically pop in a new nozzle. The nozzle sits in an indexable shoe that always comes back in exactly the same position that you took it out of.

There's video diagnosis. This -- when you seen this picture, here we see the three in holes. This is the jets. The edges are nice and sharp. We see that the particles go nicely through the center of the beam.

We see that the laser is nicely aligned with the center pin hole. When you see this, the alignment of the instrument is optimal. There's no way to get it better. Joe can go home now. Any technician, anybody can look at this, get a video screen and know that the machine is optimally aligned.

I teach people who come in my lab in five minutes how to align the flow cytometer and it will be right on the money every time if they just look at this image.

We're now adding piezo feedback elements. We have the laser steering optics on piezo elements so we can with a voltage control the position of the excitation beams. In this case we are scanning the laser beam through the jets using a piezo voltage.

You'll see that, of course, the particles that go through the stream, the florescence goes up as we -- as we have the particles better focused in our laser beam, and then goes down again as we screen out.

If we would only inspect this area here where the signals are flat, and we would vibrate the mirror a little bit in this position, and you do a regression analysis on the particles that combine, you find a nice horizontal line.

But as soon as you start falling off that optimum, you'll see the regression analysis of your intensity will be away from horizontal and the direction of the slope will tell you in which way to correct your mirrors. So this way you can dynamically, by doing a statistical analysis of your points, keep your instrument properly aligned.

And this mode of detecting instrument alignment can tell you that something is going out of whack long before you would actually notice it as a human operator.

What then would happen is that the instrument -- so the instrument -- you can put the instrument in approximate alignment. A feedback loop will then lock it into optimal alignment all the time and will let you know when it's getting out of comfortable

control range, shut down the machine, move out the sample, and tell you to take corrective actions.

So that then is the influx machine. It has its own internal cavity that's lined in stainless steel. Has a UV light to sterilize the cavity overnight when you're not running. Stainless steel deflection plates that can be opened -- to sterile tape for sorts.

The nozzle here, the disposal nozzle is in a closed cavity. All the cavity apertures are sealing with quartz or with rubber o-rings and sealed off so there's absolutely no way -- or there's very little possibility for either particles to enter the sort area or to escape from it.

There's a plexiglass door closing off the primary containment and then the whole unit is sitting inside a secondary container that can be pressurized according to optimal needs.

Very quickly, I would like to point out a few projects that are going on now, because it's not a finished product. We are developing these facilities in close association with researchers and with FDA and biosafety consultants.

We're on the verge, but we have been on the verge for several years now, of starting to build two parallel sorters for the new gene therapy lab in Seattle.

Of course, the reason why it takes so long is because we really want to make sure that all precautions and all measures are taken correctly.

I'm working with Amsterdam Academic Medical Center to build three parallel sorters for their GMP laboratory. This is the layout of the Amsterdam group.

We have -- I apologize. It's in Dutch. This is the machine room. Some of you may have -- where there's an optical table that penetrates the separation between the cell separation lab and the machine room.

There's a hermetic rubber seal that seals the optical table in this wall. Down here you have all the technological connections.

If you look from the cell processing lab, the unit looks like this. This is a simple eight feet optical bench that has three influx sort heads and attached to the windows has computer monitors and other electronic devices that can be monitored by the operators in this room.

The idea is that two of these instruments will be working in parallel and the third instrument would be in a standby mode so that when there's a critical failure, if you're operating while there's a surge, a unit then can be kicked into action and can take over.

The sorting process, I have some pictures, not very high quality, but they do give a good idea of what's going on.

This is the -- here you see the back of the machine room, the yellow lights. This is under construction. This is the slot that optical table comes through. It's supported by three levels here.

And here you see the blueprints of the influx sorter in real life to show that the part that is actually in the processing lab is very, very small. And since it's enclosed in a secondary containment really not, it doesn't affect air flows or is not a real danger for the environment in the GMP facility.

This is me in bunny suit in the cell lab. This is the cell processing laboratory here. The yellow light, again, is the machine room. If you look very carefully you'll see two electricians with their nose pressed against the glass looking at what's going on.

And then here we have a -- what we use to process the cells. And then next door are virtual manipulation rooms where the cells can be either -- before or after sorting be properly prepared or processed.

We've also started discussion with the MD Anderson Hospital. These are the blueprints that I received a few weeks ago where they are planning a similar facility in their cell processing lab where there is a clean facility that can only be entered with the proper procedures and training.

There, again, are three sort heads in the cell sorter room. But there is a laser or a machine room that can be entered in street clothes by people who do not require special training.

And are very excited that there are now several facilities, both University of Washington and MD Anderson, and perhaps others, that really take this issue of GMP sorting seriously.

I do not think it's the proper approach to say okay, we've got a machine. We've got to use it. How do we wrap it up for GMP use? You really should be looking at these machines with new eyes.

We know what they can do. We heard already this morning earlier that we really should involve GMP use at the design process of the instruments. That's what we're trying to do.

What we're building is not the ideal instrument, it's not the perfect instrument, but at least we're looking at it from the point of view of the GMP user and try to make these things as safe and as practical as possible. And I thank you very much for your attention.

DR. GEE: Can I ask a question? Baylor College of Medicine. It seems to me that the next logical extension to what you're doing is you have the level of containment in the GMP facility that you may not even need a full GMP facility to have the sort heads in by just increasing the level of containment. Is that going to be possible by putting these in some kind of a HEPA filtered cross 100?

MR. van den EGNH: I would assume it's possible, but I'll leave that up to the people who can judge what containment level is needed. I'm the insolent guy. We try to make it as solid and as --

DR. GEE: It's very impressive.

MR. van den ENGH: It's easier for the regulators to make a step back rather than to -- for the routine manufacturers to make a step forward. So, yes, of course, one can always relax safety conditions, ones that are very, very stringent.

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

MR. CODER: Could you use the microphone, please, and introduce yourself so we get a -- we're doing a transcription of this, so we need to have an accurate record.

MR. MOSTOWSKI: Howard Mostowski, FDA, CBER. I was interested in what size tube are you collecting in?

MR. van den ENGH: Well, I have not decided on what tube to collect it in. Again, I would like to discuss that with the people who do the experiment.

As you are aware, none of the applications have really crystallized yet. There's still -- we know we're going to use cell sorters in GMP facilities. What the actual applications are going to be we don't know.

The most convenient method of collection will depend on what we're going to do with the cells afterwards. But the collection mode is, I believe, fairly trivial. We could adapt this instrument to any --

MR. MOSTOWSKI: Well, that's not true, neither. Because I do the sorting where I am and I use a slow machine and I have to sort for several hours. And the wider the orifice, the more danger it is.

Also, the distance from the actual droplet to the tube itself, is that being considered?

MR. van den ENGH: Unfortunately, high-speed sorting means you have work with high velocities and you need a certain minimum path link to deflect the drops out. You can only but 60 or 70 volts in a drop. If you put more on the explode. So you need a path link just to get a few millimeters of deflection. But the way at least we've

engineered it in this machine is that we have stainless steel deflection plates that are covered with sterile tape and they are as much as possible encapsulating the sort stream so that there's as little possibility of air exchange with the environment as possible.

But I do agree that this is the Achilles heel of the whole process. We have about six inches of path there and could pick up all sorts of contaminants.

MR. MOSTOWSKI: Thank you.

DR. MARTI: I really appreciated your presentation of this desk top high-speed sorter in an upcoming issue of "Seminars in Hematology," one in that edition mentions that very soon we will see such a device, and it's amazing that it's come so quickly.

MR. van den ENGH: While the ink is dry.

DR. MARTI: Yes, the ink is -- I'm not even sure it's published yet. But have you tried in terms of the so-called ISAC guidelines in testing this environment inside the sort housing?

MR. van den ENGH: No, I've not.

DR. MARTI: It seems to me from the previous speaker that just putting a door, having a door on that sort area is that first step, that aerosols reduce significantly with that.

And then everything else that you've added, of course, are marked improvements. The fact that the whole thing can probably be sterilized.

We are a little bit concerned about the testing of the droplets, in terms of interjected phage into that system. We think more like what Scott was talking about earlier,

microbeads that can be sterilized probably would be looked upon a little more favorably. Anyway, I'm very impressed with your system.

MR. van den ENGH: The system definitely has not been run through any validation procedures and we need to that with the users and whoever is interested. I'm very pleased about this meeting, because I've been wanting this meeting for a long time. This is very, very productive.

DR. KEANE-MOORE: Hi. Michele Keane-Moore. Two quick questions. One is could you tell me what the upper particle size limit is currently for your system?

MR. van den ENGH: There's no limit size. We could make it for footballs, but the machine would look a little different. We build cells sorters with the -- of 1.2 millimeters sorting 300 microparticles. But your frequency goes down a lot. Size imperviously relates to sort speed.

DR. KEANE-MOORE: And the second question is how many parameters can you sort on?

MR. van den ENGH: Up to -- in the current version, up to 32 parameters. We could make it 64, but it would be half as slow, or half as fast, twice as slow.

MR. LAMB: Larry Lamb, South Carolina Cancer Center. You touched on something that -- I'm going to direct this question on something you said but also to Dave, if he's like to comment as well.

You showed us three sorters lined up in your facility in Amsterdam. And one of the things that has been a concern of mine is that you can have a lot of beautiful equipment, but if you're in the middle of trying to separate cells for a patient that you're

going to have a procedure scheduled for the same day, and your sorter goes down, then that equipment is functionally worthless to the individual who is supposed to be benefiting from it.

I'd like to have just a couple of comments with regard to A, what sort of requirements should we have for keeping this system operating?

Should we have two sorters, or three, perhaps, or should we have a factory-trained technologist on site. This sort of thing is practically very important for those of us who look at doing this in real life.

MR. van den ENGH: Yes. There should be an -- of risk analysis. We should know what the chances of failure are. Those should be reduced.

The three sorters are based on the Systemics experiments. Systemics ran three machines in different groups, had one in the standby mode and was operating too on the patients, so that even at an enormous calamity where two systems would go down, there always was one system, at least, that could be used to finish the sort.

So that's what we're going on. But right now, we do not have the numbers, we do not know how often these machines fail. The information we have from the research lab is anecdotal. We know they break, but not how often. When we have those, you can really make rational decisions. Did you want to --

MR. CODER: You addressed it reasonably well, you do have to have some idea of the standard measure of what's the mean time between failure of various components on it.

And somebody actually does that work and people who operate them, you get a sense of what it is. But I think the important thing that you're addressing is that if you're doing therapeutic procedures and you're trying to schedule patients, you have to have one, the assurance that you can that, and then also from the standpoint of prepping the instrument and giving the requirements of making sure that it meets the criteria as far as sterilization goes that you can now bring in the next patient's sample.

That particular time segment is going to be very important in terms of how you schedule. That's one of the critical issues that we'll need to address as well.

I just wanted to point out, we sort of schedule this and we're surprisingly, exactly on schedule. We've tried to leave the remaining time this morning for questions. And so we still have almost all of the speakers who were here this morning here.

So any questions that pop up in your mind, now would be a good time to address them. And in that context, I've got three questions here.

A couple of trivial things, but -- well, I guess we'll go to the trivial ones and then go to the more philosophical ones.

You had mentioned UV sterilization for one way of cleaning up the inside of the sorting cavity itself.

Now I know that it's very commonly used, but as a directional source, it's going to have shadows inside. And shadows, as far as UV, is not going to reach, even though it scatters considerably, is there anything known about the efficiency of that, in terms of various parts that are going to be shattered inside the instrument cavity that might harbor microorganisms.

MR. van den ENGH: Well, this is the nice thing about the closed cavity, that it's all lined in stainless steel, which is high reflective. And, actually, the lining is designed such that it reflects into the hidden areas as well.

So we really scramble the lights. The tray is stainless steel and reflects back up, so we try to maximize the eliminating of the light throughout the whole cavity.

I only see it as an extra safety precaution. We cannot use -- rely on UV to totally sterilize, but it's an extra step that comes in that is particularly appreciated by PCR people, because they really like to have UV light around in the cavity. But I do not see it was a fool proof way of sterilizing.

MR. CODER: I'm sure there's a considerable amount known. I'm sure Bob could tell us that about the wave length of UV light, the amount of energy required, duration and so on and so forth.

MR. van den ENGH: Well, this is all UV that -- what is it -- the lamps that work at the maximum absorption of DNA. It's highly effective in destroying DNA. I don't know how effective it is in actually killing everything.

MR. SAUSVILLE: I think we normally see ten to the third log reduction with UV or not much more than that, but whatever -- if you were using it as a sterilization procedure, that would need to validated.

So all the little nooks and crannies, if there were any or have organisms -- well, I mean you want to demonstrate that you can kill them in all the little areas.

MR. CODER: Well, along those lines, instead of directional radiation methods has ozone, in fact, been used -- because that would be a relatively easy way.

I read a couple of -- I think there's a company in Palo Alto that's setting up, and if you have very dry air, you simply discharge -- run a discharge through it, it creates ozone and the available oxygen in the air. And ozone is a really good --

MR. van den ENGH: Well, ozone is actually a very -- a byproduct of these lamps.

MR. CODER: Producing ozone as well.

MR. van den ENGH: Yes.

MR. SAUSVILLE: We haven't' seen too much ozone for a room, this being a very small room, sterilization. But there have been some reports of it, but we haven't seen any in the applications yet.

MR. CODER: And so there's been no validation of ozone as a method for sterilization that you know of.

MR. SAUSVILLE: The only ozone usage that we've seen is in water systems where they actually put ozone into the water system.

MR. van den ENGH: The lamps are cheap. They're 30 bucks and it's three locks for free, basically. When you can put one in, put on it.

MR. CODER: Okay. The other trivial question I had was on the plexiglass partition between the two rooms.

Now if you're running a laser through there, are you going to have a UV transmissible window as part of that?

MR. van den ENGH: A little window in there.

MR. CODER: Yes. Plexiglass would be very good for getting your UVB. So the more philosophical thing, one of your criteria you listed at the start was having absolute sterility as far as clinical sorting goes. Now you really don't mean absolute sterility, do you?

MR. van den ENGH: I mean -- you're correcting me already. It's aseptic. Is that what you mean?

MR. CODER: Well, aseptic -- what is the -- well, there are criteria like for particulates in air that you can have one colony forming unit for like ten cubic feet of air as an acceptable for I forget which level classification that is.

But there are definable criteria. And that's the thing that we really have to be moving towards. How many bugs can we tolerate per unit, whatever volume, mass, time, et cetera that will be acceptable criteria as far as "definition" of aseptic operation.

So, again, it's the whole-risk based thing again. There are no absolutes. But if we define what the baseline criteria are and we can demonstrate that successively, then we're basically done.

MR. van den ENGH: Well, it has to be pretty high. If you do 200 patients a year, none of these should be contaminated.

MR. CODER: Yes.

MR. van den ENGH: Even if you do it for a century, none of them should be contaminated. So it may not be absolute, but it will be pretty darn close.

MR. CODER: Anyone else?

MR. MOSTOWSKI: This is along the lines of trivia. I'm a little biased here, too. In the normal population, left handed people are few. In the science and math community, the percentage goes up vastly.

I'm left handed, so I'm going to be a little biased. And I find myself being a little bit clumsy and it's a little bit harder for me to get around, because of the machines are designed for right handed people.

MR. van den ENGH: Well, you'll be relieved to know that I'm left handed.

MR. MOSTOWSKI: Well, most of the science community is.

MR. van den ENGH: And I'm trying to please the right handed people in the audience.

MR. CODER: Does that mean one of the instruments requirements then is the operator has to be right handed?

MR. SAUSVILLE: I'd like to speak to that just briefly. I mean a lot of what I've seen in the last two speakers reminds me of barrier isolator technology that we see for filling operations.

And in the design of the barrier isolator, quite frequently, ergonomics is brought to bear, because they knew who's going to be operating the isolator. If it's smaller people and how they need to place the rubber gloves and things like that.

So I mean I can see that you're custom building the machines anyway. If you know who the operators are going to be, you can custom build it for them.

MR. van den ENGH: Right. There's the advantage. You have a part that you know is going to be operated by the cell tech with rubber gloves on and you're going to tailor the machine to that operation. And you don't need to worry about all of the other stuff people are wearing their street clothes.

MR. CODER: Anyone else? No. I think we're reasonably on schedule. So at this point then we have the hour break for lunch, and lunch will be served right out in the entrance to the room. And if we could have everyone back here by 1 o'clock, we could then start out the afternoon session.

I'd like to thank all the speakers this morning. It was a very good start out. Thanks.

(Lunch break from 11:55 until 1:02.)

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

1:02 p.m.

DR. MARTI: Before introducing the session chairperson for this afternoon, I too would once again like to thank the manufacturers for sponsoring the coffee break and the lunch. That's really appreciated very much.

This afternoon, Michele Keane-Moore is going to chair this session and she is the other collaborator, co-organizer for this meeting. Michele got her Ph.D. in immunology and infectious disease at Johns Hopkins University in 1994 and undertook post-doctoral training in DNA vaccines in the division of rheumatology, department of medicine -- school of medicine at John Hopkins.

And then she spent a brief time in the manufacturing world, private enterprise at Osiris Therapeutics, working with mesenchymal stem cells.

And she comes to the FDA after a faculty appointment in the Department of Environmental Health and Science at John Hopkins School of Health -- School of Hygiene and Public Health. Michele.

DR. KEANE-MOORE: Thank you, Gerry. Good afternoon. I just have a few remarks, as this afternoon's session gets underway. And I wanted to say how delighted we are to see so many people here from the flow community to participate today in this discussion, what will be an ongoing process to develop these voluntary -- a voluntary protocol for cleaning and sterilization, and to address other safety issues we move forward into sort of a new developing world of clinical flow cytometry.

It seems like at this point, a lot of people are sort of poised on the cusp of starting a clinical flow program or are thinking about it.

We have heard this morning from different -- from our manufacturers that there are many different ways of approaching these areas of safety and sterility.

And I think that that is something that we will have to deal with on our end, which is that different people are going to have different approaches. One size is not going to fit all.

And we are going to try to be receptive to that on our end, and to try to work with people.

I would encourage you all to contribute this afternoon. As Dave mentioned, we have put together a working group. However, all of you should see this also as an opportunity to enter into the dialog at this point.

The first useful tool that we will have are the transcript from this meeting. When people come to us now and say we want to do clinical flow, we will first refer them to the transcripts from this meeting.

So please if you have -- a lot of you have experience. A lot of you have been out there doing flow cytometry for many, many years. People have been flowing cells and flowing gametes. A lot of you have thought about these issues of sterility, of cell quality coming off of the machine. We need to hear what your experience is.

The focus of this afternoon's session is to basically hear from our clinical flow pioneers who are out there as we speak braving our regulatory hurdles to use flow for readministration to people.

And they're the ones who we will be hearing from, but we also want to hear from all of you who have something to add in this discussion.

So without further delay, because we have many speakers to go through this afternoon, let me introduce this afternoon's first speaker.

And I would just encourage, because we do have a tape schedule, I would encourage people to try to stay within their time frame as much as possible, as a courtesy to the other speakers, and also to everyone who's gathered here today. Thank you.

Our first speaker this afternoon is Dr. Frits van Rhea from the University of Arkansas Medical Center. And he will be speaking on just basically safety of cell sorting for flow cytometry.

MR. van RHEA: Thank you, Dr. Keane. First of all, I'd like to thank you for inviting me to speak at this meeting. My presentation today will focus on the application of cell sorting to the generation of CMV-specific CTLs on a clinical scale.

I will address also the safety issues involved in the purification of the cells and the culture system we've developed for making CMV-specific CTLs. I think you can go to the second slide.

I should add that this work was done at the University of South Carolina in collaboration with Dr. Larry Lamb, who provided of the cell sorting expertise.

Now, when it comes to making CMV-specific CTLs, you could take essentially one of three approaches. You could first of all vaccinate a donor with CMV antigen, increase the number of CMV-specific cells in the blood and directly isolate CMV-specific cells and look at and infuse them to patients.

One could also make large-scale bulk cultures with antigen presenting cells from the donor and low dose with CV antigen, add T-cells from the donor and culture until you think you've got enough antigen specificity.

Now the final approach is to do a relatively small scale culture, and then culture until you've got antigen specificity and then by flow sorting isolate your antigen-specific T-cells. Then you've got a relatively small number of T-cells and those you can rapidly expand to a clinical scale.

And that's the way we chose, particularly because the bone marrow transplant program in South Carolina focused on identical transplants and we wanted to avoid allo-reactivity at all costs, because our experience was that even infusion of small numbers of allo-reactive cells after transplant could give rise to severe GVHD. Next slide, please.

This slide shows some of the methods for isolation of antigen-specific T-cells. There's, of course, the method of T-cell cloning, which is rather arduous and cumbersome, and not suitable as a clinical routine.

It should be said that the group of Phil Greenberg has provided a very eloquent proof of principal that it's feasible to clone out CMV-specific T-cells and reconstitute CMV-specific immunity after transplant.

There are also some new developments. For instance, Miltenyi has just marked a cytokine capture reagent, which attaches to the cell and can capture, for instance, onto the gamma interferon.

So you can stimulate your cell with CMV antigen and the cells which respond will release gamma interferon that gets captured by the cytokine capture reagent, and then you can come in with a monoclonol antibody to gamma interferon and you can flow sort out your antigen-specific T-cells that way, and you could go after your TH-1 population.

And then at the bottom of the slides there are, of course -- there's some big -- there's been a big revolution in the identification of antigen-specific T-cells with HLA tetramers, and more recently dimers have been marketed. And I'll talk about that in more detail later on. Next slide, please.

Now the immune response to cytomegalovirus is rather unique in that most of the response is directed at the Pp65 matrix protein.

And in the context of HLA-A0201, a certain peptide, 495 to 503 is the immunodominant peptide. So HLA-A0201 is quite frequent in the caucasian population. So if you go after peptides, then that's a good starting point.

When I worked here at NIH, we tried to make Pp65 protein and that is quite instable. And for that reason we first started with the peptide while I was trying to solve the instability of the Pp65 protein. Next slide, please.

Here is shown the binding of the CMV peptides to HLA-A0201 in the black line and you can see that it binds in a dose-dependent fashion. And control peptides here are peptides derived from protein. Next slide, please.

So our idea was to develop donor-drived CTLs from a single blood draw and culture them to clinical scale. We would use dendritic cells, both with the CMF peptide and add CVA position T-cells from the donor.

Then we would isolate with HLA tetramers highly specific -- highly CMV specific CTLs and then rapidly expand them to clinical scale. Next slide, please.

When it comes to safety aspects, I think it's quite obvious that you need to use cytokines media and devices which are approved for clinical use and you need to have clinical grade antigen.

One might want to avoid fetal calf serum because it's immunogenic, particularly for the culture of dendritic cells. There are also some possible safety aspects involved with fetal calf serum.

One could think about the transmission of viral diseases. I think the whole of Europe is very worried about Mad Cow Disease, so I don't know whether or not they'll live. Come over to the U.S.

Then you need to have a quality control and assurance program in place and, of course, you need to meet the necessary regulatory guidelines. Next slide, please.

So how did we start? We start with a large blood draw. We transformed the cells -- transformed to a small number of cells. We isolated B cells and we treated them with CD14 ligand in order to regulate -- and accessory molecules.

Then we took CD14 cells and differentiated them into the dendritic cells, the DMCF4 and CD14 ligands. And from the CD14 negative fraction we isolated CD8 cells. Next slide, please.

So we added to this -- we posted dendritic cells with the CMV peptide, added the CD8 positive cells for the primary stimulation and used the CD14 ligand -- it's being emphasized for restimulation.

Then we have the CMV-specific CTL line, which we stained with HLA-0201 CMV peptide loaded tetramer, and we flow sorted and we were able to isolate an antigen specific -- CMV-specific T-cells which were put into a rapid expansion protocol. Next slide, please.

Now prior to flow sorting, we got very good specificity. In the top panel on the left you can see killing of autologous PHA blasts, positive CMV peptides and those cells were very efficiently killed.

The control cells, both with peptide from the 7 protein, which also binds to HLA-0201 were not killed. Neither were natural killers, sensitive to the cell line K562.

We also looked at CMV-infected cells, the MRC5 cell line, which is permissive to CMV infection, and which is HLA-0201 positive and that cell line was also killed and not infected cells were not killed.

Most -- correction. All our cells lines were CDA positive and stained to a larger -- to a lesser or a larger degree with HLA-0201 CMV peptide loaded tetramer. Next slide, please.

This will tell you a little bit of background about tetramers. The interaction between the T-cell receptor and the HLA molecule loaded with peptide is characterized by high on and off rate. In other words, you cannot stain a specific T-cell with an HLA monomer.

So Jonathan Altman in his Institute of Molecular Medicine in Oxford developed HLA tetramers. So he added to the alpha chain of the amide sequence one molecule and Burr A recognition site. If you don't add the enzyme Burr A and biotin, your HLA plus one molecule gets biotinylated.

By adding biotin macroglobulin, your peptide of choice which is able to bind with to HLA-0201, you can get a biotinylated HLA molecule.

You can form tetramers by adding strephavidin in a mode or ratio of four to one. So there you have four HLA molecules linked together and to strephavidin you can also bind a fluorochrome.

So then you can get stable interactions with your T-cell and you can visualize your T-cell which, of course, is incredibly powerful -- which is proven to be an incredibly powerful technique for visualizing T-cells. Next slide, please.

So here on this cartoon you can see HLA-0201 tetramers interacting with multiple T-cell receptors and providing a stable interaction. And you can double stain the CD8's and your tetramer and isolate very easily your antigen-specific population. Next slide, please.

For flow sorting we used a MoFlo cell sorter from Cytomation. You've heard a lot about those this morning. I think it's important to point out that the cell sorter we used principally had features related to sterility to protect the operator.

But, as you heard this morning from Mr. Christian, there will be a fume hood designed for the MoFlo cell sorter and there will be positive air pressure in the sorting chamber which

shoots it out to the protection of your sample, if you think about reinfusing material to the patient.

However, I should say that with the current version of the Cytomation cell sorter, we actually didn't encounter any problems with sterility, which perhaps pays tribute to the skills of Dr. Larry Lamb and his staff in using the cell sorter. Next slide, please.

So here is an example of what we achieved with purifying CTL lines. On the left hand panel, you have on the horizontal axis CD8 FITC, and on the vertical axis in each panel HLA-0201 tetramer loaded with CMV peptide.

Pre-selection. There is quite clearly in the lower right quadrant a population which is not tetramer positive and which is not -- which does not label antigen-specific T-cells.

After selection, you can get nearly 99 percent pure T-cell population. Then we put our cells into a rapid expansion protocol and you retain antigen specificity, although there were still some cells cropping up which were, obviously, not staining with the tetramers and that probably relies on the way we did our expansion procedure, which is shown on the next slide.

So we took our CMV-specific T-cells which have been flow sorted. We co-incubated them with EBV transformed -- autologous EBV transformed cells loaded with CMV peptide and added a three pool of allogenic feeders.

And that's the thing we possibly shouldn't have done because that may have expanded some non-specific cells. Then we added IL-2 and a submatogenic dose of OPT-3 and we cultured for up to ten days. Next slide.

The cell -- the type of cell numbers we've got you can see in the right hand column. And we got between 48 and 92 million cells.

Now when you think about the cell numbers required for EBV-specific -- EBV-lymphoma specific T-cell therapy, or at least prevention thereof, you require in the order of one to two times ten to the power of seven T-cells per liter squared. So these numbers fall well inside that range.

So if you take EBV lymphoma as our example, then this number should be adequate. Next slide, please.

Now our expanded CTLs were CD8 positive. Interestingly, we had cells which were triple positive for CD38 and 56, and that has been recently shown to be associated with the ability of exerting a high degree of cytotoxicity, especially CD56 on CD8 cells. Next slide, please.

Our cells were of memory phenotype. They were CD45 RL positive. They were activated -- that is, HLADR positive -- and as expected, they had non-regulated L-selectin. Next slide, please.

It was of interest to note that most of our cells were VLA4 positive. That's positive for the alpha 4, beta 4 interferon which has been associated with the ability to transgress endothelium.

So although we did not formally demonstrate this, this does suggest that our cells should be able in vivo to go into tissues. Next slide.

We also looked at the cytokine profile of our cells, and the cells we had expanded were secreting gamma interferon -- in tumor that comes across as factor alpha -- and not interleukin-4. They were, therefore, clearly, of TH1 phenotype. Next slide. Or TC1 phenotype, I should say. And the cells killed CMV-infected fibroblasts and not non-infected fibroblasts.

So in conclusion -- next slide -- we think that we can make with dendritic cells plus CMV peptide, for patients who are HLA-0201 positive, CMV-specific CTLs. Then we can isolate, by flow sorting and staining with tetramers, a highly antigen-specific population.

And all of this is done in a small-scale culture. And then we can rapidly expand these cells to numbers which might be suitable for clinical immunotherapy after a bone marrow transplant.

And I should also point out that we did all of this by drawing with a single blood draw from the donor. Thank you.

DR. KEANE-MOORE: Does anyone have any questions? If not, why don't we move onto our next speaker. Dr. Frank Mandy from Health Care Canada.

MR. MANDY: Good afternoon. It's a pleasure to be here. Thank you for inviting me. It's lovely to see the blossoms, tulips and cherry blossoms. We are at least a month behind you in Canada.

As you can see, I have a long title because I have very little to say. The reality is that my lab was designated as a national resource center six months ago, and we have probably the safest sorting facility in Canada. We sort fixed cells, and that's safe. We are in the process of revamping this and we will be sorting infectious cells.

So the perspective I have for you this afternoon is that if a hammer is the only tool you have, that pretty well all challenges look like nails. I'm looking at it from the perspective of running samples that come from HIV-infected individuals who may have opportunistic infections of any sort.

In addition to that, I would like to also suggest to you that the world is changing. It looks very much like that within a year, perhaps less, we going to pay attention to the southern hemisphere as well.

Drug trials and vaccine trials will start where the disease has high prevalence and frequency and some of these instrumentations, including the capacity to sort cells, will have to be set up in the southern hemisphere as well.

So the guidelines that you're going to or we're going to put together will have ramifications perhaps globally. First slide, please.

So I would like to give a little bit a historical background with respect to the disease HIV and then perhaps a little bit about current applications and future requirements and then I'll conclude. Next slide, please.

I think it was Howard Shapiro who coined this phrase that AIDS is the killer application, and it's certainly helped to put a lot of instruments around the world in the market, most of them, of course, were instruments to analyze rather than to sort. But,

nevertheless, flow cytometry mushroomed in a very short time because of the fear of HIV infection.

The clinical laboratories, of course, moved along and biosafety rules and regulations were put into place, and formaldehyde has been the sort of safety network in dealing with analyzing instruments because, of course, we have the luxury there to always fix the cells. Next slide, please.

The potentials of where these instrumentation would have application in HIV are listed here, and I don't think I will be spending much time because most of you have expert in these areas and you don't need much information from me.

One -- the last bullet I have there. One interesting application that is creeping into the reality of my laboratory, is the organization I work for is something between NIH and FDA and CDC.

So we deal with a variety of issues. And one area that flow cytometry may have some answers to question is can you do rapid screening, such as cytokines that are undesirable by products when you process cells and could flow do that?

And we think that there's a good chance that one can set up a technology based on bead solid- faced particles using beads as a way of doing multiple cytokines and get the sensitivity levels low enough that contaminants could be picked up as a screening tool for those organizations that are involved in checking on biological products. Next slide, please.

What I have in this slide and the following one is a recent report that was published, I think, a week ago in Science.

And, basically, a bunch of experience field workers looked at the successes and the lack of success dealing with this epidemic in the -- primarily in developing countries.

And the take home message from here as you go through the five areas is that research applications and vaccine development are fourth and fifth, as the field experts see it today.

And I think that the kind of initiatives that will be taken by a group as such may make that shift so that the application of sorting and the use of flow cytometry will have a far more significant impact of dealing with infectious diseases in the future. Next slide, please.

So as you can see, we would like to move the application field from fourth and fifth place higher up in the scale of things.

And I think that this is sort of coming around in circle, since the industry did profit from the disease, now it would be nice that the industry took initiative and partnership with regulatory agencies and the researchers so that we would make a contribution where we can actually arrest these epidemics. Next slide, please.

The whole idea is that in an infectious disease such as HIV, if you can manage the immune system that you are really adding a type of medication to the system, that it will be part of the solution rather than just being monitoring the disease.

To that effect, I think that some of the assumptions that we have made in the past must be revisited. The idea of looking at sodium chloride as an aerosol or a certain kind of E. coli as a phage detector is now a stale concept.

I heard today that we are talking about 40, 60, 100, the numbers for increasing a pressurized system. I'm hearing about the droplet frequency going from 20, 40 and 75 megahertz.

These are phenomenal changes, and we either will put limits on how fast you can go and still provide safety, or the corollary that you put in double protection.

We heard today already that there are ways of putting an internal positive pressure and external negative pressure barrier around the system.

If you have those systems, indeed you can check for the organisms that are suspect as contaminant. We, in HIV work, we are very much concerned about hepatitis B and hepatitis C. These are highly infectious agents and they would be the things that I would suggest to monitor in the inside of these instruments.

And then, finally, there are two ways of looking at not just product safety, but about the operator safety, is under normal operational conditions what kind of an aerosol you anticipate and under emergency breakdown.

What if 100 pounds per square inch pressure hose breaks? What are the safety features we can integrate into the system so to minimize the complications.

I think this is my last slide. Want to check? No. Okay.

So the future requirements are basically how are we going to deal with the proposed concepts that you will be hearing today.

And we are really interested in what ergonomics and sort of environmental -- more environmentally-friendly solutions we can come up with.

Obviously, if we are thinking of putting some of these instruments into developing countries, recirculating water for water-cooled lasers would be a more practical solution than trying to tap into their local water supply.

And these are some of the practical problems that I thought of that perhaps we should consider in looking at how the guidelines will shape up at the end of the day. I'll stop here. Thank you.

DR. MARTI: What would be one of the uses, Frank, of the -- for monitoring the viral load, intercellularly, of sorted cells?

MR. MANDY: No. For the time being, the applications will still have to do with fundamental research in vaccine, monitoring the efficacy of vaccines

It would be actually quite similar to the presentation just before mine, looking at CD8 cytotoxic cell productivity. Are they more ferocious than they were vaccination and so on?

These will be the fundamental proof of concept data that will be -- we will require once the vaccines are put into the field, addressing the grade of the virus that we are coping with in a particular population. And, of course, gene therapy and a whole

-- but many of those applications are not unique for HIV.

DR. MARTI: Thank you.

MR. CODER: Frank, Dave Coder, again. You're describing in some ways what is -- might be a special case as far as human cell sorting goes for clinical applications. That is the patient where demonstrated infectious disease is already present,

would you expect and -- well, in the facility that you're dealing with, where you have patients where the -- well, the immune status is will known and your infectious disease status is well characterized, that such stringent requirements are not going to be necessary.

MR. MANDY: Well, I'm also a realist. I think it will -- we'll have to reach some sort of a compromise. One of the ideas that occurred to me is that since the kind of recovery that you need, and the kind of frequency of a particular cell that you may have in a system, you may also put restrictions on the kind of pressure, high pressure that you're going to use.

So that if you are working under general good laboratory practices, you may recommend not to go beyond a certain pressure so that the aerosol could be contained and disinfected within a reasonable length of time.

Whereas, if you're going to work with 100 pounds, it's totally unrealistic to think that you can make -- you know, spray alcohol in the air and get rid of it, all the pathogens in time.

So I think we'll have to look at what is realistic, and I think we'll have to collect some data. In fact, what kind of aerosol damage are we talking about when we are talking about hepatitis C?

Because that's a disease that is rapid among intravenous drug users. So it's not just a problem for the southern hemisphere. It's a real problem in our major cities.

MR. CODER: As far as the, well, specific pathogen goes and trying to be reasonable as far as requirements go, some knowledge about the mode of infection and the

expected minimal infected dose I think would also temper what the requirements would be, as far as conditions under sorting goes.

Because some pathogens -- well, the worse case scenario would be Pastorella pestis or something, where a single organism can be infective, a variety of other organisms that takes a fairly good slug and may not necessarily be an airborne mode of pathogenesis.

And so under those conditions you wouldn't have to be nearly as stringent as far as sterilization conditions go.

MR. MANDY: Yes, I think you elegantly draw the attention to the critical dose, that there are pathogens that probably would not cause any kind of major concern to the operator if they are only five part per million, or five part per liter of air.

Whereas -- but we have to know that and we have to have data on that.

MR. CODER: Yes. Because, unfortunately, I think for a lot of these things we don't have the data right now.

DR. KEANE-MOORE: Our next speaker is Dr. Elizabeth Read, who runs the NIH's Clinical Center for Cell Processing.

DR. READ: Good afternoon. I'm going low-tech today. I have the old slides, not the Power Point on the computer. Is there a pointer here?

I'm going to talk about our cell processing facility and this is actually -- some of the slides are from a talk I gave at a GMP and cell processing workshop that was sponsored by Ice Age.

So when I put that talk together, the goal was to try to show the audience how you take the regulatory requirements, and then apply them to your own facility. Next slide.

Just a little description of our facility. We are located in the Department of Transfusion Medicine, which is a clinical center department on the NIH campus and we really evolved since 1984 to the present to serve the NIH clinical center as a core laboratory for ex vivo cell processing.

Our current scope of activities includes the operational side and then an RNV side. And on the operational side we support phase one and two clinical trials. Next.

The next three slides just show how we've changed from 1984 to the present and actually it says 2000 and now it's 2001. So things have even changed since the end of last year.

We're currently supporting about 25 active protocols, but we have supported over 50 different clinical trials from all the different NIH institutes, at least the major ones, and we cover the range of hematopoietic transplantation, to immunotherapy, to gene therapy, and more recently we've gotten into pancreatic islet isolation for transplantation from cadaveric donors to patients with Type 1 diabetes melitis. Next slide.

Also, since 1984, the complexity of our laboratory procedures has increased dramatically. We're now performing over 40 different technical methods, many of which are extraordinarily complex. We're using over 200 unique reagents and media.

We do our own assays for the most part. We have a few send outs, but we're doing our own cell counting and viability, colony-forming assays for hematopoietic progenitors.

We do flow cytometry, but I do want to say that we haven't done any flow sorting for clinical trials yet, and we haven't -- we have no immediate plans to do so, but I wouldn't be surprised if it was around the corner. We do our own endotoxin assays and we have additional assays in development. Next slide.

Along with this in the 16 years we've gone from one technologist to 16 and actually, since the end of last year I think we're up to 18.

We have more than 100 SOP's. I think it's more than 200 right now. We've supported over 40 INDs and IDEs.

And I think what's really dramatic since 1984 is really that we're now much more in tuned with standards, AABB and FAHCT standards, and also we are paying substantial attention to quality insurance, GMP's and FDA regulations. Back in 1984 it just wasn't a big deal. It wasn't an issue.

We're also now paying attention to the new proposed GTP regulations. Next slide.

So our facility was actually -- our new facility was opening in June of 1997. So we were actually operating at a lower level before then. But in 1997, we moved into our state-of-the-art facility, which probably isn't state of the art anymore, because this field has changed so rapidly in the past few years.

And as I said before, our purpose was to provide state-of-the-art, multi-use facility for ex vivo cell processing that meets evolving -- and that's the key word -- service needs and evolving regulatory requirements. And that's the key to being in this field now is that the regulatory requirements have truly been evolving.

By the time you plan a facility and build it, it's almost out of date. So you sort of have to build that flexibility into whatever you're doing.

We're located in Building 10, which is right behind here, and the design and construction were supported by the NIH, but we also had a cooperative research and development agreement with Baxter Health Care, which helped in the design of the facility. Next slide.

This is just a picture of the lab before we actually moved in, at least a portion of it. Next slide.

I think you've seen some of these regulations before in a couple of the other talks, but the regulatory framework we looked at, where the CG -- included the CGMP regulations for drugs and blood. That's in the 200 and the 600 series.

Aseptic processing guidelines, which are also published in the CFR FDA's 1993 statement on somatic cell and gene therapies, the 1997 FDA proposal for regulating tissue and cellular therapies, and then over the three years from '97 to 2,000 we also took into account the FDA's CGMP continuum. Next slide.

Oh, I thought this was the slide about the continuum, but I'll mention that in a minute. This is just to point out that buildings and facilities is just one small element of

the entire CGMP regulations, and so all of these really had to be taken into account in designing the operations of our facility. Next slide.

This is about the CGMP continuum and I'm not sure -- I guess it's relevant to this talk, or this series of talks today. We do phase one and phase two clinical trials. We don't do phase three clinical trials.

We're not trying to take a product to licensure, and I think the FDA very intelligently in public meetings presented this whole sliding scale approach to application of GMP's and cellular therapies.

And really it was done mostly in the context of gene therapy and, essentially -- and I'm not an FDA person, so I hope I'm not misstating this. But, essentially, CGMP applies to all phases of clinical trials, but full controls are not necessarily expected in phase one and phase two.

It's expected that manufacturers will think -- start thinking about them and developing them, but it really will be phase three before everything is fully developed. Next slide.

This is just a layout of our facility and this pinkish, peach kind of area here is our manufacturing area. This is our support area. We're already growing out of it, and we're already starting to take space from various people around us, and they don't like us, but we need the space. Next slide.

Our manufacturing area includes a very large central area for self-separation and processing. We have two separate tissue culture rooms that are adjacent to the central core.

We also have a freezer room and we do have a flow cytometry lab that's used for diagnostic assays. Next slide.

The features of the manufacturing area, again, are coming out of the compliance with GMP's. The surfaces are smooth and easily cleaned. The HVAC, which really comprises the majority of the cost in building one of these facilities include temperature and humidity controls, filtered air, and then positive pressure in the core.

We have an autoclave within our facility to sterilize equipment, although we really use mostly disposable supplies, but we do have several things that we still autoclave.

We do have a sterile water supply, but we really buy most of our sterile water and we have a cleaning and pest control plan, and environmental monitoring. Next slide.

Just to show you, within the manufacturing area, this feature on this slide is really what we would consider our aseptic processing area. These are contained areas. We don't actually do processing. This is the freezer room, and the autoclave's in here and then there's a downing room and I'm not even sure what that one is. That's just an extra room adjacent to the core. Next slide.

And that corresponds to the air flow. And you can see that the double positives here -- these are our tissue culture areas where we would do things like gene transductions within biological cabinets, other kinds of culture work.

And this area is really the central core where you see the positive, the single positive. The negative is -- these are the sink areas. The negative, negative, negative, the freezer room and an autoclave room.

This is the flow cytometry diagnostic lab. So, basically, it's like zero pressure. So the pressure -- air pressures are set up to correspond to where we're doing aseptic processing. Next slide.

Aseptic processing guidelines, I think, one of the first speakers referred to these and they're in the CFR way back in June of 1987. And I think the person said they were undergoing some revision now and they were going to be published again, which is terrific because it's been a long time.

Also, there is a federal standard, 209E, from September, 1992, that outlines many of the same standards.

And essentially, we can break our facility into a controlled area, controlled areas and critical areas. In controlled areas we're trying to achieve a Class 100,000, in terms of particulate counts. And this would be non-viable and viable particles, and then air flow.

Class 100, again, would be what we would try to achieve in the critical areas. And, again, there are requirements for non-viable and viable particulates in air flow. Next slide.

I need about two hours to really read every word on each of these slides. In our facility, therefore, the controlled areas are at class 100,000, and this is what we used for closed-system processing.

And that consists of all open spaces in rooms within our manufacturing area. So that whole peach area that I showed you is really at class 100,000.

But when we have any open system processing, transfers, sampling, we, essentially, would go into a biologic cabinet within those controlled areas and there we're achieving the class 100 air.

We actually have measured the air in our facility. We do it on a regular basis and we're actually better than class 10,000. They actually over-engineered our facility, which is wonderful.

And so we easily get to well below -- you know, well below 100 in the biologic cabinet. Next slide.

I'm going to take you back in history to the 1980's when we first started cell processing. I'm going to show you some of the horrible open systems we used to use.

Now, this isn't a biologic cabinet, but imagine it -- even it's not a biologic cabinet, if the air isn't really being monitored, this is really -- this could spell disaster.

But these are -- this is an old way of bone marrow processing that used manual phycogradients and pipettes sticking out of the tubes here. Next slide.

These are roller bottles. This isn't so bad. The roller bottles really are open systems when you have to start pouring and transferring. Next slide. That was the -- the roller bottles were from the early days of lack, which we started working on with Dr. Rosenberg back in the 1980's.

This is the first gene vector that we used in a clinical trial ADA Supernate, and it came in this little plastic vial, plastic bottle with a screw off -- screw top cap.

These were frozen, so when you actually thawed these you would get all sorts of condensation and things underneath the cap. This is not a nice, closed system and, actually, we have gotten our vector manufacturers to put all of them into bags with tubing that we can sterility dock. Next slide.

Again, this was the first clinical trial with ADA deficiency and gene therapy. This is from 1990 -- '90 or '91. That's Ken Culver and these were 24 well plates.

He's working in the hood, but this was before we had our nice facility. And I will tell you that we did have some contaminations and had an awful lot of scrubbing down cabinets, incubators and throwing out cells. Next slide.

Now we're getting into some of the nice closed-system features. I'm showing this because there may be some people in the audience who just haven't kind of walked through a modern lab to see what we're doing. This is very simple minded, but these are essentially based on blood bags. This is blood bag technology. These are culture bags.

These are lymphocytes, T-cells being grown -- this is the incubator shelf and they're being grown in bags. Next slide.

This is an apheresis device, which we can actually use not connected to a patient to do cell separations. And again, you can have a really nice closed system. It looks a little messy but, in fact, everything is closed. So everything that the blood product or cell product touches is sterile and all connected. Next slide.

This is on the CS3000, which is a cell separator. This was back in the days when we were doing bone marrow processing a lot more frequently, and this was a -- just a mononuclear collection from a bone marrow.

And, again, it's in a sterile bag. This was hooked up inside this device with the centrifuge on but, essentially, can take this heat seal off and you've got your product in a -- it's been in a closed container the whole time. Next slide.

This is a sterile docking device, a sterile connecting device. And we have probably ten of these in different parts of our lab. They're very expensive, but we would not be able to do what we do in closed systems without this device.

Essentially, you can take two pieces of tubing and weld them together in a sterile fashion. Next slide.

This is a device that we use a lot for cell processing for peripheral blood stem cells for CD34 selection and T-cell depletion. That's an Isolex device.

And, again, this is the device, but what's nice about this device, and there are several other devices out there, they basically provide a whole sterile, disposable kit that you kind of hook up to this whole system. This is a little spinning membrane.

These are, basically, blood bags with either reagents or cells or will eventually have your cellular product or waste in them and it's all hooked up as one piece.

And you can even take these apart with the -- you can hook them up with sterile connecting devices or you can heat seal things off. So it's essentially one nice, closed system. Next slide.

So that's what we're -- we're really trying to go to do mostly closed systems in everything we do, but there are necessarily some openings in the system.

And one of the most common ways of opening the system is just to take a sample. We're constantly taking samples for assays. When we do that, we go under the biological cabinet and take that sample. Next slide.

I just wanted to show you why even if we had perfect closed systems, that we would still be potentially set up for microbial contamination.

And this is a picture of a bone marrow harvest and the bone marrow is being pulled out of the iliac crest of this person and into syringes, and then it's being squirted into this bag.

Well, guess what? This is open and this is open. So this was an attempt to improve on the old method of bone marrow harvesting and processing and it is more closed than what it was before, but it's still not completely closed. The other thing is donors and patients aren't always completely sterile. Next slide.

This is just an apheresis device. We're getting most of the cells that we processed by apheresis now and, again, most of the time these are sterile collections, but sometimes they're not. Next slide.

I thought about our lab in terms of closed systems and opened systems, and I think there are two procedures that we currently do that still concern us that aren't completely closed. We just can't -- we can't close them up.

And one of them is a elutriation and the other is our pancreatic islet processing. And I just wanted to show you pictures of it just to kind of get your minds working.

And I don't have any real good conclusions for these, but this is one of our technologists doing the elutriation. This is like a big centrifuge. There's a rotor in here. Next slide.

And with elutriation, you've got this plastic chamber that you're putting cells into and then it's in a rotor, and you're applying a flow under a fair amount of pressure, similar to flow cytometry, and then you've got it in a centrifuge.

We can sterilize this, but we have to pull it apart and then put this whole set up together with these tubes and these various mechanical parts, similar to your flow cytometer.

What we do in this situation is we actually construct it, put it all together each time and we do a cold sterilization in place.

That concerns us. I know it's concerned some of our regulatory people. We'd like to close it up, but there's nothing really available now right at this minute that will substitute for that. Next slide.

The other thing in our pancreatic islet processing, I think we've tried to close this up as much as possible, but this is the cadaveric pancreas that we obtained from the procurement folks.

And we obtain it in a series of bags. There are layers and layers of bags. So it's as sterile as it can be, but frequently, its not sterile even coming from the cadaveric donor. We take it under the biologic cabinet. We do a dissection. Next slide.

And then we have to do this very elaborate mechanical and enzymatic digestion. And I'm showing you this, and I'm not going to walk through this because that's a long talk in and of itself.

But, basically, we've got beakers, and we've got the stainless steel chamber, and all these tubes. And this is within the biological cabinet.

But, basically, a lot of these pieces -- all these pieces are sterile, but it's not a closed system.

At some point when we -- the digested islets are coming out, they're coming out and dripping into a beaker. It's not a beautiful closed system.

So these are things we're working on, and I think the goal -- I don't have experience with flow sorting for clinical purposes, but I think the goals of trying to get things as closed as possible are really, really critical, and then if you can't get it closed, then you have to figure out good, reasonable ways of containing what isn't closed at some level of air quality, and some level of aseptic technique. Next slide. I think that may be it. Yes, that's it. Thank you very much for your attention.

DR. KEANE-MOORE: Our next speaker is Dr. Adrian Gee, from the Baylor College of Medicine.

DR. GEE: Good afternoon. I'd like to start by thanking the organizers for this opportunity to present what we've been doing at Baylor related to therapeutic cell sorting.

What I'm going to talk about is some of the work that's done -- been done primarily by April Dureti, in our facility, who runs the flow area, and my main responsibility is for QA and GMP compliance.

We're both at the Center for Cell and Gene Therapy. We have about an 8,000 square foot GMP facility in Houston, consisting of an area for clinical grade vector production and then nine laboratories for GMP cell processing, which we use for hematopoietic stem cells, cytotoxic lymphocytes and vaccine production.

What I'm going to focus on today is the use of flow cytometry and some of the protocols that we have open, and also a more general ranging discussion about some of the ways we've tried to address some of the concerns that you've heard about this morning.

In summary, I'd like -- in overview, what I'd like to do is look at some of the advantages for why we've selected cell sorting for particular applications.

To mention some of the disadvantages of the technology for therapeutic cell preparation, some of the special concerns, again, relating to some of the things that you heard about this morning, to give you a little view of some of the applications for which we're using cell sorting and then a summary.

The advantages are, obviously, that this technique has the potential to produce cell preparations at very high purity.

We can use multiparameter sorting in order to get an even greater degree of purity in cells with exactly the right characteristics that we require for the particular application.

We can, potentially, also recover all of the cell populations if we're doing a simple sort into two populations. If we want, we can recombine both in various proportions in order to get a very highly-defined population.

And if we are sorting and using the unlabelled cell population, we have, essentially, a very minimally-manipulated cell population that hasn't been exposed to a lot of reagents or antibodies.

The disadvantage are, obviously, in cell numbers and speed as you've heard today. And, obviously, relating to that is time.

The potential problems of open systems, of reagents -- and we really haven't heard a lot of talk today about some of the problems related to reagents that we're using and, particularly, obviously, the various antibodies that are being used for cell sorting. Many of those present certain concerns to us if we're going to use those cells for therapy.

Also, viability concerns. And as you've heard, a large part of this talk is also going to deal with regulatory issues.

If we deal first of all with speed and time concerns, generally, for therapeutic doses of cells, we're looking at anywhere from ten to the three to ten to the sixth cells per kilo.

But we're starting with initial cells numbers. I'm very interested in the change in fonts that's going on. So everything's new to me when I look at it.

But initial cell numbers for things like hematopoietic stem cells, we may start with a population of ten to the nine to ten to the eleventh cells and want to end up with ten to the three to ten to the sixth per kilo. And to do that solely by cell sorting becomes almost unmanageable.

So we have, obviously, a very prolonged sort time if we want to undertake this type of manipulation by cell sorting alone.

So, in general, there is often some up- front debulking. And that immediately gets you into the area of manipulating cell populations and gets you into the whole other area of regulatory concerns.

Some of the debulking methods that are used are, as you've heard, density gradient separation, so you may make a mononuclear cell preparation if you're dealing with blood or bone marrow.

You may use magnetic depletions. And Frits talked about using magnetic particles for debulking and selecting populations, and also ex vivo culture.

And for a lot of the population, again, as Frits referred to, we may then take the sorted population and expand it up. And, again, that presents a particular regulatory concerns.

We have found that certainly high sort rates can result in cell damage. We do not sort at 60 psi because we've had problems with that. So we, generally, reduce the pressure down to 30 psi.

Although we can recover cells, we found that at 30 psi they retain all of the functional characteristics of unmanipulated cells, whereas, as we increase the pressure, we may be able to retain the viability, but lose some of the functionality.

We also have generally reduced flow rates to seven to ten times ten to the three events per second.

There is obviously the issue of prolonged sorting, which in itself can cause cell damage, risk of contamination of the product, and if do you any kind of premanipulation using magnetic particles, or using some kind of density gradient, you may also affect the sorting characteristics of the opulatoin as they go through the flow cytometry.

If we look for open system concerns, obviously, there is risk of exposure of the operator. And you heard, in particular in relationship to HIV-infected cells, but I think it's a very good point.

As you've heard from Elizabeth Read, we will often get -- well, not often, but we will get cell populations to sort from patients who have positive markers for infectious diseases, or for the preparation to autologous cell products, and we are then required to use those cells. So we have to have the ability to work with contaminated products.

There is, obviously, not only the risk of exposure of the operator, but also the risk of product contamination. This is a concern to the FDA and many of the new regulations, GTP, for example, are focused on the risk of contamination of products.

We've talked about this morning about the issue of cleaning and sterilizing the sorter, and having a procedure that is validated to show that that actually achieves the level of cleaning and sterilization that is required.

And also, decontamination of the sorter. If we know we're going to be sorting a positively- infected population, then we have to be able to show that we can adequately decontaminate the sorter afterwards.

The way we've addressed this in Baylor is, as I mentioned, the GMP cell processing facility is rated at class 10,000. Within that facility we have one room, the cell sorting room that is rated at a class 1,000.

We do a complete clean on that room before use, which means it's completely stripped down, the floors, walls, ceilings and everything are cleaned by specially-trained cleaning crew.

We do extensive monitoring of the facility, that includes static and dynamic monitoring, involving doing particle counts within the room, doing viable counts within the room and doing RODAC plates.

For the staff -- now, we do do infectious disease monitoring on all of the donors of the products, so that we at least have a heads up as regard to the infectious disease status of any product that comes into the facility and we also insure that the facility is secured, so that only certain people are allowed into areas that are involved with handling products.

But for protection of the staff, we ask that -- we require that staff use tie back suits or scrubs when they're working within the facility.

They wear fluid proof lab coats. They are required to have shoe covers, a hat, latex gloves and mask. This is shown with a surgical mask, but we'd also require people to wear face shields.

In terms of the sample, we have a MoFlo cell sorter, and we have the containment device. We also do sterile processing of the products prior to doing cell sorting.

So in many cases, the cells will have gone through some up front manipulation. Those will be done in the class 10,000 rooms, in a class 100 biological safety cabinet.

We then clean the sorter, and I'll describe that in a moment. We have the containment system on the MoFlo that you heard about this morning, the negative pressure sample containment device.

For cleaning the sorter we've used the following system. We flush the system four hours at a minimal with a ten percent solution of bleach.

We then run 70 percent alcohol for two hours and we can, if possible, leave it in the system overnight. We run sterile PPS for two hours prior to sorting, and then we do calibration.

Now the problem is many of the calibration reagents are not sterile themselves, so then we have to repeat some of the resterilization. It would be very nice if the manufacturers could consider coming up with GMP grade sterile calibration reagents for this type of procedure.

In order to get over that, after that we've run the calibration, we do one hour each of bleach, ethanol and sterile PPS.

I mentioned to you the concern about reagents. We're, obviously, very concerned about what antibodies we use. There are certain antibodies that are supposed to be of GMP grade material.

And, obviously, that is the kind of minimum standard that we should want. We do ask -- and some of these are mainly primary conjugated reagents, but also it's the availability of secondary reagents. We're often using newer types of antibodies that may not be available in a conjugated form.

There's also the issue of many of the reagents that we would like to use contain sodium azide. That, obviously, is potentially toxic if one is going to use the cells therapeutically.

We do ask for certificates of analysis for all of the reagents that we receive into the facility and we bar code all of our reagents in order to keep track of them.

In some cases, we will do additional testing of various antibodies to meet a product specification and, obviously, we cross check lots of the antibodies from batch to another so that they meet specifications before they are used for the particular application.

The other issue is there is residual material that may be left in the sorter product that my be float face antibody, for example, that may carry through during the staining procedure. You have other reagents.

And those really need to be removed if they present any potential hazard to the patient. And then if you are going to remove them, you have to validate that your removal procedure will work.

There's also -- if there is going to be residual material in there, such as mass antibody that may cause a reaction in a patient, you may have to be prepared to measure, for example, human anti-mouse antibody that may be produced in response to receiving cells that have been coated with antibody.

In addition, if you are interested in this area, there is guidance available from CBER. And these are just two of the documents that deal with monoclonal antibodies, not in specific relationship to this particular field, but in relationship to the therapeutic use or drug use of these types of reagents that give you a lot of information about the kinds of concerns that you should at least raise before you use them.

There's also the issue of buffers and sheath fluids. The type and composition of the sheath fluid that you decide or buffers that you decide to use during both the preparation of the cells and their passage through the sorter, the use of preservatives, other reagents that you use in fusible grade.

If you're going to directly take that product and put it into a patient, do you have certificates of analysis for these reagents?

Are there going to be any downstream effects? For example, if we take cells and then go to use them in another manipulation, are these buffering reagents going to have adverse affects on the cells for the next part of the downstream processing?

And, again, if you have harmful reagents in there, can you validate that you removed them prior to using the cell populations.

We heard a little bit this morning about viability issues. I think, obviously, the effects of speed sorting and time of sorting can, obviously, have adverse affects on viability.

We also have to think of longer term effects and what are the most effective or the most useful viability assays. We may get one immediate measure of viability post sorting, but does that truly reflect the functional capability of the cells when you go on to do additional manipulations or culture?

So what is the best type of viability measurement for these populations?

What are the effects of cells being sensitized by the antibody that is being used by sorting? Does that adversely affect future functional requirements for that cell population?

And is it possible, in many cases, to use positive or negative cell sorting to overcome some of those disadvantages so that one selects out a population by virtue of the fact that it does bind the antibody as opposed to the fact that it does?

Very briefly, obviously, you've heard the regulatory responsibility. The FDA has paid a lot of attention to this and CEBA has been particularly active in this area.

There are a number of documents -- and, again, I don't have time to go into these -- and I believe they're listed at the back of the handout this morning, that do give you a lot of guidance for the preparation of therapeutic cell populations and it is very unlikely that you are going not to be affected by some of these regulations because of the manipulations required prior to sorting, the actual sorting itself and post-sorting will put you into a category where you will have to address these kinds of issues.

You can see, for example, in the somatic cell therapy documents, there is information required on cell identity, on viability, sterility, purity. So those are the kinds of things that you have to build into your protocol as you work on -- start working in this area.

One of the particularly useful documents is on human somatic cell and gene therapy. And this covers any autologous allogeneic and xenogeneic cells, as manipulated or processed ex vivo. And it would include selection, and so it would include sorting approaches as well.

Again, I want to go through this fairly quickly, because this is the type of information that you can pull and look at to see how it works.

But particularly, in the case of -- as you've heard about the tiered idea of risk and that the greater the potential risk that the cells are put to, or the donor, or the recipient may be put to, the greater the degree of regulation.

And so if you're working with cells that are considered to be more than minimally manipulated, and that's not easy to say. I wish I would have come up with something a little bit easier. But it's anything that alters -- potentially alters the biological characteristics of the cells or their function or integrity. It includes expansion and genetic modification.

And you've heard today about some of the applications for cell sorting and involving gene therapy and expansion of cell populations. And for those you would require an IND and I think that puts us fairly closely into most of the protocols that are open now for therapeutic cell sorting.

Again, just to go quickly, you would require an IND application, quality control of manufacturing, definitions of methods and reagents.

If you're developing cell banks of sorted cell populations, you would require QC and you have to show that your product was reproducible in terms of from one lot to the next.

All of this information, again, can be found in the CFR. And, again, I'm not going to go into any of that, but how to prepare IND applications.

Other general requirements, data showing reasonable safety and rationale. Additional data, as you've heard, collecting information during a phase one trial that can be used to support later things -- the type of things we're talking about there are quantitative assays of bioactivity and product stability, so how stable are cell populations following these types of manipulations.

Again, I think for the sake of brevity, but some of theses things do pertain directly to cell sorting protocols, collecting cells, giving the appropriate information on how those cells were collected from the donor, how the donors were selected.

In general, as I've said, we've used blood donor standards. If you collect from a donor who doesn't meet these standards, you have to have justification of why you've done so.

Again, the major interest being on infectious disease screening. You may be required to do HLA typing of the donor and be aware of possible cellular interactions, and you must have details about the collection procedure and the location for the collection procedure.

For cell culture again, which is often -- proceeds or follows cell sorting, you need full QC of manufacturing and of the reagents, of the media, acceptance criteria, lots and source information, components with potential to cause sensitization.

You've heard about fetal calf serum. Growth factors have to be well characterized and you should avoid the use of antibiotics. So we can't do a dirty sort and then try and clean it up with antibiotics.

Other issues, again, are adventitious agents, document conditions that minimize contamination, periodically test your product. And we screen our products for bacteria, fungi, microplasma, endotoxin and adventitious viruses.

Implement controls to look at the culture identity and heterogeneity and try and avoid cross contamination by the use of appropriate controls.

You have to be able to identify very clearly the population that you are dealing with by using, for example, cell surface antigens, define acceptable limits for that population, try and use functional assays for the cells to show that they have retained functionality after sorting, for example.

You may be required to verify the matching of the donor and the recipient. You'll establish the stability of the markers that you're going to use for identity testing and verify, where possible, some type of biological functionality of the cell population after manipulation.

And then there's a whole series of things, which again, I won't read out but, basically, the same kinds of controls have to be undertaken for control of the materials

that you would use during your processing or your upstream or downstream manipulation of the cell populations.

And, for example, there you see the use of animal serum in certifying that calf serum has been obtained from countries where BSE is not an epidemic.

Again, characterizing the product. What are the acceptable concentration limits for things that may end up in that product? Provide methods and data on removal and potential toxicity of any residual components by using an animal model.

The recent publication in January, the eighth in the Federal Register, was on good tissue practices, which is a kind of supplement to GMP relating specifically to therapeutic cells.

And, again, without going into a lot of detail here, it is intended to prevent the introduction of communicable diseases through the use of these practices.

So you have to insure that the products do not contain a communicable disease agents, that they do not be contaminated -- become contaminated during manufacture and that the function and the integrity are retained during that manufacturing process.

And just to show you the kinds of things that are in there, you have many of the things that we've discussed earlier in this talk. A quality program, organization, procedures, facilities.

So you can see class 10,000 and class 100,000 and class 100 are but small components of the overall approach to good manufacturing and good tissue practices.

I think I'll try and skip this one. I'll just have to step through it. Just to end on the applications, obviously, the major application is in the separation of rare cell populations.

There has been a lot of interest in primitive and progenitor cell populations and we have therapeutic protocols open on separation of very primitive stem cells by cell sorting.

There is a lot of interest in separating tumor cells. I think we've moved, to a great extent perhaps further away from purging applications, trying to remove tumor cells from hematopoietic cells grafts.

We have used tumor cell sorting for gene therapy, so we use the flow cytometer to sort out tumor cell populations, which are then subject to gene therapy.

They have -- they're transvected with genes for interleukin-2, CD40 ligand, lymphotoxin, for example. So that is the major sorting of tumor cell populations that we do.

We also look at activated cells, particularly trying to remove allo-reactive T-cells from grafts and trying to purify specific T-cell populations, for example, those with particular types of activities, as you heard from Frits, for CMV and for anti-tumor activity.

Just to give you an example, for separation of allo-reactive cells, to give you a kind of flow chart of what kinds of things will happen before and after, we would separate recipient, peripheral blood mononuclear cells.

We would then culture and generate lymphoblastoid cell line from those cells, collect and separate donor peripheral blood mononuclear cells, expand those in

culture, radiate the recipient cultures, co-culture the two populations together for six to nine days and then we would do the sorts.

So there's a lot of up front manipulation here. We would then sort activate it, CD3 positive, CD147 positive cells and we would expand those in cultures.

So there's a lot of manipulation in these protocols, hence, the emphasis on somatic cell therapy. Using this kind of protocol, what we can see here is we have about 26 percent CD147 positive cells, 38 percent negative cells prior to the sort.

We would then sort using ABX-CBL and anti-CD147, and separate the allo-reactive population from the non-alloreactive population, and then culture the non-alloreactive cells for infusion.

In summary, then I think we are actually seeing increasing use of new applications for cell sorting, for particularly immunotherapeutic and gene therapy protocols.

We still have a lot of reagent limitations and we focused a lot today on machine limitations, but there are still reagent limitations that we have to overcome.

There is the concern, obviously, about validated decontamination protocols and cleaning protocols for the machines. I think it is particularly heartening to see new designs in machines, instead of trying to clean up the ones that we have. It's nice to know that there's a whole new kind of generation potential of sorters becoming available that will address many of these concerns.

We have done many cell sorts now, not all for therapeutic, and we have yet to have one come up positive as screened by bacterial, fungal, viral, microplasma and endotoxin.

I'm not sure if we're been lucky, or whether just our attention to the kinds of controls that we've put into place explain that.

And I think it's certainly with increasing experience at our center and a number of centers that we can truly evaluate the risks that this type of manipulation poses to both the operator and to the sample. Thank you very much.