FOOD AND DRUG ADMINISTRATION

Center for Biologics Evaluation and Research

ALLERGENIC PRODUCTS ADVISORY

COMMITTEE MEETING

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Daniel Ein, M.D.
Te Piao King, Ph.D.
Nancy Sander

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P R O C E E D I N G S [8:39 a.m.]

Agenda Item: Opening Remarks

DR. FREAS: Good morning. I am Bill Freas, the executive secretary for the Allergenic Products Advisory Committee. I would like to welcome everybody to this, the 16th meeting of this committee.

Today's entire meeting will be open to the public. At this time, I would like to go around and introduce the members of the head table to those in the audience.

I will be starting on the right hand side of the table and I would ask the members to raise their hand not that there is anybody in the back of the room, but if there was somebody in the back in the room, so they could see who you were.

I will start with Dr. Betty Wray, committee member. She is professor of pediatrics and medicine, Medical College of Georgia.

Coming around the table is Dr. Sam Lehrer. Dr. Lehrer is a new committee member. Welcome to our committee. Dr. Lehrer is research professor of medicine, Tulane University Medical School.

Coming around the table is a former committee member, who is participating as a temporary voting member for today, Dr. T. P. King, associate professor,

Rockefeller University.

Our next committee member is Dr. Gail Shapiro, clinical professor of pediatrics, Northwest Asthma and Allergy Center.

In front of the podium is Dr. Andrew Saxon, professor of medicine, UCLA School of Medicine.

Next is our chairman, Dr. Dennis Ownby, professor of pediatrics, Medical College of Georgia.

At the corner is Dr. Maria Soto-Aguilar, a rheumatologist, allergy and immunologist in private practice from Florida.

Next is Dr. Henry Claman, Distinguished Professor of Medicine and Immunology, University of Colorado Health Sciences Center.

Next is another new committee representative, our consumer representative, Dr. Dolores Libera, director of publications, Allergy and Asthma Network and Mothers of Asthmatics, Incorporated, Fairfax, Virginia.

At the end of the table is Dr. Dale Umetsu, Chief, Division of Allergy and Clinical Immunology, Stanford University.

Again, thanks to everybody for coming this morning. I would also like to thank the committee management specialist, Pearline Muckelvene, who is at the table outside, who was responsible for organizing this

meeting, the administrative aspect of this meeting.

I would now like to read into the public record the official conflict of interest statement for this meeting.

"The following announcement addresses conflict of interest issues associated with this meeting of the Allergenic Products Advisory Committee on February 10, 2000. Pursuant to the authority granted under the committee charter, the director of the Center for Biologics Evaluation and Research has appointed the following participants as temporary members: Dr. Daniel Ein, Dr. T. P. King and Ms. Nancy Sander.

"To determine if any conflicts of interest existed, the agency reviewed the submitted agenda and all relevant financial interests reported by the meeting participants. As a result of this review, there are no COI disclosures for the public record at this time.

"In the event the discussions involve other products or firms not already on the agenda, for which FDA participants have a financial interest, the participants are aware of the need to exclude themselves from these discussions and their exclusion will be so noted for the public record.

"With respect to all other meeting participants, we ask in the interest of fairness that you

state your name, affiliation and any current or previous financial involvement with any firm, whose product you may wish to comment upon.

So ends the reading of the conflict of interest statement.

Dr. Ownby, I turn the meeting over to you.

DR. OWNBY: Thank you.

I would like to welcome everyone, especially our new members and all of you attending for the first time and for those members, who are staying on as temporary voting members, thank you for all your time and effort on this.

Hopefully, we can have an open and free discussion. I don't intend to force any major time constraints on this meeting. I think we have enough time to adequately cover the agenda. So, for the committee members, if there are questions or things that come up, please feel free to ask them at the time.

Our first speaker today is Dr. Jay Slater, chief of the Laboratory of Immunobiochemistry. We will try to follow the agenda that you already have from here.

Jay.

Agenda Item: Laboratory of Immunobiochemistry

DR. SLATER: Dennis, thank you very much. Welcome to all the committee members. I appreciate

everybody coming from such a long distance to hear us present our work and our questions to you today.

As you can see from the agenda, we are going to have several parts of this presentation. In the first part we are going to be talking to you about the operations at the Laboratory of Immunobiochemistry, what we have done operationally over the past year, what we have done in terms of our research activities over the last year.

In the second part, Dr. Richard Pastor and I will present to you some regulatory proposals that we have that we would like the committee to discuss and give us the committee's opinion on after the break. These proposals involve expanding our definition of the release limits for lots that are sent to us for certain allergen vaccines, in addition to a formalization of the allergen standardization procedure that we have been working on that we would like to move forward with.

Finally, we will be talking about some specific new standardization targets that we would like to propose in view of the Department of Health and Human Services recent asthma initiative. What Dr. Ownby said goes for me as well. Certainly, if there are questions that come up during the presentation, we can entertain them. I will also be happy to entertain questions after the

presentation as well.

The Laboratory of Immunobiochemistry has had a very good year. It has really been a very solid year for the lab in many ways. Certainly operationally and from a research point of view, we are getting off to a very good start again, building on what has been done before and really expanding in terms of the research program that I described to the committee last year.

We now have what I would consider to be, at least for the work that we currently have, a full complement of individuals. I am the lab chief. I have been in this position for the last year and a half. Dr. Lyudmila Soldatova is a visiting scientist. She has been with the laboratory for the past two and a half years. She actually had been a Orise(?) Fellow before that time, but just since the summer, she was converted to a visiting scientist position.

We have four biologists now working in the laboratory. Our senior biologist is Maneesha Solanki. She has been with the lab for about four years. Clearly, she is the most experienced of all of us and has continued to be a terrific team member and a terrific research for the rest of us.

We have two very new biologists in the lab. Kristin Morrow came to us in August from the University

of South Florida. Kristin has a master's degree in microbiology with a special emphasis in molecular genetics and we are really planning on using her expertise extensively.

In addition, Melissa Catena came to us just a few weeks later. She has a master's degree and came to us from the University of Maryland. Her area of study had been in animal and avian studies, with a special interest in endocrinology. Again, she has been heavily involved in the scientific research in the laboratory as well.

Mona Febus is a microbiologist, who actually has been with the Center of Biologics for many years, most recently working in the Laboratory of Biophysics, but she has transferred over to the Laboratory of Immunobiochemistry and will be assuming large amount of our lot release activity responsibilities.

Beth Paupore, who had been full time when I spoke to you last year and had come to FDA with me from Children's Hospital, has now decided that she wanted to learn more about immunology and, in spite of all my best efforts, decided to go back to school half time. She is in the Hopkins master's program of immunology and public health, but she is continuing to work with us part time and is devoting her activities to some of the research

that I will be talking to you later.

Gerry Poley was my fellow at Children's Hospital before I left. He followed me to FDA as a guest worker in order to keep doing his research. He is now a full attending at Children's Hospital and is continuing to do his research about half time in our laboratory.

Now, looking at the staffing in a static way doesn't give the full picture. So, I put together this slide indicating some of the fluctuations that we have had in our staffing. And the bottom line take home is in spite of the fact that things are looking very well now, we have had some down periods where we have really only had two rather than four or five people working in the laboratory.

In spite of that, we have been able to build up and we are now at, I think, a very good level of staffing, which I hope to maintain for most of the next year.

In terms of operational improvements within the laboratory, I presented last year to you the validation studies that we had done with the competition ELISA. These validation studies will be referred to a little bit more in one of the later talks. But basically we made some minor improvements in the competition ELISA. We revalidated its accuracy and we distributed that

information to all of the manufacturers so that they could at their discretion, institute some of the changes that we instituted in the competition ELISA.

We, I think, have improved the speed of feedback to specific inquiries from the manufacturers. We try very hard to get back to the manufacturers within hours, if possible. I have really tried to have as much of an open door policy for all representatives and the manufacturers to talk to me about any specific questions that they have.

In addition, as we discussed last year, I have instituted an aggressive proactive reference replacement program that I will be talking to you about in just a few minutes.

One of the important administrative features of the past year for all of us has been the merger of the Division of Allergenic Products into the Division of Bacterial Products. You heard about this last year from Dr. Thomas Hoffman, who introduced the concept to the committee and that merger actually did take place on September 1st, 1999.

We are now part of a larger division that is called the Division of Bacterial, Parasitic and Allergenic Products or DBPAP. Our acting director is Drusilla Burns. Our deputy director is Carolyn Deal.

All change is anxiety producing and I will not lie to you and tell you that I had nothing in my mind that caused me any concern beforehand.

But I can honestly say that really within -- let's see, the merger happened by Friday -- by mid-week the next week, I was thoroughly reassured about the merger. We were clearly the beneficiaries of a very highly developed regulatory division that, if anything, has really contributed incredibly to our ability to do our regulatory job better.

So, in fact, this was a merger of unequals. We were a smaller division being merged into a larger division. There are lots of concerns about that. I am here to tell you six months later it has worked out better than I would have hoped, spectacularly well. We have clearly had increased access to regulatory resources. We have had increased access to administrative resources to help us do our job better.

We have continued to have a very high level of program support. The Laboratory of Immunobiochemistry Allergenics is in a very strong position at this point. So, I would not be -- I really think that any concerns that we might have had beforehand have been amply allayed by our experience so far. And furthermore, the research regulatory balance that I have and that my people have in

the lab has been maintained and we are doing quite well with that as well.

So, what are the regulatory activities of the Laboratory of Immunobiochemistry? We have a large number of very routine regulatory activities. These are our bread and butter activities that we do day in and day out all the time and they include what is on this list. Protocol review, this is when lots that are -- of standardized allergen extracts that need to be released. The protocols and samples are sent to us for us to review and approve before the lots can actually be released.

Now, our lab is not the only unit that reviews the protocols. There are other units that review the protocols as well. The protocols have to go through checking and signing off by several different units within CBER and a certain number of them actually are tested by us and by some of the other labs as well to confirm the manufacturer's data.

We also were involved on a frequent basis with reference development. We certainly are involved with reference distribution and reference maintenance, including semi-annual checks and replacement. In terms of protocol review, in 1999, we reviewed 477 protocols. That means 477 lots of new standardized allergen vaccines were sent to us for approval prior to release.

One of them was withdrawn simply on review. In other words, we just looked at the protocol. We identified some irregularities. We called the manufacturer and they withdrew the product.

Four of them failed on potency testing. This was testing where we tested them. They were outside of limits and they were failed. Two lots failed on glycerol testing. In other words, when our laboratory -- not our laboratory, but when the laboratory that tests for glycerol content, and that lot was withdrawn as well.

In terms of reference distribution, in 1999, we distributed 1,983. We distributed nearly 2,000 vials to the manufacturers of reference material in 104 separate shipments to the manufacturers. So, the take-home message is we do a lot of shipping or reference to the manufacturers, that we are the source of these materials and we send them out.

And our reference materials are maintained with semi-annual checks. We go through all of them. We do gels, potency assays, just to make sure that they are up to the requirements.

Last year when I spoke to you, I introduced our aggressive reference replacement program and the genesis of that was that when I arrived at the lab, we found that of the references that we had in stock, the 24 references

that we had in stock, 20 of them appeared to be beyond the three year dating period that had been provided by the manufacturer, based on stability studies.

So, formally speaking, they were out of date. In fact, there is no particular reason to believe that there is anything wrong with using those references because we do do six months checks on them. We were monitoring them. But from an administrative point of view, I certainly wanted our references to be as current as possible.

So, we began a reference replacement program, the idea to bring the full inventory to within the stated date and our target completion date for that program was August 2001. The procedures were fairly straightforward and obvious. We identified the references to be replaced. We select candidates from recent submissions by manufacturers to replace the references. We do our own initial testing in the laboratory.

We then select based on our extensive testing a provisional reference. We then ship that provisional reference out to all of the manufacturers for them to actually review and test and compare to the previous reference.

We usually give the manufacturers about 60 days to respond, send us back their data and then with a

really large amount of data, not only our extensive testing but the testing from the manufacturers, we can decide either to go ahead and select that as our replacement reference or as happens sometimes to go back to Step No. 2 and try again. It is a very time-consuming process, but it is a process that we need in order to keep our materials up to date.

So, in 1998 --

DR. OWNBY: Jay, on that question, do you actively submit -- ask the manufacturers for extracts to be candidates for replacement or are these lots that come in under protocol for routine review.

DR. SLATER: Second. In other words, we like to pick lots that have been fairly recently manufactured so they will have a good long life as a reference if we select it and we go back and look over, say, the last six months of products that we have screened and identify candidate materials that would be a good reference.

The first thing we actually do is call the manufacturer and find out if they much of this stuff left because sometimes they have sold out of it by the time we indicate an interest.

So, no, we don't actually actively solicit specific products for reference.

DR. OWNBY: Thank you.

DR. SLATER: So, in 1998-1999, we replaced four references, the pooled serum for D.pteronyssinus and D.farinae, cat, cat hair and D.pteronyssinus references and we are currently working on a series of grass references, red top, orchard, meadow fescue, sweet vernal -- actually these three are in the midst of the process already. This one is just starting on the process. We are also going to be replacing our short ragweed serum, which is a sheep serum, simply because it is running out.

DR. SAXON: Why do you have cat and cat hair? I mean, it may be historic or is that for some intellectual reason for having those two different --

DR. SLATER: Cat pelt and cat hair. So, the cat pelt contains albumin.

DR. SAXON: So, manufacturers will either make -- have made one of two kinds of products then?

DR. SLATER: They will make both products.

DR. SAXON: Is there any scientific reason? Is one different from the other or better?

DR. SLATER: The scientific reason is that a certain small percentage of cat allergic individuals have significant cat albumin sensitivity in addition to their Fel d 1 sensitivity.

DR. WRAY: I can confirm that some patients react to one and not the other.

DR. SAXON: You have people who react to cat hair and not to cat pelt? Dr. Shapiro, is that your experience? I just was wondering. I didn't realize you had both standards as one --

DR. SHAPIRO: I think of cat hair as an inert sort of substance rather than active stuff that was attached to the hair. Maybe that is where the issue is. What do you mean by cat hair? Just the keratinized material or all the stuff attached to the keratinized material?

DR. SLATER: Both of the extracts are standardized according to their Fel d 1 content, but the difference is that on the immunoelectrophoresis, the cat pelt, one would expect to see an albumin band in addition to a Fel d 1 band. We don't standardize it according to the cat albumin content, but because there is a minority of individuals, who are significantly allergic to the cat albumin, you want to make sure that that product is available for use.

DR. SAXON: I understand.

DR. LEHRER: Jay, the question is are there any patients that are positive to the cat hair and not the cat pelt?

DR. SLATER: You mean, why not just make cat pelt as the product? You know, in essence, I don't know

the answer to the question and I am not going to attempt to answer it, other than --

DR. WRAY: It seems logical but yet we have seen some that react only to the hair and not to the pelt.

DR. SAXON: That wouldn't surprise me if, in fact, what is on the hair is a little dust mite and a bunch of other things that the cats have picked up. I was just interested. I didn't realize they both existed. I don't want to make a big deal of it. I just am interested, didn't know about it.

DR. UMETSU: There is still a question, though, what do you use to treat patients with?

DR. SAXON: It is pretty obvious at UCLA we don't use cat hair.

DR. UMETSU: But if it based on the content of Fel d 1, isn't that what you want to treat patients with?

DR. SAXON: They both are standardized by Fel d 1. So --

DR. UMETSU: They are both standardized on the basis of their Fel d 1 content. But the cat pelt contains cat albumin as well.

DR. SAXON: And the other may contain other things.

DR. UMETSU: They are both the same in terms of

their content of cat and the Fel d 1 dominates, but if you look at these on IEF, you know, the pelt has a huge amount of albumin compared to the Fel d 1 content.

DR. SLATER: We are actually going to see a gel of a cat pelt preparation.

DR. SAXON: I know. I saw Olman's(?) work many years ago showing it and they were concerned it would make people sick but it didn't. Have you ever done the opposite, take the cat hair and measure it for dust mite?

DR. SLATER: I don't know whether that was done in the initial --

DR. SAXON: I was just asking because Dr. Wray says she sees people react to the hair and not the pelt and I was wondering, gee, if it was something special about the hair, which I find antithecal to my thinking, but I assume it could pick up other things. Curious.

DR. LEHRER: Are there some allergens in the air that are greater concentration than the pelts? That would explain it.

DR. SLATER: You mean aside from Fel d 1?

MS. BRIDGEWATER: Jay, Jennifer Bridgewater. Part of the issue, I think, is the way that they are collected and the manufacturers can correct me if I am wrong, but the cat hair they actually get, I think, by shaving the hair off the pelt. So, you really wouldn't

have a lot of albumin in there where the pelt is. They actually cut strips of the pelt.

DR. SAXON: Great. Understood.

DR. SLATER: I don't know the answer about other cat allergies that may be in higher concentrations.

One of the lessons of our reference replacement program is not that only that our current activities to bring it up to date have been very resource consuming in terms of our time, but that even future efforts to keep it up date, if we are continuing to deal with 36 month expiration dates, are actually going to be very costly in terms of our time.

Therefore, one of the things that I talked to you about last year was to initiate a reference, a lyophilize program whereby for the next few years, we are actually going to purchase about 20 percent more reference material from the manufacturers than we actually think we will need, lyophilize that material, study the potency of that material at time zero and then follow that material out, hopefully, beyond the three year dating period to see whether it is comparable to the glycerine aided material that we use as our current references.

Our assumption is that this material will be more stable and if we can develop references that will

have a ten year dating period as opposed to a three year dating period, we will considerably reduce our resources that we need to develop to this problem.

So, the plan was this year and next year, to lyophilize a portion of the new reference extracts and then for the next three years, to assess the stability and reliability of the lyophilized products compared to the current references. Once we have the results, we will distribute the results and samples to APMA membership for their comment prior to instituting any changes.

Agenda Item: Research Report

That leads me into the next part of my talk, which is the research report because that is actually going to be the first research project I am going to tell you about.

We have had a good year in the lab in terms of research activities. We have had a good year in terms of beginning several projects and many of the projects that I am going to talk to you about have just started over the past three or four months.

So, we are not going to have any conclusive publication of quality data for a number of them. But some of them have really come along quite well. We have done well in terms of abstracts submitted. We have done

well in terms of papers accepted in refereed journals. So, you have seen this research program summary before. It hasn't changed since last year.

The two areas of major interest that we have are the areas of allergen structure and function and, in addition, the area of immunomodulation. Within allergen structure and function, we have been studying the stability of lyophilized references, which is the first part that I will talk to you about. We have also looked at these other questions that I will go to in order as we come to them.

So, the first allergen that we attempted to lyophilize was a D.pteronyssinus allergen vaccine. What you see here is an STS polyacrylamide gel of the glycerinated material in the right hand column, compared to lyophilized materials, compared to material that was lyophilized in the presence of mannitol. Mannitol is a bulking agent for lyophilization. It is thought to confer some added stability to the products.

We should note, of course, that before lyophilizing the product, we have to dialyze the glycerol out of it, which adds a level of concern that you might be losing some essential allergens. So, our plan was to do SDS polyacrylamide gel electrophoresis on these and you can see here pretty clearly that there is no visible

difference in this gel between the glycerinated material, the lyophilized reconstituted material and the material that had been lyophilized in the presence of mannitol.

You can also see clearly the bands that accordingly to their molecular weights, we are guessing are Der p 1 and Der p 2 and those bands do not appear to be changed in terms of their density after the lyophilization process.

Yes, sir.

DR. LEHRER: I wanted to ask you a question about the lyophilized material in terms of the amount of material that you place on the gel because some believe that there are some denaturation with lyophilization and that you are -- you may not be able to solubilize all of the material. So, I think it is really crucial in terms of how -- the amounts that you place on the gel. If you redissolve the lyophilized material and then get a protein concentration and then adjust them so they are all the same or if you calculate how much material you have initially in the lyophilized material and you place an equivalent amounts, that would account for any loss due to lyophilization.

DR. SLATER: Right. It wouldn't compensate for any loss. The first metric would compensate for loss. Right. So, we use the second method. In other words, we

really wanted to see what happened when we took the original material that we had and in the end reconstituted the same amount of stuff in the equivalent volume.

So, we did not do a protein concentration. We didn't compensate for losses along the way. So, if there were losses -- so, not only is there an issue of precipitance that you can't resolubilize, but there is an issue of selected protein loss on the dialysis tubing and through the process.

So, no, we did it the second way because we really wanted to see what kind of losses you actually experienced. Remember, even if we had seen losses initially, it wouldn't have invalidated the approach because really what we are looking at now is sort of the zero point in terms of the potency. Let's just say that by lyophilizing it, we lost 20 percent of the protein across the board.

If you still had a material that was 80 percent of potency that stayed completely stable for ten years, you would still have a reagent that was of value. What we are more concerned about is do we lose -- does potency deteriorate over time in the lyophilized state relative to the glycerinated product?

In addition, we are concerned about the

possibility of losing minor allergens; in other words, changing the composition of the product by the processing. And, again, this is basically the zero point. This is not a stability study at all. These relative potencies were done within just a few days of the lyophilization process.

What you see here is that the relative potency of the glycerinated product, the lyophilized products, were all statistically indistinguishable from one, if you at the 95 percent confidence intervals, you can see that they bracket one and not only that, they bracket each other in terms of their content.

So, this was reassuring in terms of the competition ELISA and suggests that the lyophilization process itself doesn't appear to cause any visible compositional changes or relative potency changes in the product. The plan now is to follow these lyophilized products out over the next three years, along with the glycerinated product that is currently the standard.

DR. LEHRER: Could I ask you another question about lyophilization?

DR. SLATER: Sure. Of course.

DR. LEHRER: Do you plan to look at other allergenic extracts?

DR. SLATER: Oh, absolutely. Absolutely. The

plan is to serially just go through everyone that we do. It is just a matter of getting started.

We also did this on cat pelt and you can see here quite clearly that the cat albumin band, at about 68 kilo daltons is really quite prominent. You can see the fel d 1 band here. It is definite. It is clear. But it is not prominent. It is really clearly a minority of the allergen in there. But, again, the product is standardized for its fel d 1 content.

Now, what is interesting here is you actually can see a difference between the glycerinated product, which is here and the lyophilized product and you can see it -- there is a triplet band here and the heaviest of the triplet disappears completely in the lyophilized products. We don't know what allergen that is, but we definitely can see that something in our lyophilization product doesn't agree with this particular extract.

Now, is this an allergen band that we need to be very concerned about? We are not sure yet but we have to study that.

DR. KING: When you have a protein mix, you lyophilize it and it is usually stable, but when you have a purifier protein, you lyophilize it and it is the end of it. The best one I myself personally know is this antigen and ragweed pollen. If you have purified protein

lyophilized, you store that and it gradually goes to pot. It is true in many other enzymes, too. Once they are purified, they cannot be lyophilized. When you have a mixture extract, it is perfectly fine. You lyophilize stores or lyophilize power.

DR. SLATER: So, we should be in luck with these.

DR. KING: I would just like you to be aware of the fact that lyophilization would not -- may, in fact, in this particular case, this protein may be not stable on lyophilization. That is why you see glycerinated farther but you don't see it in lyophilization.

DR. SLATER: Good point. Thank you.

DR. KING: The other question is you do all these analyses by comparing SDS gel electrophoresis. I know I would do the same thing, too, because that has been our best resolution, but as you know very well, in fact, they are inactivated in the SDS gel electrophoresis.

So, yes, the protein band is there, but is it really the immunoreactive band is another question.

DR. SLATER: I think that is a very good question. I think with some of these extracts, we are going to have to do some allergen-specific measures as well, as we move along with the study.

Again, just following up with the cat pelt lyophilization study, here is a comparison. We measure Fel d 1 by radioimmunodiffusion. So, we measure the radius of diffusion. As you can see here, these are indistinguishable and, in fact, the unitage(?) of Fel d 1 was indistinguishable among those three as well.

So, in terms of the Fel d 1 content, not the rest of the composition, we did not see any deterioration at all. Another very important part of -- and I should say that the work that we are doing with the stability of the lyophilized references is being handled by Kristin Morrow, who is one of the biologists that really just started with us a few months ago and she has been involved in this and in other projects, but this has been one of the projects that she has been working on.

In terms of glycosylation of allergens, this is a very important part of our laboratory's activity. We are trying to address several important questions that really impact very heavily on the regulatory work that we do and that we are going to be doing.

Is the decreased antibody binding of the non-glycosylated antigen primarily a function of impaired folding? What is the biochemical anatomy of glycosylation requirements? Can non-glycosylated allergens equal native allergens in immunotherapy and

most importantly, how can non-glycosylated products be evaluated by us for diagnosis and therapy.

Our model of looking at the role of glycosylation allergen structure and function continues to be looking at bee venom hyaluronidase and bee venom acid phosphatase. These are studies that have been run mostly by Lyudmila Soldatova, who has published in this field already and I discussed some of her results last year. I will show you some of them again now.

She is moving forward with this project and, in addition, Kristin Morrow, who, as I said, just joined the lab in August, is going to be taking a chunk of this project and working with it herself. It is a big project. There is a lot of work to do.

Our approach is, I think, fairly ambitious. First of all, we are going to tool up the current enzyme assays because we really do need to have very good, precise, quantitative measurements of these enzyme activities. We need to express glycosylation mutants in insect cells and we need to study the antibody binding and enzyme activity of the native materials, the recombinant materials and the mutant proteins.

You are aware of the important allergens in honey bee venom phospholipase A2, hyaluronidase, acid phosphatase. Our focus has been for the most part on

hyaluronidase, which as part of Dr. Soldatova's studies, she expressed in E.coli, as well as in the bacula(?) virus system.

Just to summarize her previous published work, she was able to show that the material that was expressed in E.coli, here in the open squares, was significantly lower in terms of RAST in addition with pooled human sera, than the bacula virus expressed material, which is in the open -- yes, in the filled squares here.

In fact, the bacula virus-expressed material was indistinguishable from the native material, which was in the open circles. So, this was indicating that in terms of antibody binding to human antibodies, it was really a dramatic improvement when the recombinant material was expressed in the cell system that was capable of supporting glycosylation. And in terms of enzyme activity, she found that the E.coli material had a low specific activity in units per milligram of material compared to the bacula virus expressed hyaluronidase, which as we saw in the previous slide was fully comparable with the native hyaluronidase in terms of enzyme activity.

Yes, sir.

DR. SAXON: Jay, let me just make sure I understand. I think I understand but I put it on the

table. The concern is that when people make recombinant antigens, are they going to be equivalent to native, right? So, appropriately the concern is if you take the sugars off, will they be less potent. Right?

I just want to add there is another twist to that, I see now, and that is, in fact, when using in vitro tests, a lot of the cross reactivity against glyco -- sugars, is probably epi phenomenon and does not have biologic activity in vivo. That was best worked out with nuts and seeds where, in fact, a lot of the cross reactivity, which is in RAST testing, does not show up on skin testing and a lot of the cross reactivity with latex in vitro assays is probably an epi phenomenon.

So, just as another side to it so that, in fact, things without sugars, just because the in vitro test doesn't bind as well, doesn't mean in vivo they might not be biologically potent. Right?

I just put that on the table.

DR. SLATER: Absolutely. And, of course, when we are working allergen vaccines, we are not only concerned about skin test reactivity -- in fact, it might be desirable to have one that has less skin test reactivity, but if it is fully active as an immunomodulator, it might be perfectly acceptable. So, we really need to explore all of those features as the

native and recombinant materials.

In collaboration with colleagues, Dr. Soldatova has obtained several mutated clones of hyaluronidase. She has obtained clones that are mutated in the four putative endoglycosylation sites, which are shown in aqua and, in addition, she has mutants that have been mutated in the active site. She and Kristin Morrow are now in the process of expressing these clones. They are going to develop systems for expressing them and for studying them and characterizing them and hopefully through these site-directed mutants, we will be able to come up with some real answers as to what the specific requirements are for these particular glycosylation sites, both in antibody binding and enzyme activity.

Another important enzyme in acid phosphatase, which Dr. Soldatova has been studying over the past year to year and a half, her studies were initiated as an attempt to sequence the CDNA obtained from dissected honey bee glands. Unfortunately her first attempt using degenerate primers to obtain a sequence from the messenger or from the CDNA was unsuccessful. So, she went back to the genomic DNA, was able to get out a very nice segment from which she could then develop non-degenerate specific primers, went back to the CDNA and was able to come up with about a 70 percent sequence for

this material.

She is continuing to extend the ends of these CDNA fragments and continuing to sequence the acid phosphatase message. What was interesting is even from her preliminary results, we now know something about the event of acid phosphatase that we didn't know before.

From the earlier tryptic digests and the limited sequencing that had been done, it had been assumed that the bee venom acid phosphatase was homologous with acid phosphatase from prostate, which is the best studied form of acid phosphatase. It turns out that from this more complete sequence information, we can see that it is not homologous with prostatic acid phosphatase, but rather with Drosophila acid phosphatase and with the human lysosomal phosphatase precursors.

It is interesting because we know a lot about prostatic acid phosphatase and we know very little about the lysosomal acid phosphatase precursors. So, this sequencing and characterization work has an opportunity not only to teach us something about a bee venom allergen, but also to teach us something about an enzyme that hasn't been particularly well studied up until this point.

Another area of interest is the area of enzyme activity in allergens. Enzyme activity is extremely

important in several allergens. We are concerned about the relationship between allergenicity and enzyme activity, in terms of the antibody binding, bioavailability and antigen processing. The specific regulatory applications, of course, high minoptera(?) for which several of the important allergens, of course, have enzyme activity. In fact, for high minoptera, the enzyme activity is our standardization method.

In other words, that is what we actually measure in terms of lot release. But for dust mites and latex, as well, several of the allergens have enzyme activity. Our model -- and this is a study that is being conducted by Melissa Catana, who started with us in September and she has been working very hard at this, but a lot of the methods have needed to be developed from the start. So, she is getting a good experience in methods development right now.

But our model for this is to look at the event of hyaluronidase and phospholipase A2, again, to validate and refine the current enzyme assays to develop good immunoassays for hyaluronidase and phospholipase A2 and to compare the enzyme activity and antibody binding in fresh, old and inactivated materials. This is just one of her preliminary studies in which she took serum that we had obtained for phospholipase A2 and was able in

relatively short order to develop a good competition ELISA, comparing a current venom standard to two manufacturers products and to obtain a relative potency that is statistically valid based on that antibody.

DR. CLAMAN: Is your aim eventually to replace the standardization method so that you don't rely on enzyme activity? Enzyme activity may be convenient, but perhaps not biologically the best.

DR. SLATER: I would be open to the results going either way. I think that it is certainly plausible to hypothesize that enzyme activity is convenient, but probably irrelevant in terms of most immunologic functions that we want an allergen to fulfill. On the other hand, we might find that enzyme activity correlates really well, in which case enzyme assays are awfully easy to do. So, it might -- really the science could take us in either direction, but I think it is very important, especially since we have this dichotomy in the way we approach the extracts.

One group of extracts, most of them we do immunologic evaluations. The other group we do these enzymatic evaluations. I think it is important to compare them head to head and this is a very good model for doing it because we have the antibodies. We have animal models that we can use and we have measures of

enzyme activity that seem to work. So, it is a nice model and what I am hoping to do is to be able to draw some conclusions as to the validity of this in terms of following allergens.

If it looks great, we might really want to explore looking at that for future standardization procedures. But I am open for the data taking me either way on that.

We are concerned with identification methods. We spend a lot of time identifying allergens, using mostly immunoelectrophoresis. We have been looking at exploring the possibility of using physical chemical methods for measuring allergen content. Again, as you heard, we use immunologic methods mostly. We use some enzyme activity methods, but we wanted to explore the possibility of using physical chemical methods. And this is a project that Beth Paupore, along with Bob Boykins, who really runs our MALDI-TOF mass spectroscopy unit in the division, has been moving forward with.

I talked about MALDI-TOF last year. Our model is to take ultimately all of our standardized allergens and to evaluate these allergens in sequence with SDS-PAGE and with MALDI-TOF mass spectroscopy.

In the initial phase and we are still very much in the initial phase of the study -- it is really what I

call the normative phase -- I really just want to look at what they look like to get an idea of what kind of diversity there is among the products that come out, what we can identify, what are bands that you always see, what are bands that you never see.

That is definitely where we are right now. Ultimately, I would like to identify specific allergens, correlate it with the SDS and with the MALDI-TOF and see what kind of results we get. This is just by way of showing a standard SDS-PAGE of venoms. This is a hornet venom, a wasp venom. You can see the allergens that I have identified. This is probably antigen 5, possible lipase A1. This is a fairly faint band for hyaluronidase in these.

This is good data but it is sort of the data that we have been looking at all along and there are limits to the resolution that you can get by doing these kinds of gels. In contrast -- and this is fully to the credit of Bob Boykin's excellent work and mastery of a very, very costly piece of equipment -- the MALDI-TOF analysis of two venoms -- this is a honey bee venom; this is a vespid(?) venom -- really shows the power of this particular technique.

Not only can you clearly see phospholipase A2 in this honey bee venom, you can see the hyaluronidase

peaks, which are low. In both of the venoms, you can see the antigen 5 peak in this venom. You could also see -- notice I have an arrow here and there is nothing at the bottom of it and there is nothing at the top.

You can see that this particular vespid venom doesn't appear to have phospholipase A1 in it and we don't measure that, but there is no phospholipase A1 in this particular vespid venom. What you can also see -- well, maybe you can't see, but I am going to try to convince you that this is true, is that you can see not only phospholipase A2, but you can see the multiple glycosylation isoforms of phospholipase A2 here.

So, not only with MALDI-TOF can you get a fairly nice, specific fingerprint, if you will, of these particular products, but you can actually look at the fine detail of their glycosylation patterns in terms of their molecular weight. You really don't know what the glycosylation units are, but you really have the opportunity to develop a quantitative profile of the lateral materials, but you also have an opportunity to use MALDI-TOF to carefully access the recombinant products that we know we are going to be receiving.

When the recombinant products come in, we need to know how glycosylated they are. We need to know what the consistency of manufacture is. And this is a tool

that I am now convinced we are going to be able to use to look at those products as they come in and make sure that they have the consistency that we require.

I introduced you last year to studies that we had done with lipopolysaccharides. This is work that had begun previously while I was at Children's, looking at latex and lipopolysaccharides. We have extended it since then. Maneesha Solanki has really taken hold of this project and has done a large amount of work.

Why are we interested in LPS and allergen responses? We are interested because LPS is clearly ubiquitous. It is not only present throughout the environment but it is also present in the allergen extracts that we market. So, it is important for us to understand what the immunomodulatory consequences of co-injected LPS might be. We know that LPS elicits broad immunologic effects in all systems that we look at.

Now, in mice, as we had a discussion last year, in mice, LPS has a peculiar in vitro feature of being a very strong B cell mitogen. That feature is not present in humans. But in both humans and mice, LPS appears to ask as a strong adjuvant. It seems to elicit both TH-1 and TH-2 responses in both humans and mice and it is clear from old studies that were done actually in the early 1970s, late 1960s, that the effect of LPS on

antibody production in mice appears to be independent of this B cell mitogenesis feature.

It may not be fully independent but the bottom line is it doesn't happen in nude athymic mice. If you take nude athymic mice, whose B cells when you take them out will actually respond to LPS and you inject them with LPS and antigen, it does not function as an adjuvant in those mice.

We, of course, know that LPS has multiple immunologic consequences that really are complicated and depend on how you model the system. In our previous work that I had talked to you about last year, we were able to show that LPS co-administered nasally with the latex allergen Hev b 5 led to accentuated IgE and IgG responses to Hev b 5 and to spleen cell responses to both Hev b 5 and the carrier protein, maltose(?)-binding protein.

Our current model is to continue with airway immunization in mice and to verify the Hev b 5 results using ovalbumin. I would like to continue studying Hev b 5, but it is kind of a limited product and once we decided to go ahead and study the LPS phenomenon, we were just as interested in starting with a more readily available allergen as well.

It turns out this was a very good choice and I will show you why in a few minutes. We also wanted to

begin to assess functional responses and we have wanted to identify, if we could, the anatomic specificity and the anatomic requirements of this sensitization. As you will see, we have really accomplished some of this, but by no means all of it.

Once again, we see that LPS added to the ovalbumin clearly accentuates the IgG and IgG 1 responses. In this picture, time after immunization is indicated on the abscissa, the normalized antibody titer that is the comparison to a hyperimmunized animal is indicated on the ordinate. The large open arrows indicating the immunization periods -- we immunized the mice for 12 days, initially nasally and then about seven weeks later we repeated the immunization with three more immunizations at that point.

As you can see, the dark brown line, there is a significant increase in the IgG against ovalbumin in the animals that received the ovalbumin in the presence of lipopolysaccharide. There is no response in the other animals. Now, I can't expect you to remember last year's slide with the Hev b 5, but with Hev b 5, which I can only interpret by the fact that Hev b 5 is such a strong allergen, that Hev b 5 actually elicited responses in all animals after the first immunization, but it was significantly greater with the LPS. Once we gave a

second immunization, all the animals have the same immune response anyway.

So, we could see a difference between the LPS-primed animals and the ones who just received Hev b 5. The Hev b 5 mice had a significant response without the presence of lipopolysaccharide. Likewise, we see a response in the specific IgG 1 and in addition, we see a definite response with ovalbumin specific IgE in these mice. So, again, the mice that received ovalbumin in the presence of LPS have a strong response, specific IgE response to the ovalbumin; whereas, the other mice, including the one that received ovalbumin alone, don't appear to have any response at all.

We had less good fortune in measuring airway changes. We are using the Buxsco(?) whole body plethysmograph and our model was to do methacholine challenges in the various groups of mice and what you can see here is a general tendency for the mice that received LPS in the presence of ovalbumin to have greater methacholine responses, but these pesky error bars get in the way and the data really are truly not statistically significant at all.

So, we are going to address that in the next phase of the study in a couple of different ways. One is in terms of the plethysmography, we are going to measure

responses both to methacholine and to antigen, which I think probably give us different results.

We are also going to attempt to separate upper and lower airway delivery of the antigen. I would like to see whether what is really important here is concurrent antigen delivery or not. We are going to be looking at cellular responses, using bronchoalveolar lavage, measuring both cellular responses and cytokine responses to the product.

DR. CLAMAN: Question. Sorry I wasn't here last year. I was at the moment in an endotoxin-rich environment; namely, India. There is a little bit of deja vu. This subject brings me back to some experiments I did about the time you were in kindergarten, which I will not bore you with. But is the implication that there is sufficient endotoxin in allergenic extracts to modulate the immune response itself? I don't know what the endotoxin LPS content is. Is it biologically active and relevant or is that one of the objectives of your research?

DR. SLATER: That is one of the objectives. One of our objectives is to try to develop where we can see how much endotoxin is really necessary. Now, Dave Piedon(?) has published an article in the last two or three months in the JACI in which he has been introducing

1 microgram of LPS into the noses of volunteer subjects and -- humans, I am sorry -- and demonstrated that in the atopic individuals, the 1 microgram of LPS is sufficient to cause measurable increase in the insinital(?) influx to the nose; whereas, in the non-atopics, it makes no difference. So, clearly, I think, what you are saying is the dose response is key here. I couldn't agree with you more. We are heading in the direction of doing dose responsing, but we are not there yet.

DR. SAXON: How much endotoxin is there, are there, in extract? I remember the old days when they worried about dust mite was basically endotoxin, you know, the mixture. But today in the cat hair, how much endotoxin do those bacteria on the -- is that something that is known for all your standards?

DR. SLATER: No, it is not measured. It is not determined.

DR. LEHRER: There must be some information on it because you have alluded to the fact that endotoxin is in there.

DR. SLATER: We know that it is in there, but I don't know what the recent data are. I don't know what the --

DR. SAXON: So, manufacturers or the agency does not monitor any endotoxin levels in the product?

DR. UMETSU: It seems like it should be.

MS. BRIDGEWATER: Bridgewater, FDA.

They are exempted from endotoxin testing under the regulations.

DR. SAXON: Why is that?

MS. BRIDGEWATER: Well, considering those were probably written in the sixties, I couldn't tell you that exactly but I --

DR. SAXON: It was bad in the sixties. I don't want Henry to tell everybody how old he is, but even in my memory, there was that whole business where dust mite allergens were at, you know, zero to huge amounts and what people were reacting to was the endotoxin in it. There were enormous differences. It may be the agency --

[Multiple discussions.]

That is what I meant. House dust. I am sorry. I meant house dust. House dust was endotoxin. I mean, basically what you were injecting was endotoxin. So, it seems like maybe the agency should just think about, you know, is it worth looking?

DR. OWNBY: I thought there was a requirement that there was a pyrogenicity in rabbits for extracts or is that something that we were doing that was totally out?

DR. TURKELTAUB: There was an old paper looking

at pyrogenicity of extracts. There is no requirement, obviously, for FDA. And they are all pyrogenic, the ones that were tested way back when.

DR. LEHRER: But has anything been done since the sixties on that?

DR. TURKELTAUB: Not by FDA.

DR. SAXON: Would it be difficult to do?

DR. LEHRER: The problem with endotoxin is how do you assay it and, although most people are using the limulus(?) lysate assay, I believe, that is still the method of choice because it is in a kit and it is easy to do, I myself question the specificity of that assay and certainly it doesn't demonstrate endotoxin biological activities as we know them, such as pyrogenicity or some of the other activities that we see in man.

So, I think in addition to, you know, concerns about endotoxin, there would be concerns about the assay. Probably most people would support the limulus assay, but I am not so sure that other carbohydrates or like materials that may have activity in that --

DR. SAXON: Would it make you feel better, though, if it was very low in the limulus assay? If it is high, that doesn't mean it is a big problem. But should there be some kind of at least screening to see what the situation is?

DR. LEHRER: I tend to agree that it would be useful to have some information about these extracts. I would tend to feel that at least biologically we haven't heard a lot of information. I mean, actually the clinicians could tell us more about that in terms of the adverse reactions to -- endotoxin-like reactions. So, it may not be a serious problem, but I think we need to know. I think it was much more of a problem when I was in kindergarten -- I wasn't in kindergarten. I was probably in college with house dust because house dust was such a crude material and yet it was so active and nobody knew --

DR. SAXON: I understand. I can think of -- I mean, in the vaccine story, people are now coming back and saying in the 1940s there were things in vaccines. They knew they were there. You know, this is unrelated to allergenic products. It seems like maybe at least a little scan of the situation to see if it is enormous or not.

I mean, if they came out enormous on the limulus assay, then you might want to have a more formal look. If they are quite low, probably everybody would say you have shown due diligence.

DR. UMETSU: And there also may be significant differences between lots and so you might want to

standardize it so that the amount of endotoxin is at least pretty stable between lots.

DR. HAUCK: I am Pete Hauck. I am with Center Laboratories.

A couple of things from a manufacturer's perspective. What a manufacturer is obligated to do is make sure that they are not adding to the endotoxin load that is in the source material that we are obligated to do. The source material does, even including pollens, does seem to be rich in endotoxins.

Another concern -- and Dr. Lehrer alluded to it -- actually there was some work done about 20 years ago by Darrell Lu of FDA, suggested that a lot of the endotoxin tests would give you false positives if you have a lot of enzyme activity. We know there is a lot of enzyme activities in these extracts.

So, before you run off and try and measure these, you have to look at enzyme interference with these kinds of tests. That is probably the reason there are no endotoxin limits on these products.

DR. OWNBY: Thank you.

DR. EIN: Dan Ein. Just speaking as a clinician, clinicians tend to be sort of bottom line types. Sam said we ought to know what the clinical implications are and speaking as somebody who does a

reasonable amount of immunotherapy, I can't say that we observe a lot of endotoxin-like reactions. From time to time you get a lot of extract that seems a little problematical.

I don't know what individual differences there are in reactivity to endotoxin. We talk about an endotoxin problem, but from the data that was suggested here, it may not be a problem. A little bit of endotoxin may be a good thing.

DR. SHAPIRO: Is there a correlative between measurement of Der p 1, for example, and skin test reactivity that is done through the agency? I mean, when you standardize for the active antigen, do you also do some skin test assays to see if there is comparability between products that way?

DR. SLATER: Well, the standardization is based on the skin test originally, the skin testing assay originally, and then at that point, a surrogate is chosen that correlates well to the skin test reactivity. In the case of dust mites, the surrogate that was chosen was the competition ELISA, which looks globally at reactivity to all allergens. We didn't select one particular allergen.

DR. SHAPIRO: Well, if would look at something like Fel d 1, where there is one antigen that you are using for standardization.

DR. SLATER: The exceptions to that were the ragweed allergens and the cat allergens in which the surrogate is, in fact, the specific allergen measurement in terms of Amb 1 or Fel d 1. But for the grasses and the mites, the assay is based on the aggregate reactivity to cooled sera of highly allergic individuals as measured in the competition ELISA.

DR. SHAPIRO: So, what I am trying to get at is whether the endotoxin piece can be sorted out by looking at a correlation between the biologic reactivity and the actual molecular identity in the case of the situations where you are looking at just Fel d 1 or --

DR. SLATER: Right. I think what you are saying is if there were substances among lots of endotoxin level, you might -- and endotoxin itself had its own skin test activity, you would pick that up by correlating the competition ELISA, by failing to correlate the competition ELISA value to the skin test.

DR. SHAPIRO: And you don't see that -- I mean, I am assuming that --

DR. SLATER: That is not an ongoing activity. That was done with the initial standardization --

DR. SHAPIRO: So, that is not done on a yearly basis for antigens.

DR. SLATER: No.

DR. SHAPIRO: Okay.

DR. SOTO-AGUILAR: I would like to know from the clinical standpoint the content of endotoxin in any particular extract. Would it be more likely to cause an ateest(?) type phenomenon due to IgG reactivity rather than IgE when we see these big swollen arms or should we expect either IgE type reactivity or IgG?

DR. SLATER: I don't know the answer to that.

Okay. We are working our way rapidly towards the end.

The last area of interest in terms of our immunomodulatory part of the lab's activities is looking at epitopes of specific allergens. Specifically, we are continuing to look at the epitopes of Hev b 5. This is also a project that Beth Paupore had carried over with her from Children's.

This is our initial study that we performed that showed the various T cell and B cell epitopes of the latex allergen Hev b 5. This work was not only confirmed in our laboratory in mice. It was confirmed in Don Beeshold's(?) laboratory in humans and rabbits and confirmed by our collaborator, Robert O'Hare, in Australia in her human studies.

So, we have a pretty good idea of what the T cell and B cell epitopes of Hev b 5 are. To define that

further, what Beth is doing is she is attempting site-directed mutagenesis at three specific sites, three specific motifs that we think may be the important antibody-binding site.

She is attempting to mutate the lysine to proline in three different locations, 15, 23 and 28, and then is going to express those products and look at their antibody binding in the model that we have developed. What you can see here is she has been successful at producing the p28 mutant of Hev b 5. We have sequenced it. We know it has the insert just in the right location and it seems to be in frame and we have been able to express the p28 mutant in E.coli.

So, we are going to be moving forward with these studies, which, again, are really just getting off the ground in many ways. But hopefully, we are going to be developing a series of mutants of Hev b 5 and we will be able to identify the critical antibody binding sites, again, looking toward the possibility of immunotherapeutic approaches either with DNA vaccines or with epitope specific immunotherapy.

DR. LEHRER: How were the initial epitopes identified?

DR. SLATER: We identified them with hyperimmunized mice, looking at their serologic response.

In addition, we identified them with mouse screen cell responses, looking at fragments.

DR. LEHRER: [Comment off microphone.]

DR. SLATER: Actually, it was both. We did it both ways. We had synthetic peptides and fragments of the molecules.

DR. LEHRER: You were looking at IgG reactivity in the mice. Now, Don Beeshole using the same fragments was looking at IgE reactivity in humans and Robin O'Hare, using our material, was looking at T cell reactivity in allergic humans in Melbourne, Australia.

So, the studies really come from geographic diversity and species diversity as well. We have some pretty good ideas where we are.

So, that is the summary of the research program. I just wanted to go down each of the research activities and identify for you what the sort of functional objectives are. It has been my position since taking this job that I wanted the lab to do good basic research but that all of the research that we did had to have something to do with our regulatory function and I think you can see it pretty clearly, but just to drive the nail in, certainly the initial study that I talked to you about, Kristin's study on the stability of lyophilized extract, this is of critical importance to us

in being able to maintain good U.S. standards of reference in a reasonable manner.

The glycosylation studies that Dr. Soldatova and Melissa Catena are -- I am sorry -- Dr. Soldatova and Kristin Morrow are working on, again, will be critical as we start to evaluate these recombinant allergens that are coming through. We need to have ways of determining how to measure the allergenicity and immunogenicity of these allergens.

Those studies that Lyudmila and Kristin are doing are absolutely critical for us.

The enzyme activity assays that Melissa Catena is doing, again, as we said before, we use enzyme assays for some of our lot release activities. We need to know whether that makes sense. We need to know whether it makes more sense than the immunologic assays. Potentially, it is an important lot release tool. Potentially it is a lot release tool we should abandon. This is the kind of study that we need to do in order to do that.

In terms of the identification methods, using the physical chemical identification methods, it would be wonderful if we could use physical chemical methods in order to do our lot release, especially as we get to allergens that have engineered or modified allergenicity.

So, certainly in terms or current products, it would be nice, but for future products, I think it is essential that we explore these methods and work them out well and figure out what we can identify using the MALDI-TOF and other methods.

The epitope study, clearly one of the directions in which immunotherapy is going is to do epitope specific immunotherapy. Hev b 5 is increasingly obvious it is an important latex allergen. So, this work is critical in terms of moving forward in this important field.

Finally, the lipopolysaccharide studies, we know that LPS has adjuvant activities. We know that it is an immunomodulator. We know it is in the products that we regulate. We need to understand how that happens in order to do a better job of assuring the safety and efficacy of the products that we regulate.

We have had a very successful year in terms of abstracts and publications. We have five abstracts in at the academy meeting in San Diego. We are going to have a very busy time there covering the full range of our activities. I won't go through each of them now because time is short.

Dr. Soldatova and I attended the Paul Erlich(?) Symposium in September and both of our papers

will be published in the proceedings of the Paul Erlich Symposium, which I am optimistically indicating might be published in the year 2000.

Finally, we have had four papers published or accepted in refereed journals. The first is the paper that Beth and I and Robert O'Hare authored about the B cell and T cell epitopes of Hev b 5. That appeared in Molecular Immunology towards the end of last year.

The house dust stability that I am going to be talking to your about in about an hour, authored by Dr. Soldatova, Dr. Pastor and I. It is going to be appearing in the JACI within the next month or two. The work on the novel methods of determining equivalent doses of allergens is also going to appear in publication, the JACI probably in March, and finally a paper from Robert O'Hare's lab, in which I was a collaborator on the human T cell epitopes of Hev b 5 will be appearing in the spring as well.

Thank you very much for your attention. I apologize for running over. I will turn it back to Dr. Ownby.

DR. OWNBY: Are there any further questions? Dale.

DR. UMETSU: I just had a question about the T cell epitopes with Hev b 5. Are there single major

determinants for that for many humans or how does that work?

DR. SLATER: Yes. There are two major determinants that Robin identified in Hev b 5. Her study was, I believe, of 13 or 14 latex allergic health care workers. She didn't study any spina bifida patients. These were all adult health care workers at medical centers.

DR. UMETSU: But does that go across many different HLA types? I mean, getting back to the same problem that -- when people initially identified major epitopes for Fel d 1, it probably wasn't really the major determinants.

DR. SLATER: Right. There was definitely heterogeneity of HLA types among the individuals that she studied. I don't remember exactly what the breakdown was.

DR. OWNBY: Any other questions right now?

We are scheduled for a break. Why don't we go ahead and take a ten minute break and we will try not to run too far over for lunchtime.

Thank you.

[Brief recess.]

Agenda Item: Regulatory Proposals -- Potency Limits for Standardized Allergen Vaccines

DR. OWNBY: Our next speaker -- I finally figured out what he is doing here today -- is Dr. Richard Pastor, who is chief of the Lab of Biophysics. That seemed a little removed from allergy, but Jay was just informing me that he was the previous interim chief of the Laboratory of Immunobiochemistry. So that he has been dealing with these issues and thinking about them.

We are happy to have him here. Thank you.

DR. PASTOR: Thank you.

I will be speaking about the limits that we set for these products. Basically, you got part of this talk last year but in the course of the year we learned more stuff and had a broader picture of the whole issue. So, I think it is worthwhile to go through it again. So, here goes.

So, in the first part of my talk, I will just talk about the general issues we have here and then I will actually speak about these new limits, which bottom line -- which we are going to make the manufacturers keep to their old limits and then the receiver will do the lot release test and if they are in between a relative potency of .5 and 2, they will actually get -- they won't fail. So, it is a broad thing, as you will see.

After I have gone through that, I will speak very briefly about some of the -- now, with this data, we

can look at the non-standardized products, but what is the rationale for not having a protein content any longer. The underlying theme of this may be a little new to say in the FDA, but it has been around for awhile and now we want to make it more formal.

What we really want to do when we set a lot release specification is to make a balance between the manufacturer's risk and the consumer's risk. What the manufacturer's risk is that is the risk that the manufacturer will make a perfectly acceptable product but because of the variability of testing and consistency of manufacturing, that acceptable product will fail.

That is something you don't want to happen a lot, but it is part of the process in which there is the converse of that, a product that is on the borderline because of the variability of testing will like sometimes pass. So, you can always ensure that there will never be a product on the borderline by basically failing every product you get.

That is ludicrous, but that is an extreme. So, one has to balance these things and as we go out of the research lab, where you get gel, you get MALDI-TOF, you get ELISA results. At some point you have to make a decision to say, well, here is the spec. This fails; this passes and this is the process by which we are

trying to do it.

I am a biophysicist, so I don't use power points, slides and that stuff. I just like draw my curves, but now I am kind of caving in a little bit. So, now I do them like this.

So, what are the like three issues that we use in general and then more specifically for these extracts. One is that does the stuff work. Presumably, there is an efficacy range versus potency and at very low potencies, you don't get anything. At very, very high potencies, it may keep working, but it may drop off for reasons we don't know. We are going to presume somewhere in the middle there is some range at which the stuff works.

So, if one were to take a range for ELISA limit, say, you might consider using here to here or, you know, here to here. So, that is one way to get a range. Another range would be to for a given potency, to just look at the reaction rate, anaphylaxis and so on.

Now, obviously, at very low doses, there won't be any. At very high doses, you will get way too much. If that is somewhere in the middle, but, in fact, we are going to rephrase that slightly and say, well, with allergen extracts, one of the big issues is if you switch a bottle. So, if you get a -- because a bottle runs out, you actually get a new bottle. Obviously a patient is

going to get a dose from bottle 1 and then a dose from bottle 2.

We can ask, well, what is the maximum dose change one can tolerate. Obviously maximum is also something that you can work at, but, you know, you don't want the adverse reaction rate to be too high for a given change.

Lastly, there is just the issue of manufacturing, of consistency. Again, on just writing potency and now here my x axis is time and as you go along, you see everything is more or less, you know, the same and then all the sudden you get this guy here and then you might go back and then you might want to say, well, you don't quite know what happened wrong. You just know it is really different and you want it out.

So, you might imagine setting a limit something around here so that you won't allow this product in. The bottom line on this is that the safety requirements are actually not that flexible. We can't go above a certain reg. This, like manufacturing practices is more flexible. It is assumed that the products are safe, you know, once you have passed the clinical trials and so on.

Now, these limits, where you will pick can actually vary. For instance, say you do a bunch of these tests and you reject like 1 percent of a lot at each

time. If you keep on doing them, you will keep on like rejecting lots until you do a hundred of them. You might get rid of almost all of them.

So, once again, you have to be a little cautious and you have to think about how to balance stuff like manufacturer's risk as you set these limits, especially for here.

With these extracts as Jay didn't get into yet, but you can see where the 95 percent confidence was of some of the numbers, they are actually quite broad, something you have to take into account.

So, with our current ELISA method, if you just do a single replicate and you measure like relative potency of the reference itself. So, you know that has to be 1 right, but as you measure it, it could be quite broad. In fact, it does turn out to be quite broad because of the variability assay. The 95 percent confidence intervals for this are, you know, basically between .5 and 2, not exactly, but, you know, in other words, you know, in 95 out of 100 cases, something that is 1.0 can be anywhere from .5 to 2.

Now, that is quite broad so, in fact, what -- the way one narrows that down is to take more replicates. We use 3. That is a convenient number if it fits on an ELISA plate, sensible and the 95 percent confidence

interval is .7 to 1.4; so, about a factor of 2. You see where your assay is, a factor of 2.

This is a big improvement over the RAST method, where the standard deviation was about twice as large. So, we have really like narrowed it down by switching from RAST to ELISA. It was done a little while ago. How do we accept or reject lots now. Well, we actually rejected at the 98 percent confidence because we were doing the retest and it worked out that it was 98 after we did the analysis. So, that means any lot that is like outside of the range, .654 to 1.53, fails lot release.

Curiously, I mean, this follows that because it is 98 percent confidence, about 2 percent of lots that is the relative potency is 1.000. In other words, it is the reference itself actually fell. So, that is the manufacturer's risk that is just thrown right there. There is actually somewhat more risk -- as I said, a lot that was perfectly acceptable, well, is .9 acceptable, is, you know, 1.1 acceptable. Well then it is broadened.

So, basically you just have to take into account that the manufacturer lots are actually not 1.000. Rather they have a distribution themselves and, in fact, this is a famous formula for those who know it well and, so, basically the standard deviation squared of the observed values that you get is actually equal to the

variability of the assess plus that of the sample. So, it is broad.

So, now the situation is we have this broadened sample that we are working with and then the problem is -- sort of can be stated in kind of a down home way, what if a lot was really .7, you know, and the way you ascertain that is by doing a thousand replicates. You know they are doing something with .7 and the manufacturer tested at .8, passes it, seems reasonable. CBER gets it. It tests at .6 and rails it. And one of the things I asked when I started this -- I am a biophysicist and, you know, I didn't have any -- I hadn't thought about a lot of allergenic extracts. I said, well, is that okay?

You know, people said, well, that is how we are doing it now. We can't change it. You know, one has to think, well, you know, maybe you have to think about changing it. So, how do you go about changing the fact that this would happen, that this would fail? You can't just say make it .5. You know, you have to do some thinking here.

So, in fact, I am going to take you through in the next little bit just sort of our thought process by which we went through these four things.

First, you actually have to analyze by clinical

data. If you recall like CBER files, which were done with skin testing Paul Turkeltaub, were mostly to establish the reference standard. There wasn't that much on what the safety range was. It was just to say this is 100,000 BAUs.

The next thing we have to do is to revalidate our testing method again because the original validation was just testing multiple lots of reference to reference. So, we never tested those extremes. You want to know that, right?

Next, we actually looked at the variability of the manufacturers' lots after the standardization. We had a lot of data. So, we could actually do that. Then, lastly, we had a little math problem that was kind of cute. You say, well, if you change bottles, what is the sort of relative -- the change in the relative potency and you can actually work that out. We will show you how to do it.

So, you heard this last year a little bit. It is now an official draft guidance document. We have tried to cut it a little closer, but we weren't able to. So, it got out on the 8th. You can find it on the CBER Web site under Guidelines.ACM or the actual file is this one here.

This is also -- the scientific reasoning for

this was actually -- is in this paper, which will be published, hopefully, in a couple of weeks. In the validation and the protein work is in this book chapter with Jay, et.al.

Jay was the one who looked at the clinical data. There is way to much on this slide to read right now. I just want to point out that it is in your handout. Basically, Jay looked at a number of studies done by a number of groups and got some results.

So, let me tie in specific sets. This is an easy-to-read slide. So, first, let's look at efficacy. Remember, the first thing we want to see is what is the range for potency and next, what is the range for safety. So, it is efficacy.

So, with this -- verbally, it is just right here, is that apparently in this study doses were worked for a thousand range. Here, this person only did -- this group only did two that worked, 6 and 12. Of course, so -- so, it is possible that if they had done three it would have worked as well. But we can just say if 6 and 12 work, your range here is at least a factor of 2 and if you do the same sort of thing for all the other ones, you get factors of 14, 12 and like 30. So, you see there is like a wide range of like potency appears to work for efficacy.

To summarize that, my little slide here, so we can say, well, we aren't sure if this goes down or up or down, but we can say that it is like a plateau that is roughly a factor of 10. So, that is point No. 1. Now, the next thing we have to do is to say, well, how about potency change. Is this factor of 10, if you work back and is it 5 percent, is it 10 percent or, you know, is it all the way up here? We have to find out where this is.

Alternatively, you can say, well, if we only want a 5 percent change, we can work backwards and find it here. So, we don't know yet.

So, we isolated the studies of that, you know, big table, which emphasized -- which we looked at safety. I am not going to go into this, but these are the three and, you know, you can read the sort of stuff that happened.

I will just show you one example of the analysis that we did. Because it is at the low end of the curve, you can -- it is reasonable -- it is always good when you do this sort of analysis to do a couple of different kinds. So, we did a log linear. We looked at the log dose, looked at the percent of adverse reactions and you can see that this data for here gives, you know, reasonably straight; .91 is not so bad. We did logarithm of dose versus the logarithm of the probably of adverse

reaction over 1 minus that probability.

That his a more standard way to look at adverse reactions and the line is better. So, again, we are -- we did that for like each of the studies. We combined them we could.

Now, there are two points to make about this. You know, one is that the lines are reasonable. The other one is there is only three points. You would like to have a lot more. You would like to have a lot more studies with a lot of different allergens, but, you know, you have to start somewhere. Hopefully, this will spur it on more this sort of data.

In any case, let me just -- for purposes of time, let me just concentrate on this last line here, this third column. So, we wanted to ask the question, which we could do, once you have that bit of data. It is what dose increase is associated with a 5 percent increase in adverse reactions. We felt that was a reasonable way to say it and the reason why we used these sort of studies is to say, well, if a person is using an extract of a certain bottle, that person is presumably every time he gets a shot, he is not having adverse reactions. So, you say, all right, we are going to -- now, we will like raise this dose a certain amount. What is the probability that then he will get an adverse

reaction?

Obviously, that is a spread. Some might get it. Some might not. So, you say, well, if you analyze all the data and say, well, we want to keep that probability under 5 percent. That will set an outside limit. You might want it less, but 5 percent is what we pick. When you do that, you can see that effectively from the different data you get different results, but it is about -- you know, if you average these things, about a factor of 4.

Now, different data -- we use per patient data, so, it is a little tighter. We don't necessarily think that is, you know, the optimum way to do it. If you use different allergens, you might get different numbers. But at this first stab, this is how we get that factor of 4.

So, now, we were -- so, so far, we got the factor of 10, which is, you know, larger than a factor of 4. So, we say, okay, we are going to pick the smaller one. It has got to be a factor of 4. It can't be larger than a factor of 4. Even though a factor of 10 works in terms of efficacy, it implies that there is like extra associated with that factor of 10, which we don't want to do.

Now that we have the factor of 4, you know,

these things are kind of happening at the same time, but then we want to say, okay, now let's see what our ELISA does when the relative potency varies by a factor of 4. It is not obvious. You hope it does.

The real good news is that it works quite well. So, let me just do just this one grass. I mean, if you take the -- extract this .5, after 24 observations, we got, you know, .516. One is still working. Two -- you know, it is 2.00. We get, you know, 2.01. So, basically, you know, our tool is working.

The original assay had a standard deviation of this .1375 and you can see that it is in the same ball park. So, we have got the range. We have got the tools. Now let's see what the distribution of these lots actually is. This is a little complicated. So, I will try to, you know, kind of sketch it.

But after you look at -- we did both grasses and, like, mice. We looked at 412 lots which were submitted by the manufacturers. Of those, 12.4 percent failed, 29 and like 22 low. So, that -- using that, we can work backwards and say, ha, ha, now let's work out what the variability of that lot is. Now we just assume that, you know, half are high and half are low. Pretty reasonable.

So, in other words, if 12.4 percent, you know,

fail, that would mean that under this assumption, 93.8 percent were actually -- were like below the upper limit. Therefore, you can now write the distribution of the observed relative potencies, which consist both, remember, the assay and the manufacturing. If you integrate that, it is .938.

Therefore, from that equation -- therefore, you can just work out and it is not so bad that the standard deviation of these observed is .120. Nw, remember, your variability of your assay is -- you know, is .1375 divided by the square root of 3. We would use three replicates. We got this. We plug this into this equation and now we know that the variability of the sample is, you know, .09.

Now, these are just numbers flying around, but, oh, like tie them into stuff real soon, but I think it is a really great thing that we could just work this out. I found it very satisfying.

We actually see that the variability of that sample is actually smaller than the grass. That actually makes sense. Remember, the lots of grass were actually lots which were in support of the PLA. So, there were some ones that weren't so good; whereas, the lots of the grass were actually standardized products so the manufacturer really had the thing down right.

So, it is really also nice that you actually got this slight change here. These guys are doing a good job.

This is just a plot of the way that looks now. So, you know, here is the .5 to minus .5 and -- well, log relative. So, our -- this is in log RP. A relative potency of .5 as a minus log RP and minus .3. So, you can see this is through the mite, that the reserve for the mite is well within the factor of 4 and if you actually work backwards and say what is the sample for the mite, which is the green guy, this is well within that.

So, you see where now we feel safer that these manufacturers are turning out lots that are well within this factor of 4. We need to go a step further. This is the last of this quartet.

It is solvable. So, now let's just think of a little problem you might give your kid or something. It depends on the kid. But if you have distribution and you want to pick two samples from that, what is the range of samples that you expect to get?

Well, qualitatively, you kind of expect to get, you know, one kind of in the middle and another one off to one side. So, you know, expect something like the standard deviation of samples, about. What is less

likely is, you know, one way or one end and one here and what is not likely at all is to get one from the far left and like one from the far right.

No, how do you solve that problem? You know, you just do it. It is like you are trying to crank basically. So, what the result ends up being is that your average range, which one calculates as an average time to density itself is given by this and then we actually define a maximum range that is worth just spending a second more on.

This is like saying, okay, what is the range such that 95 percent of the changes are smaller than this are prime. So, now we say all right, we want it so that 95 percent of these bottle changes are less than this number. Then you can work it out in terms of the -- you have very simple expressions for the range, where this average range is, you know, .8 sigma, like I said, about a standard deviation and this maximum range is about 3 sigma.

So, it should be kind of comforting. It is inherently reasonable. You would have guessed that by yourselves.

Now we can look at what are the ranges of our extracts. If we actually look at the sample range now, just plugging in the numbers from the grass pollens,

remember, we had this .09 in log relative potency. You can now calculate your average range and average R prime in terms of log RP, but, you know, I think it is good to think of it in terms of what kind of change in like of relative potency you have got.

But the grass pollen is -- you know, you send like two bottles out to the population and ask for what is the change in that, your average change is around like a 20 percent, just like maximum. Ninety-five percent change is like less than a factor of 2. If you look at mites, it is even smaller, only 12 percent, and about 50 percent. So, in other words, the only -- I realize often it is hard to listen to math, but the bottom line is that the change in the relative potency for all these x axes is much less than a factor of 4. That factor of 4 is what we determined was like good for safety.

So, now we are track. So, now let's go back to that original question. If you had a relative potency of a lot that was really .7. The manufacturer tested it at .8 and passed it. We get .6 failing. Now we can answer the question was this all right. The answer is "no." There was overly much risk.

So, we have to do something because we already show this factor of 4 in terms of safety is fine. Now we are failing lots of the manufacturers. So, we need to do

something. So, we came up with this. There are various things one can do. We thought this was pretty reasonable.

Basically, we have the manufacturers maintain their limits because they are already doing it anyway and we have shown that with these limits, we get a nice tight distribution. Because their distribution is tight, CBER can like widen it. So, now we would say, okay, forget about that .654 stuff. You get .52. So, we haven't compromised public safety and we have reduced the manufacturer's risk.

The advantage of this is that you will actually still get the every once in awhile lot that, you know, something slips through. The darn stuff is .3. You have to sift that. But, basically, we can act more of as a sieve to the really extreme lots and actually -- as opposed to like rejecting a lot of stuff, you know, it is probably okay. We haven't compromised safety.

We will actually certainly reduce the number of lots we reject, you know, the acceptable lots. Then this one last one is kind of interesting because if a manufacturer does a better job at making the stuff, there is even a lower probability that it will failure CBER release. And here is how.

So, a manufacturer's test with n equals 3 on

the ELISA assay and say he gets .699, which is a 95 percent confidence interval of that, if that lot is passed over CBER, there is only about a 10 percent chance that that lot will like fail. That is reasonable, I think, because, remember, if it is tested at .699, it sort of could be lower. Right? So, you want to weed out stuff.

However, he tests n equals 6 and if it turns out to be .7, then the chance of failing it is only like 7 percent. You can look at the various numbers. They are in the guidance document. Then there is some fine print explaining how we do this, you know, why that is true.

So, just a couple of slides and sort of last comments and, hopefully, look at it now from a bigger picture. So, what have we done? Well, we like placed our limits so that this red one gets like filtered out. See, these limits now are .5 to 2, which in log relative potency is plus .3 to minus .3. Now, just look back for a second. Remember, the observed relative potency for the graph was .12. The observed relative potency for the mite is .1. So, effectively we say we are going to set our manufacturing goals at 2.5 sigma observed for the grass and a 3 sigma of the mites.

So, that is, you know, fairly FDA-wide policy

anyway. So, it is sort of interesting that it worked out that way. We came through it with a different way. I would also like to say, though, this is science driven. We weren't set on widening these limits.

If, in fact, the -- or for a new case, not just a new standardized product -- say if the relative potency of this material had been very broad -- I just made something up here, where this is, you know, a good chance to be .5, a good chance to be 4, what do you do then? You can't try this thing of anything from .5 to 2 makes it because something that tests at .5 could be .3. Something that tests at 2 could be 2.5.

So, what we would do instead is do what we were originally doing. We would draw a line between .7 and 1.4 and we would sort of sift it that way. So, there are different ways you handle different materials. You could say, well, gee, how come you like did that, did this equivalence now, you know, then and how come you just didn't do the .5 to 2 earlier? Because we didn't have the data. So, now because we have the data, we can widen it.

Lastly, I just think it was interesting. We actually looked at the labels of the manufacturers just to see what the range of potency for the unstandardized things were. This is just on the labels. You can read

them yourselves. Go buy a couple of bottles. Open them up. There we can actually look at that safety range again. So, it just lets us do one quickly. You can see that here for the water extracts, the range of like relative potencies is actually a factor of 9.

Now, for the glycerinated, it is 5. As you go down this road, you can see there was a factor of very high, 83. So, here is this like range. So, you can basically see this is a vindication of actually what we did. We actually reduced this in a significant manner.

It is almost reasonable after you have done something to say did we really have to do this. We could ask now the question, this ELISA, could we just have used the protein content, which is, you know, pretty easy as opposed to the immunological assay, which is, you know, actually not more about the site and a lot of work.

So, one of the ways you can look at it, again, now that we have the standardized results, we can actually plot the value of the relative potency. Now, these all had a relative potency, which were in the confidence limits of like 1. So, these are all equivalent to 1.0. So, we can see that for lots of this relative potency was 1. The protein content, you know, goes from, you know, something like .3 to 12, a huge number for -- that is 172 lots of grass, but 188 lots of

mites, we have the same sort of result, you know, about a factor of 10.

So, in other words, it is shown pretty clearly for grass and mites. It might not be true for something else. In fact, you really do have to use ELISA not protein content.

In conclusion, I hope I have shown you where our thinking is in terms of these risks. By widening limits, we have lowered this without changing this. Last, I think I have -- you know, we have tried to tell you that the variability is unstandardized and then this is the way we will do things for each of these new products and put it through the same kind of thinking and, you know, hopefully, we will get things right.

Thank you very much.

DR. OWNBY: Thank you.

Are there any questions for Dr. Pastor at this time?

Dale.

DR. UMETSU: It seems if I understand this correctly is one of the problems is due to the fact that the ELISA assay is not as reproducible as we would it to be. So, you are saying that instead of doing the assay three times or in triplicate, you can do it in six duplicates to get a better value. Why not just do it

nine times to get a much more precise value so that you can still maintain relatively small specifications for each lot?

DR. PASTOR: Because to do it nine times is a lot more work. With every ELISA you do, you actually have to use reference standards. If you do nine, it is actually really like three times as much work as three because, you know, the way the plates are is you get kind of three per plate.

So, you can say do you add significantly to the safety of the product by doing nine as opposed to doing three. The answer was "yes" and you really -- then you say, yes, we really have to do it but if doing nine doesn't really change much, then you don't have to do it. It is really a balance all the time. When one does testing and lot release testing in manufacture, you have to say what is the benefit.

DR. UMETSU: Well, I guess I can understand that. If your main assumption is safety, you are saying that you can tolerate a fourfold difference in lot variation, but if you are really trying to say we want a product that is standardized so that every lot is as close to the last lot as possible, you might want to sort of use a different parameter rather than safety. Safety is important, of course, but --

Remember, we actually did look at two. We said in terms of efficacy, the first analysis can be a factor of 10. So, safety was a factor of 4. If efficacy had been a factor of 2, then we would have chosen that. You really have to always say -- you know, it is a freshman chemistry kind of thing. When someone gives a result in significant figures, you have to say, well, first of all -- usually with freshmen chemistry you say the answer is "no," but with this you say do you have to do the experiment such as you have six significant figures? The answer is "no."

On your car, if you are driving -- if the speed limit is 30, 35, you have to have, you know, 34.99968. 35 or nothing. You know, I had another analogy with cars. If you are setting a limit, what do you need? Do you need a speed bump, a stop light or a toll booth. You know, so you have to work these things out so that there is a balance. N equals three is plenty.

Jay might want to add to that. It looks like he might.

DR. SLATER: -- higher level of precision and that is when you are replacing your reference. In fact, when we replace the reference, we end up doing between 24 and 50 tests if you accumulate all the data that we get from manufacturers in order to achieve as great a

precision as possible and, in fact, we can achieve a high level of precision because if when you are switching your reference, which is your standard material that you are going to use. If you allow drift that would be clinically acceptable to occur, it could keep occurring in the same direction and then you might enter an unacceptable zone pretty quickly.

But I think one of the key things here is the therapeutic index of the product is broad enough so that even though a higher level of precision would be satisfying from a measurement science point of view, it is really not justifiable from the point of view of the products themselves.

DR. CLAMAN: That was a good presentation. I get stuck with the sigmas and the stuff like that. I am debating whether I would buy a used car from you or not and at the moment that jury is out.

I would like to ask manufacturers' representatives how important changing these limits really is.

MR. HAUCK: I can say, as far as Center Laboratories is concerned, I think it is a pretty good idea. We have kind of been in support of it. In fact, most manufacturers, I believe, are now using the n of 6 at some point during their testing. So, I think the

fourfold limit is very reasonable from their perspective. It is not reasonable from our perspective. We can't use it, but it makes sense when they are reviewing our data and our products.

DR. OWNBY: If there are no other burning questions, I would like to move on. I would remind the committee we have plenty of time this afternoon to come back and rediscuss the ins and outs of this and I think there are some other ramifications of it that we may want to discuss. We would also like to hear from the manufacturers' representatives, their position and any public comment on this before we try to come to any conclusions.

I believe next on the agenda is Dr. Slater again. Not quite a one-man show today.

Agenda Item: Regulatory Proposals -- Allergen Standardization

DR. SLATER: Thank you.

In your hands you have a package entitled "Allergen Standardization Algorithm, January 2000." I suggest you pull that out. That will be helpful as we go through this.

I am going to make some preliminary remarks about allergen standardization and I am going to take you through this new algorithm,, which is our effort to take

allergen standardization forward. But before we can take it forward, we need to look at where we have been. That is part of what is a process that really started nearly a year ago in terms of a critical review of the allergen standardization process.

I think that it is safe to say that allergen standardization in the United States has been a major public health success. We have, as you know, many hundreds of allergen extracts marketed in the United States about which little if anything is known about their actual true biological potency.

As a result of CBER's Allergen Standardization Program, which just for the record entirely preceded by many years my appearance in this lab. It is clear that we now have 19 products that have been well-standardized, that are safer for which their potency is assured. As I will come back to over and over and over again, the standardization process was essential not only for the clinician and the patients; it was essential for the scientists as well. You can't do good science with these extracts until you know what is in them.

The potency, the allergen standardization process has been critical for this. This is a listing of the currently standardized allergens and the tests that are currently used to assess their potency.

When we began to consider going forward with allergen standardization into the future, we wanted to look backwards carefully and see where we had been. By way of organizing ourselves, we decided to as a group in the division look at the scientific results of allergen standardization, the so-called QC/QA results of standardization to decide where we were going to go in terms of building our experience and how to move forward with planning next steps.

Clearly, the most important lesson that was learned scientifically from allergen standardization is that protein does not equal potency. If there is a single take-home message of the standardization effort, it is that the unitives that have been used for decades prior to this was not a good indication of biological potency.

This is a slide that Rich Pastor showed you just a few minutes ago, in which we actually look at standardized grass pollen and mite allergens; the grass pollen at a hundred thousand BAU per ml potency, the mites at 10,000 AUs per ml potency and we looked at the protein content of these and we saw a tremendous scatter of protein content.

If you eliminate these outliers, the mite situation is definitely tighter than the grass situation,

but both of them clearly indicate that there is no question that we shouldn't go back to the system of looking at the protein as a measure of potency. It simply isn't.

Furthermore, again, this is a slide of data taken from the manufacturer's package insert that clearly shows that in the prestandardization days that there was a tremendous variation of potency, especially among the aqueous extract, but also among the glycerinated extracts that you can see for a wide range of products.

One of the other lessons that we learned is that clinical testing is essential. You can't construct an allergen standardization program unless you introduce material into humans and see what is going on. You have to do something that relates to clinical activity in order to standardize product. The prime example of this is the Bermuda grass pollen extracts, which prior to the clinical studies no one really understood that the maximal potency of these products was significantly lower than it is for the other grass pollen extracts.

That labeling has now been incorporated into the labeling for the standardized Bermuda grass pollens. We also learned that in vitro tests can serve as surrogates for skin tests and this seems sort of obvious now. It is clear that you can do this, but it wasn't

necessarily obvious that these tests, the last inhibition tests, the competition ELISA test would really serve as good surrogates for biological testing, that they have been.

We also learned that different in vitro tests may be optimal for different allergens. For some allergens, for cat and for short ragweed it is okay to look at a single allergen. That is a good measure of potency. It is a good surrogate for potency measures; whereas, for the others, for mites and for grasses, you need to look at a more global approach, using cooled allergic sera in the competition ELISA assay, looking at multiple allergens at the same time.

So, we know that we can't prejudge the situation. We don't know what tests are going to be best. We have to study each specific allergen extract and draw conclusions from that. Again, this has been known for decades that glycerol improves the stability of extracts. Standardization confirmed that the potency was clearly maintained better in glycerol solutions.

Another more recent study that we actually did in our laboratory suggests that the stability assessments may actually be method dependent. In other words, you have to be careful what methods you use to look at stability. This comes from a study that was done in the

lab prior to my arrival, by Dr. Lin(?), along with Teresa Lu(?) and a follow-up study that was started by Dr. Soldatova in collaboration with several people in the division to look at the stability of mite extracts in solution.

The original study that was done looked at this but it was noticed shortly after it was completed that the control that was used, the 4 degree control, was not really the optimal control, that we really wanted something that was more stable than 4 degrees to see whether the 4 degree specimens were stable as well.

So, the design of this study, which was begun before I arrived on the scene was to take lyophilized mite extract products at time 03, reconstitute them in 50 percent glycerol, to store them for up to 12 months at minus 70, minus 24 degrees and, believe it or not, at 37 degrees, as well. And then to check the relative potency specific allergen content measures and immunoblocks at 6 and 12 months.

These are data that I presented in part last year and I am going to go through them relatively quickly. The bottom line was that for all of the extracts that we examined and if we looked at the relative potency, using the standard competition ELISA, what we found was that at minus 20 and at 4 degrees, the

extracts were indistinguishable from the reference.

In other words, there didn't seem to be any deterioration at minus 20 and at 4 degrees in any of the extracts that we reconstituted after 6 and 12 months, compared to the lyophilized material, which was freshly dissolved at the time of the assay. So, these data were very reassuring and clearly suggested that the materials were stable.

However -- and I am sorry for the way this comes out. This is the one failure of power point to be able to take a scanned blot, but, however, when we looked at it by two other methods, we found different results and I am going to take you through these shadows and this is going to be an indication of how much you trust me after this whole morning.

What we see here is that the -- this is the minus 70 sample. This is the minus 20 sample and these two are the 4 degree samples. What you can see here is the loss of these bands and of these bands as well in the 4 degree specimens. The 37 degree specimen you lose everything, but nobody here is particularly surprised by that. Likewise, this is another batch. We also lost this allergen completely. This is the Der P 1 allergen in both cases -- Der f 1 allergen -- I am sorry -- in both cases by immunoblock.

Mind you, by relative potency we didn't lose any activity in these extracts. So, this is a divergence of the answers that you get depending on how you look. Then when we used monoclonal antibodies to measure Group 1 and Group 2 allergen content at 12 months, we also found some heterogeneity in that especially in the Der f specimens, there seemed to be a decrease of 40 percent, 50 percent, again 40 percent, in both the minus 20 and the 4 degree specimens, compared to the lyophilized material.

Remember, the lyophilized material is not shown on this graph. The lyophilized material is the hundred percent one. However, in the Der p 1s, these were much closer to a hundred percent and less of a problem with deterioration.

We actually plotted this in a somewhat novel way in order to try to explain a lot of data that was a little contradictory, to look at the relative potency using pooled allergic sera plotted against the relative potency that was allergen specific. That is a new parameter. Okay? Normally, when we talk about relative potency, we are talking about pooled allergic sera, the standard way that we do this.

But we also took the allergen specific potency, which was simply in one example the Der P 1 content of

the stored material divided by the Der P 1 content of the lyophilized material that was freshly dissolved for assay at that moment.

So, it is simply a ratio of the potency of the test material versus the standards. And there are a couple of observations to notice here. First of all, the Der f, the D.farinae specimens, the closed figures are the D.pteronyssinus specimens. The first thing to note is this is a true scatter plot. There is no relationship here. The slope of these lines if you tried to draw one is not statistically different from zero. There is no correlation between these two values. That is observation No. 1.

Observation No. 2, though, is that, gee, it is interesting that all these Der f 1s are down here and all these Der p 1s are up here. There is, in fact, a significant difference between the two populations. What does that suggest? Well, it is not earth shattering from a regulatory point of view yet. We haven't really worked this out yet, but it suggests that in D.farinae extracts, that specific allergens appear to degrade relative to the relative potency of the material at a faster rate than the Der p 1s, the Der p extracts.

Conversely, it may simply mean that there is a better correlation in D.pteronyssinus between the content

of these two specific allergens and the overall relative potency, than there is in D.farinae. We don't really know that yet.

But the take home message from our point of view is that there is a discrepancy between the results that we get, depending on how we look. The relative potencies look perfectly stable at minus 20 and 4 degrees, but there were specific allergens that appeared to degrade significantly at 4 degrees and some of them even at minus 20 degrees. These are other results of the study.

Protease inhibitors did not appear to contribute to stability. We know now that lyophilized extracts appear to be more stable in these particular examples than even the glycerinated ones. But it is clear that the stability of glycerinated extracts is enhanced at lower temperatures.

Again, looking backwards at allergen standardization, what have we learned? Okay. From a QA/QC point of view, clearly the most important result that we have from the standardization effort is the development of U.S. standards of potency. We do not have in-house references. This is not consistency monitoring. There is a single U.S. standard of potency with a common, industry-wide unitage for each allergen extract.

What are the advantages of that? Well, it clearly increases safety and consistency of the products. It clearly facilitates scientific studies. If you look at the scientific studies that we were able to cite when we were trying to plot out the dose responses, it is no accident that most of them are Amb(?) a 1. Those are the only ones you could do it with for awhile, because if you don't have a standardized material to work with, you simply can't do dose response by these. You can't learn about the extracts in an organized quantitative way, unless you have a U.S. standard or some standard that you are comparing these with.

Certainly, having a U.S. standard, facilitates scientific studies that can be done in Seattle and replicated in Bethesda with reasonable assurance. Furthermore, having a single U.S. standard clearly enhances product choices for individual practitioners to go among different manufacturers. In addition, QA/QC results include the development of standard operating procedures for testing methods. We now have SOPs for testing all of these allergens and that allows us to have true quality assurance and quality control of the development of the potency measures.

In addition, we now have stability monitoring programs in place for these allergens. So, looking back

on allergen standardization, what is it that we clearly want to incorporate as we build on our experience and move forward. Well, clearly, we want to continue the standardization effort. I am sure that is not a shock to anyone in the audience that I would say that.

But, clearly, we want to move forward with allergen standardization and we want to move forward with the development of U.S. standards of potency. In addition, we clearly want to keep clinical testing as the gold standard by which we measure all other tests. How do we move forward?

Well, the first step is to try to build consensus, to construct a transparent process, which I hope you will feel what you have in your hands is a transparent process. We want to identify specific decision points at which we would be able to change our minds. In other words, as we embark down the road of standardization of product, one of the things that we wanted after much discussion to build into it was an intrinsic way in which we might decide for either good reasons or bad reasons to abort a particular standardization campaign.

I will go into that in more detail later. We certainly want to consider an increased role for industry and for other collaborations. We want to make sure we

choose the most stable references possible for reasons that I shared with you early on. Given the studies that Rich and I have done, the analyses that Rich and I have done that you have already heard about both this year and last year, we certainly want to begin the process of considering clinical data in setting rational release limits.

Finally, we would like to consider for future allergens to consider enlisting the help of the U.S. Pharmacopeia in handling and distributing references. So, the purpose of the algorithm that you have in front of you is to build upon our prior successes, which are substantial. We want to establish priorities and procedures by which we can go forward as quickly and as efficiently as possible.

If I had to identify some new aspects for this algorithm -- I mean, a lot of what you will see in the algorithm is just what was done already, but if I had to identify something new in the algorithm, I would say that we are making an attempt to state clearly at the outset what our criteria are for allergy selection. We are going to attempt to delineate responsibilities for different parts of the allergen standardization process.

Finally, we are going to be able to set exit points for reasons that I will outline momentarily.

Let's talk about the impact criteria. The impact criteria were the subject of a great deal of discussion and the important thing that I want to state about the impact criteria is that they are not meant to be exclusionary. The idea here is to give us an organized way of talking and thinking about allergens and I suppose the most obvious way to think about this is if you want to look at one impact criterion, think about two extracts about which everything else was equal. We would like to focus on that one in which they unequal and make a decision based on that.

What are the impact criteria that we agreed upon? First of all, the availability of stable, preferably lyophilized materials for use as long range reference extracts. Second, the consistency of currently marketed products needs to be taken into consideration. Again, that probably requires a little explanation.

All other things being equal, if one product is remarkably consistent across the market, as we observe it in the unstandardized state and another product is dreadfully inconsistent, such that switching from bottle to bottle would pose a true safety risk to individuals that might get it, we would clearly want to focus on the inconsistent products. But that doesn't mean that is the only thing we would look at. It is certainly possible

that a fully consistent product across the market might be, in fact, mediocre and we might need to standardize it to bring the level of the product up.

We certainly want to consider how widespread the product is used. Clearly, if we are establishing priorities, we want to standardize products that are used a lot as diagnostic and/or therapeutic reagents in the United States. We want to know about the number of manufacturers producing a product. We want to consider the potential use in immunotherapy for which we would give it a higher priority or perhaps just a diagnostic reagent which it would have a somewhat lower priority.

We want to know what the public health impact of the correct diagnosis and/or adequate treatment would be. Again, just to emphasize the point, there is nothing in here that is intended to be excluded. For instance, one could easily imagine with peanut, for instance, which is not currently a therapeutic reagent, but just a diagnostic agent, the decision might be made that it was of such importance to go ahead and standardize it, that we would do that in spite of the fact that it is not currently used for any other therapy.

Furthermore, we are very sensitive to the issue that when you standardize a product, you can encourage the science that can lead to further studies, that can

lead to expanded usage of the product under appropriate procedures. So, I just want to make sure it is quite clear that these are -- that none of these is intended to exclude anything.

Theoretically, we could even decide to standardize a product that nobody makes, but that we know that it is of such public health importance that if we standardize it, manufacturers would come in and would be willing to produce the product. So, there is a short form of the algorithm and a long form of the algorithm. The short form is a lot easier to look at.

Basically, it starts out with the preliminary region of identifying target allergens, using the impact criteria. We go into a laboratory phase, a clinical phase, a manufacturing phase and a post approval phase. At both the laboratory phase and the clinical phase, there are ways that we can decide that we don't want to proceed with this allergen that I will go into in greater detail.

This is completely useless to put up except to say that I am now going to talk about the first part of the algorithm. In the preliminary stage, we identify the target allergens using the criteria that I discussed. At that point, CBER would ask to be sent multiple lots of the target allergen by each manufacturer. Three lots is