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Office of Vaccines Research and Review (OVRR)

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Zoom Video Conference

(Session II)

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Members

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Jay M. Portnoy, MD	Professor of Pediatrics, University of Missouri – Kansas City, School of Medicine, Director, Division of Allergy, Asthma, and Immunology, The Children's Mercy Hospital	Kansas City, MO

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Mark S. Dykewicz, MD	Raymond and Alberta Slavin Endowed, Professor in Allergy and Immunology, Professor of Internal Medicine, Chief, Section of Allergy and Immunology, Division of Infectious Diseases, Allergy and Immunology; Department of Internal Medicine, Director, Allergy & Immunology Fellowship Program, Saint Louis University, School of Medicine	St. Louis, MO
Paul Greenberger, MD	Professor Emeritus, Medicine (Allergy and Immunology), Institute for Public Health and Medicine, Northwestern University Feinberg, School of Medicine	Chicago, IL

Guest Speaker

Thomas Platts-Mills, MD	Professor of, Medicine, Division of - Asthma, Allergy, and Immunology, Department of Medicine, University of Virginia School of Medicine	Charlottesville, VA

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Trenna Repp	President, Allergen Products Manufacturers' Association (APMA)	

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Vinayak Prasad, MD, MPH	Center Director, CBER, FDA	
David C. Kaslow, MD (Presenter)	Office Director, Office of Vaccines Research and Review (OVRR), CBER, FDA	
Karin Bok, MS, PhD	Deputy Director, OVRR, CBER, FDA	
Sudhakar Agnihothram, BPharm, PhD	Associate Director of Office Regulatory Initiatives, OVRR, CBER, FDA	
Jerry Weir, PhD (Presenter)	Director, Division of Viral Products (DVP), OVRR, CBER, FDA	
Zhiping Ye, PhD	Chief and Principal Investigator, Laboratory of Pediatric and Respiratory Viral Diseases, DVP, CBER, FDA	

Sharon Tennant, PhD, MPH	Acting Director, Division of Bacterial, Parasitic and Allergenic Products (DBPAP), OVRR, CBER, FDA	
Ronald Rabin, MD (Presenter)	Chief, Laboratory of Immunobiochemistry (LIB), DBPAP, OVRR CBER, FDA	
Michael Brad Strader, MSc, PhD (Presenter)	Staff Scientist/Biologist LIB, DBPAP, OVRR CBER, FDA	

Designated Federal Officers

LCDR Cicely C. Reese, PharmD	Designated Federal Officer, VRBPAC, CBER, FDA	
CDR Valerie Marshall, MPH	Designated Federal Officer, VRBPAC, Alternate DFO, CBER, FDA	

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Call to Order

Dr. El Sahly: I would like to welcome the Committee again for the Topic II. Topic II will be to discuss and make recommendations on advancing CBER's Allergen Standardization Program. I hereby call the meeting to order. I would like the four allergy experts on the Committee to raise their hand so I can see where they are and I can call them to introduce themselves.

LCDR Reese: Oh, excuse me, Dr. El Sahly. We're going to go through-- I have to go through the Conflict of-- Introductions and Conflict of Interest Statement again, so I'll go ahead and pick that up and add them in there, if that's fine with you.

Dr. El Sahly: Okay, sure. It had me doing this on the annotation, but that's great. Thank you.

Roll Call and Introduction of the Committee

LCDR Reese: Thank you. My name is Cicely Reese, again, and it is my honor to serve as the Designated Federal Officer for Topic II of the 190th meeting of the Vaccines and Related Biological Products Advisory Committee. On behalf of the U.S. Food and Drug Administration, the Center for Biologics Evaluation and Research, and the Committee, I'm pleased to welcome you back and to extend a warm welcome to those who are just joining us for the second portion of the meeting.

We will now take a brief roll call to reconfirm attendance for those who have been with us since the morning, earlier this morning, and then invite our recently joined allergen experts to introduce themselves for the record. When called upon, please turn on your camera, unmute and introduce yourself by stating your first and last name, your organization, and your expertise or role. Once finished, you may turn your camera off so

that we can move to the next person. If you could please display the slide. Thank you. And we'll begin again with our Chairperson, Dr. Hana El Sahly.

Dr. El Sahly: Good afternoon, everyone. Or good morning still. My name is Hana El Sahly. I'm a Professor of Molecular Virology and Microbiology, Baylor College of Medicine in Houston, Texas. My expertise is in infectious diseases and clinical vaccine development.

LCDR Reese: Thank you, Dr. El Sahly. Dr. Bernstein.

Dr. Bernstein: Hello, everybody. My name is Hank Bernstein. I'm a Professor of Pediatrics at the Zucker School of Medicine at Hofstra North Shore and Cohen Children's Medical Center in New York. I'm a general pediatrician with expertise in vaccination and policy, and I do primary care pediatrics.

LCDR Reese: Thank you, Dr. Bernstein. Dr. Durbin.

Dr. Durbin: Hi, my name's Dr. Anna Durbin. I'm a Professor of International Health in the School of Public Health at Johns Hopkins University and Adult Infectious Diseases and Internal Medicine in the School of Medicine at Johns Hopkins. And my area of research is vaccine-- Early vaccine development and evaluation. Thank you.

LCDR Reese: Thank you, Dr. Durbin. Dr. Kollmar.

Dr. Kollmar: I'm James Kollmar. I'm a Pediatrician by training and a Scientific AVP in Global Regulatory Affairs and Clinical Safety, Vaccine and Infectious Diseases at Merck & Co. I'm the Industry Representative.

LCDR Reese: Thank you, Dr. Kollmar. Captain Meyer.

CAPT Meyer: Hello. Hi, I am Sarah Meyer. I'm a Pediatrician and Medical Officer at the CDC. My areas are pediatrics and vaccines.

LCDR Reese: Thank you, Captain Meyer. Dr. Monto.

Dr. Monto: I'm Arnold Monto. I am an Epidemiologist at the University of Michigan School of Public Health, interested in respiratory infections and control with vaccines and antivirals.

LCDR Reese: Thank you, Dr. Monto. Dr. Omer.

Dr. Omer: Hi, I am Saad Omer. I'm the Dean of the O'Donnell School of Public Health UT Southwestern in Dallas. I focus on interventional and observational studies of vaccines and infectious diseases.

LCDR Reese: Thank you, Dr. Omer. Dr. Perlman. You may be on mute, Dr. Perlman. We can come back to Dr. Perlman-- Is everyone hearing me?

Dr. El Sahly: I can see him. [Indiscernible - 03:08:33]

LCDR Reese: [Indiscernible - 03:08:33] on mute? We can come back to Dr. Perlman, but if we can see him and he'll join-- Doctor-- Oh, there we are.

Dr. Perlman: Sorry, I was on mute or I muted myself here. Yeah. I'm Stanley Perlman, Pediatric Infectious Diseases at the University of Iowa with expertise in Coronaviruses.

LCDR Reese: Thank you, Dr. Perlman. Dr. Portnoy.

Dr. Portnoy: Good afternoon. I'm Dr. Jay Portnoy. I'm a Professor of Pediatrics at the University of Missouri, Kansas City School of Medicine. I'm an Allergist Immunologist at Children's Mercy Hospital in Kansas City, Missouri.

LCDR Reese: Thank you, Dr. Portnoy. And Dr. Rubin.

Dr. Rubin: Eric Rubin at Harvard, the Brigham and Women's Hospital, and New England Journal of Medicine. I'm an Infectious Disease Doctor who studies tuberculosis.

LCDR Reese: Thank you, Dr. Rubin. And we will now join in with our allergen experts for the first time today. Dr. Assa'ad.

Dr. Assa'ad: Hi, I'm Amal Assa'ad. I am a Professor of Pediatrics at the University of Cincinnati. I am the Director of Clinical Services in the Division of Allergy Immunology at Cincinnati Children's Hospital. My expertise is allergy and immunology.

LCDR Reese: Thank you, Dr. Assa'ad. Dr. Davis. You may be on mute. Do we have Dr. Davis's audio? We can come back if we're working on that. Dr. Dykewicz.

Dr. Dykewicz: Hello, I'm Mark Dykewicz. I am Professor of Allergy and Immunology and Internal Medicine at St. Louis University, School of Medicine in St. Louis. And my expertise is allergy and immunology.

LCDR Reese: Thank you, Dr. Dykewicz. Dr. Greenberger.

Dr. Greenberger: Hello, everyone. Paul Greenberger. I'm an Internist and Allergist Immunologist, Professor of Medicine Emeritus at Northwestern University, Feinberg School of Medicine in the Department of Medicine in Chicago. My expertise includes work with *Aspergillus fumigatus,* primarily in the context of allergic bronchopulmonary aspergillosis, and here-- Not that many years ago, cat allergy accelerated immunotherapy through the Immune Tolerance Network's study called CATNIP.

LCDR Reese: Thank you, Dr. Greenberger. We're going to try to go back to Dr. Davis and see if we have her audio. If not, we can work on that and at a break, we can have her introduce herself. We're working on the audio. We'll come back to her. We may be having some audio difficulties. So, I'll go on to the next portion of the meeting where I'll read the Conflict of Interest Statement again.

Conflict of Interest Statement

LCDR Reese: The FDA is convening today's meeting of the Vaccines and Related Biological Products Advisory Committee, also referred to as VRBPAC, under the Federal Advisory Committee Act, FACA, of 1972. The VRBPAC will meet in open session to discuss and make recommendations on advancing CBER's Allergenic Standardization Program.

With the exception of the Industry Representative, the members of the Committee are either Special or Regular Government Employees and are subject to federal conflict of interest laws and regulations. Accordingly, FDA has reviewed the financial interests of the Committee members for compliance with federal ethics and conflict of interest laws. We have screened the members for potential financial conflicts of interest related to today's meeting agenda, both their own interest and their-- And those that are imputed to them, including those with their spouses, minor children, and employers. Based on the agenda for today's meeting and all financial interests reported by the Committee members, FDA has determined that all members of this Advisory Committee are in compliance with federal ethics and conflict of interest laws and as a result, no conflict of interest waivers under 18 U.S.C. 208 have been issued in connection with this meeting.

Dr. Archana Chatterjee has been recused from the meeting based on today's agenda and her financial interests analyzed by FDA. James Kollmar of Merck & Co. is participating in the meeting as Non-Voting Industry Representative acting on behalf of regulated industry. Consistent with Commissioner Makary's April 17th, 2025 statement, FDA is only including Industry Representatives in Advisory Committee Meetings where required by statute. FDA is required to include an Industry Representative in today's meeting under 21 U.S.C 355 (n)(3)(c). Industry Representatives are not appointed as Special Government Employees, nor are they Regular Government Employees. Industry Representatives serve as Non-Voting Members of the Committee. Non-Voting Industry Representatives represent all regulated industry and not any particular association, company, product, or ingredient, and bring general industry perspective to the Committee. Under FDA regulations, although a Non-Voting Member serves as a representative-- In a representative capacity, the Non-Voting Member shall exercise restraint in performing such functions and may not engage unseemly advocacy or attempt to exert undue influence over the other members of the Committee. Dr. Jay Portnoy is serving as the Consumer Representative for this Committee. Consumer Representatives are appointed Special Government Employees and are screened and cleared prior to participating in the meeting. They are Voting Members of the Committee.

There are speakers-- There are guest speakers at today's meeting and an organizational speaking-- Speaker who will be giving a presentation to the Committee, answer questions from the Committee, and return the Committee back to the Chair. They will not participate in the Committee deliberations, render advice to the FDA, or vote. The speaker and organizational speaker participating in this meeting are presenting the views of their professional societies and not their personal views. The following

guest speaker has been screened and cleared to participate in this topic for the meeting. And that is Dr. Thomas Platts-Mills. In the interest of transparency, FDA asks speakers and guest speakers to disclose any personal financial involvement with a firm, product, or other entity affected by the Committee's discussion to allow the audience and the Committee to objectively evaluate their presentation. Today's speakers and organizational speakers have not reported any such relevant interests. FDA asks that all other participants, including the Open Public Hearing speakers, advise the Committee of any financial relationships that they have with any affected firm, its products and if known, its direct competitors.

We would like to remind the members that if the discussions involve any products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participant needs to inform the DFO and exclude themselves from the discussion and their exclusion will be noted for the record. Thank you.

Dr. El Sahly, I will now turn the meeting back over to you to commence the Open Public Hearing for Session II.

Open Public Hearing

Dr. El Sahly: Thank you, Cicely. So, now is the Open Public Hearing Session for Topic II.

Welcome to the Open Public Hearing Session. Please note that both the FDA and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the Open Public Hearing Session of the Advisory Committee meeting, FDA believes that it is important to understand the context of an individual's presentation. For this reason, FDA encourages you, the Open

Public Hearing speaker, at the beginning of your written or oral statement, to advise the Committee of any financial relationship that you may have with the sponsor, its product, and if known, its direct competitors. For example, this financial information may include the sponsor's payment of expenses in connection with your participation in the meeting. Likewise, FDA encourages you at the beginning of your statement to advise the Committee if you do not have any such financial relationships. If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking. My understanding is that there are no one who registered for the Open Public Hearing Session. So, that concludes the Open Public Hearing Session.

Replacement of Radial Immunodiffusion (RID) Assays of Currently Standardized Extracts with ELISA or Aptamer-based Enzymatic Assays

Dr. El Sahly: Next on the agenda is Dr. Rabin. Dr. Rabin is Chief, Laboratory of Immunobiochemistry, Division of Bacterial, Parasitic and Allergenic Products, Office of Vaccine Research and Review at CBER, FDA. Dr. Ronald Rabin will give us an overview of the replacement of radial immunodiffusion assays of currently standardized extracts with ELISA or aptamer-based enzymatic assay. Dr. Rabin. We cannot hear Dr. Rabin.

Dr. Rabin: Okay. Can you hear me now?

Dr. El Sahly: Yes, we can.

Dr. Rabin: Okay. Terrific. Thank you very much, members of the Committee, those of you who were in the morning session for staying for the second half of the session and thank you very much for the group of allergists who have joined the Committee meeting for this session. As you have seen from the agenda and the briefing document,

we've basically broken this presentation-- This topic up into two presentations. The first one pertaining to the enzyme-linked monoclonal antibody and aptamer-based assays that we are currently developing. Next slide, please.

The desired outcome for today's meeting is the updated methodologies for potency measures of allergen extracts that can be represented by one or two allergens, and to apply these methods towards increasing numbers of standardized allergen extracts. The problems that we're addressing is that the current method for measuring the major allergen content is outdated and should be replaced, and that non-standardized extracts are a regulatory gap that compromises clinical reliability. Next slide, please.

Just to remind you that the questions that are relevant to this talk are the scientific soundness of mass concentration measurements by ELISA or a similar assay using aptamers rather than monoclonal antibodies and the appropriateness of these revised assays for CBER's Allergenic-- To expand our Allergenic Program-- Standardization Program. Next slide, please.

What I'm going to do is discuss allergenic products in the regulatory framework for the regulation. To the allergists in the group, I apologize because we have a lot of people on the Committee who do not think about allergic disease and allergen and immunotherapy from day to day. I'm going to go over some of the basics so that everybody's on the same page. I'm going to discuss current potency measures of allergen extracts; progress in the ELISAs and aptamer-based enzymatic assays to replace outdated potency assays in current use; a need for expanding the list of allergen extracts-- Standardized allergen extracts; and the idea, the concept, that the adoption of the ELISA that we're working on would serve as a template for validation of new assays and tech transfer to manufacturers to use for lot release. Next slide, please.

So, just to remind you of what I'm sure you all know, that asthma and food allergy are common chronic diseases. Over one in four American children have allergies, which for the purposes of this statement could include atopic dermatitis, allergic rhinitis, conjunctivitis, asthma, or food allergies. And the graph on the right is from the recent MAHA report showing the increase in prevalence of food allergies over a period of about 30 or 20 years or so in children, either 0 to 4, or 5 to 9, or 10 to 17 years of age. Next slide, please. So, allergen immunotherapy is the only disease modifying treatment for allergic disease. And in 2009, it was estimated that about 3 million Americans received allergen immunotherapy. Immunotherapy for environmental, that is, non-food allergies, is most often administered as subcutaneous injections of allergen extracts. And the basic recipe for these allergen extracts has been used for over a hundred years. The next slide, please.

Now, the FDA has not changed its approach towards standardization of these allergen extracts in the past 30 years, but advances in science really present an opportunity to bring regulation of allergens into the 21st century. And that is why we are having this conversation today. Next slide, please.

So, what are allergen extracts? Allergen extracts are sterile aqueous extracts of plant or animal proteins used to diagnose or treat allergic hypersensitivity. These are crude preparations, and the source materials may be pollens, food, or animal hair or dander. Now, it's important for me to state that the questions before the Committee concern these licensed products that are used to aid in the diagnosis or treat allergic disease and that are licensed for administration by the percutaneous intradermal or subcutaneous roots. The presentation and discussion are not relevant to the licensed sublingual products, the licensed oral immunotherapy product for peanut allergy, or any novel

products in our regulatory pipeline. We are only referring to these allergen extracts-- These aqueous allergen extracts. Next slide, please.

Now, the regulatory framework-- Our authority to regulate these extracts derives from two laws enacted by Congress, the Food Drug and Cosmetic Act of 1938, and the Public Health Service Act of 1944, regulations that specifically address allergen extracts appear in part 680 of Title 21 of the Code of Federal Regulations, although other parts of 21 CFR also apply to allergenic extracts as well. And what these regulations say is that CBER determines standardization methods and potency units, and that once CBER standardizes an extract, it must be distributed by the manufacturers as a standardized extract. Currently, there are 19 allergen extracts, and they were standardized between 1987 and 1998, and they're standardized by either measurement of a major allergen or overall potency. And I'll go into those in more detail in a few moments. Now, for the extracts that aren't standardized by CBER, they're considered non-standardized, and they're described as-- Their concentrations are described as being protein nitrogen units or weight per volume at time of extraction. And it's important to point out that for these non-standardized extracts, there's no indication as to whether a protein is intact. Now, one salient feature about the standardization and how we regulate it is a difference between how we do it here and how it's done, for example, in Europe. In Europe, the different companies standardize their own extracts and use their own unitage and what that means-- But here we do that, and the reason for it is because, unlike the European market, by using reagents and potency units that are common to all manufacturers, healthcare providers can infer from that, for example, that a Timothy grass extract labeled 100,000 BAU/mL from manufacturer A is qualitatively and quantitatively identical to a Timothy grass extract labeled 100,000 BAU/mL from manufacturer B.

This is an advantage that we feel-- A strong advantage for regulation and standardization by FDA rather than individual manufacturers. Next slide, please.

Now, as I mentioned, some of the extracts are regulated by the major allergen. Now, a major allergen is currently defined as a protein in which more than 50% of the allergic patients are sensitive to. And we have two-- This talk, at least, concerns two particular allergens extracts that are regulated by major allergens and that that major allergen is measured currently by the radial immunodiffusion assay. Now, a picture of the radial immunodiffusion assay is shown on the left side of this slide in which you have a slide, a glass slide with agar, and then you have holes that are punched into it. The agar has in it a polyclonal antiserum against the allergen, and then the allergen at different concentrations are put into the punched wells and after a period of time these precipitant rings are formed, and the diameter of the precipitant rings correlates with the concentration of the allergen that was placed in the center in that hole. And you could see a curve, a calibration curve in the center, and you could see as well the machinery that we use to measure these precipitant rings. And the important point here is that while this technology works, it's antiquated, it's old, the precipitant rings do not form these ideal edges, discrete edges that I'm showing you here in the picture. And the instrumentation that you see on the right is no longer being manufactured. And so, if it were to fail, then we would be in a bit of a pickle. Okay. Next slide, please.

Now, the allergens which-- This talk doesn't-- This talk will not really center on the allergens that are standardized by overall potency. But for contrast, I am going to go over how that was done back in the 80s and the 90s when it was done. And the reason that these allergens were standardized is by overall potency is either there were no major allergens that could define them or at the time at least there were no known major allergens that could define them. Of course, in the last 30 years or so, we've learned

quite a bit about all of these allergens. But the way that it was done was with this procedure called IDEAL testing in which you had subjects who were highly allergic and they received these intradermal injections of serial 3-fold dilutions of the allergen extract, and you see sort of how it was mapped on their back on the right. In response to these injections, they would develop the little wheal, which is the little oval in the center, and then the flare, the redness, which is the outside oval. And the flare, the redness, the erythema that would be measured-- The orthogonal diameters. And when the orthogonal diameters were 50 millimeters, that was just arbitrarily referred to as an extract that was 100,000 bioequivalent allergy units. Next slide, please.

Now, obviously a problem with the IDEAL testing is that it was useful for measuring and determining potency, but it wasn't an assay that you could use for lot release. So, a surrogate assay had to be developed and that assay is the competitive ELISA for overall potency. And for those of you that aren't familiar with it, it's simply that you have a-- In plate-bound you have antigen or in this case an allergen, a reference allergen in which you have the known concentration that is bound to the plate in your 96-well plate, bound to the bottom surface. And then you have your test allergen in solution. And these two allergens are competing as shown in the center part of the cartoon there for antibodies, in this case IgE from pooled human sera, 10 to 15 allergic subjects. And then, the antibody itself is either linked to an enzyme or there's a secondary antibody that's linked to an enzyme, and you wash away everything that's in solution, and what's left is the signal from what's bound in the plate and the more allergen there is in solution, the less antibody there is bound to allergen in the plate. And it's a reasonably good ELISA, it works reasonably well, but it does have some limitations. It doesn't detect compositional differences between extracts. And of course, since we're using serum pools from paid donors, these pools can vary because of

differences amongst donors. We don't get them from the same donors each time. The donors of course are not identified. Next slide.

So, as I mentioned, there are 19 standardized extracts and hundreds of non-standardized extracts, and this is just to show you how they kind of break out. The ones on the left, the grass pollens and the house dust mites are standardized according to overall potency by BAU for the grass pollens or AU. And they're really the same thing for house dust mites. And then on the right, short ragweed pollen and cat, hair or pelt, by their major allergens. Those are the ones that we're discussing today. And the venoms also by their major allergens, but we're not going to be discussing those today. Next slide, please.

What I've shown you so far is that allergen extracts are crude preparations that are safe and effective, and modulate allergic disease. There are 19 allergen extracts that are standardized for potency, some for major allergens, some for overall potency, and there are remaining hundreds of extracts that are non-standardized. Next slide, please. But there's some drawbacks for major allergens. These assays are outdated. We have an instrument that can't be replaced and it would be difficult to expand the standardization program using these sorts of assays. Next slide, please. Next slide, please. Next slide. Yeah.

For overall potency, that relies on some assumptions that I'll be discussing more in the next talk, but the assumptions are that the extracts from the different manufacturers are qualitatively similar and that allergic patients react similarly to the same set of allergens. If either of these aren't true, then standardization may not actually represent the product. Next slide. And then the-- Next slide, please. And then the remaining-- And then, as far as those that are unstandardized, there's no indication as to

whether the protein is intact and so therefore they can have lack efficacy, and as a diagnostic product they can lead to misdiagnosis. Next slide, please.

So, the rest of-- This part of my talk then pertains directly to this first question, the scientific soundness of mass concentration measurements as used from-- as obtained by ELISAs or a similar assay using aptamers. Next slide.

So, as I mentioned, the way that standardization works in the United States is that we develop the standardization assay and that the assay then can be adopted by the manufacturer. So, the manufacturers don't have to use our assay, they want to use their own, that's fine, but they have to demonstrate that it's either equivalent or superior to ours. But we devise, we qualify, we validate these potency assays for allergenic extracts. We distribute the reference reagents at no cost to the manufacturers for potency testing for lot release of the standardized extracts, and we do so in our laboratory, the Laboratory of Immunobiochemistry, which is the laboratory that I manage. And this is an ISO 17025 certified laboratory, at least right now for the competitive ELISA, and for any other assays that we will integrate into the lab, we would intend to obtain ISO certification as well. Next slide, please.

Before I continue, for those of you who don't think about allergic disease every day, you might not be familiar with the nomenclature of these allergenic proteins. The nomenclature of these allergenic proteins is fairly straightforward. The first three letters pertain to the genus, the second letter pertains to the species, and then the number pertains to a group number of allergen, which can be-- Is often just numbered according to their order of discovery. Although within a group of species of plants or animals, if there's a number of an allergen-- If there's a particular allergenic protein that has high homology amongst the species, they'll all have that number. So, a cat for example is

felis domesticus, and we're talking about Fel d 1, by far the most important allergen from cats. Next slide, please. And that is the most important and allergen from cats. And then ragweed, we're talking about Amb a 1, which is by far the most important allergen for a short ragweed. Next slide, please.

Now, what is Fel d 1? It's considered a universal allergenic protein because almost all cat allergic patients are sensitive to it. It's a secretoglobulin protein complex produced by salivary and sebaceous glands. It's a tetramer comprised of two disulfide linked heterodimers (chains 1 and 2), and the chain 2 is an N-linked glycoprotein. It's an extremely stable and sticky protein. It's found almost everywhere in homes that have cats, in homes that don't have cats because people who have cats will visit homes that don't have cats. It's found in schools because it sticks to clothing. It's really quite remarkable. And even if you get rid of your cat, you'll have Fel d 1 in your house for a good six months after the cat is gone. It originally was assigned in Fel d 1 units, and it was sort of later determined that maybe a unit was four micrograms of Fel d 1. The literature isn't really clear as to how they got that number. And then it's also-- The extracts are required to have 10 to 20 Fel d 1 units per mL. And then they're assigned this BAU unitage, which is a little bit odd to me, has always been, in which case where you had an extract that was 5-9.91 Fel d 1 units, it was just called 5,000 BAU and 10 to almost 20 units it was 10,000 BAU. Next slide.

Now, this is just a picture of the heterodimer and you could see that they're linked by their sulfur and there's some calcium molecules, ions in there are involved in the linkage. What's important, what this reminds me to share with you is that the IgE reactivity to the heterodimers has been shown to be greater than the sum to either of the monomers. So, we can infer from that that some of these IgE epitopes are

conformational rather than linear. And so, the stability of the heterodimer is very important to its allergic properties. Next slide, please.

Well, we had some in-house monoclonals for Fel d 1, and we worked with them for quite a bit, but we couldn't really get them to work with the kind of precision and accuracy that we needed. And it became apparent over time that laboratories, one academic laboratory and then another in private industry, were cloning allergen specific IgEs from allergic donors. And this was very attractive to us to use as reagents because they're biologically irrelevant just by definition. And what this figure shows is just a schematic of how that was done, that they would enrich the B cells and do single sort on the B cells, RN-seq to determine which were plasmablasts or memory cells, or naive cells, clone the immunoglobulin, determine its content. And what you could see from this is probably the most interesting take home message is the bottom right graph in which you could see that unlike any of the other immunoglobulin types, almost all the IgE secreting cells were plasmablasts. And we know that that's the case with allergic disease. The allergic disease is such a problem because these IgE secreting plasmablasts are long lived. Next slide, please.

And then what they do is they take these antibodies. In this case they found four antibodies that reacted to Fel d 1, and they do these sandwiching or they do these competition assays on a surface plasmon resonance chip, in which case they have one of the antibodies bound to the chip. They have the Fel d 1 bound to the antibody, and then they put in the other three antibodies, and the antibody either binds to the Fel d 1 meaning that it recognizes a different epitope and can be used as a sandwiching antibody or it's blocked, and that means that it can't be used as a sandwiching antibody. And they found two pairs of sandwiching antibodies and we licensed one of those pairs. Next slide, please.

These are just SPR assays showing all four of these monoclonals. And I should point out that we're referring to them as class-switched because this company IgGenix is using their business model to use antibodies as a therapeutic modality. So, they class switch them to a stable IgG4, and that's what they really are. But at any rate, that obviously doesn't affect the binding affinity to the target allergen and what you could see here is that these all have sub-nanomolar affinities. Next slide, please. Well, we use these antibodies to develop the ELISA and we could show that it was certainly reproducible and reasonably precise. The EC50 of the relative potency shown of two particular cat allergen extracts, which is shown in the upper left hand corner, was a very tight distribution along that line over 142 measurements. And the distribution of one of the extract potencies-- We found that the reference value was 14.7 Fel d 1 units/mL, and that the mass per unit of Fel d 1, we determined to be about 3.12 micrograms. Now, this you can-- Next slide, please. Now, this was a reasonably good assay and it was good enough to publish, but we realized that it really wasn't good enough. We hadn't really gotten it to perform well enough in the regulatory domain. And so, we're working very hard on that and we've changed some of the parameters of the assay. And what you could see here is some preliminary data in which we have six replicates of the assay in which the EC150 is very tight with confidence limits +/- 6%. So, that's really what we're shooting for before we start using this assay as a regulatory tool. Next slide, please.

So, what about when high affinity monoclonals aren't available for all allergens? Well, another possibility is to use DNA aptamers. Next slide, please. And what are those? These are synthetically produced oligomers that bind to target molecules with high affinity. They're often referred to as chemical antibodies. They're selected from a starting pool of about 10 to 14 randomly sequenced single strand DNA oligomers. There

are affinities and competitions are determined with a technology like surface plasma resonance, and there are some advantages to it because there's no animal or human blood that is necessary. And once the optimal pair has been chosen, synthesis of DNA-- Single strand of DNA of course is relatively cheap. Next slide, please.

This is a schematic of how these aptamers are selected, and it's a little bit complicated, but I'll just describe to you the salient features. Briefly, the aptamers have these primer binding sites on each end and random sequences in the middle, and then the little site that docks them to beads. If the DNA aptamers bind to the target protein, they separate from the beads and then those aptamers can be amplified by PCR clone and sequenced, and then tested for binding affinity with the technology again, such as SPR. The best pair then are selected and then we develop the ELISA or the aptamer enzymatic assay. Next slide, please.

What I've done is I've described to you where we are with these newly developed assays. I can tell you that the aptamers are now in the process of being selected. I actually just received an email from the company yesterday that they've got the pairs and they're moving forward with it. So, that's happening. And I just want to remind you again that the Fel d 1 and both the Amb a 1 are in units. They're currently in units, for the Fel d 1 that's Amb a 1 units and then BAU. But I want to communicate to you that this is done because we use as the reference material an extract. That we use an extract, so we are always comparing one extract to another extract. But since really this was put together 35 to 40 years ago, there have been highly purified preparations that are commercially available of these allergens and that allows us to measure their absolute concentrations, and that way we can discard any kind of ambiguous unitage and simply state the concentration of these major allergens and micrograms per mL.

That of course, is the first question that we are posing to you this afternoon. Next slide, please.

Now we'll move to the second slide, which is the appropriateness of these revised assays for CBER's Allergenic Program-- Standardization Program. Next slide, please. So, I mentioned to you that non-standardized allergen extracts can be a problem and they can be a problem because there's no indication of potency. It's simply protein nitrogen units or weight per volume at time of extraction. And it can be a problem if the protein in the extract is not intact. And in fact, we did have that problem. In 2022, four lots of peanut extract were withdrawn because of false negative skin tests. Some children were misinformed that they were not peanut allergic, and they subsequently reacted to peanut containing foods. And then it happened again in 2023 that pecan extract lots were recalled because of false negative skin tests. And what you see below the text are four images in which-- This is a typical ELISA that was done by a lab in Vanderbilt that has human IgEs to these four peanut allergens: Ara h 1, Ara h 2, 3 and 6. And you could see the red and the violet with the inverted triangles to the right showing that unlike the other three extracts, these concentrations were really quite low in these recall extracts. So, this was a real problem and it had real consequences for patients who depended upon these assays for a diagnosis of food allergy or to be informed that they were not food allergic. Next slide, please.

From that, there became-- People advocated that we should expand our standardization program, and the main advocate was our, of course, stakeholders, those who are food allergic and those who are concerned with the medical care of patients who are food allergic. And probably the largest and most predominant advocacy group, Food Allergy Research and Education (FARE), had a workshop last year teamed-- Put in tandem with their annual development day seminar in which we from FDA,

manufacturers, academicians, and of course representatives from the FARE itself discussed standardizing food allergen extracts and we all pretty much agreed that this was a worthy endeavor. And so, this is something that we're going to be doing, that we intend to do with your concurrence in our reference reagent lab to develop ELISAs or perhaps aptamer based assays to replace obsolete assays and also to expand our standardization program. Next slide, please. But we could do that beyond food extracts, and what we've learned about allergen has really helped facilitate a strategy that we could use that might make some of these antibodies very potent tools. And so for example, a tree pollen allergy is actually a big problem, and none of the tree pollen allergen extracts are currently-- They're all non-standardized. Oak, birch, cedar, these are all very prevalent in North America. Next slide, please

And what I'm showing you here are ribbon diagrams of six related trees: birch, hornbeam, chestnut, European beech, white oak or alder. And these are all proteins that belong to the pathogenesis-related protein-10 or PR10 family of allergens. You see, they all share the number 1 because they're all very, very similar and they look very similar. And if you go to the next slide, please, you can sort of gander-- I mean, I realize we're not going to give you a lot of time for close inspection, but there is a fair amount of similarity and identity amongst these six proteins. And so, it's quite possible that we can use-- With a minimum number of reagents, that we can standardize all these tree pollens with one hit. And of course, standardizing tree pollen, such as white oak pollen, probably means that we could standardize the major allergens from red oak pollen and other pollens at the same time. And it's really a very effective strategy, at least theoretically it's appealing. Whether or not it'll go as smoothly as we hope, of course we won't know until we do the experiment, but I would say that cross reactivity of

patients who are tree allergic to many of these trees suggest that it's a viable option. Next slide, please.

So, here we are. We're replacing the Fel d 1 one, and the ELISA is progressing towards qualification and validation. The aptamers are being selected. And the most important-- And then the work on the Fel d 1 ELISA will serve as the template for qualification, validation and tech transfer. Next slide, please.

Now, what about validation? The validation is really an important part of this process. It's absolutely required by 21 CFR Part 211, a Good Manufacturing Practice, and 21 CFR Part 610, General Biological Product Standards. And there's a number of documents here and the reason for showing you these documents is not to really discuss them, but to point out that if you're not really familiar with assay setup, there's a huge amount of work and body of literature as to how to go about doing this. Next paragraph. Next slide. Assay validation is the foundation of quality control. The objective is to prove that the assay is fit for the intended purpose, appropriate for the stage of development of the product, and can be trusted for critical regulatory decisions. In other words, that it's accurate, precise, reliable, and reproducible, and therefore supports the concept that the product is safe, pure, potent, and effective. The assays are the foundation for quality control and an unvalidated or poorly validated assay can give misleading results that can affect safety and efficacy of the product. Next slide, please.

There are a number of performance characteristics to test and document in the process of validation. The ones on the left are critical, the ones on the right are also addressed. Accuracy, specificity, the limits of qualification, linearity, range, and precision. All are defined not only in the lab, but the results are statistically quantified.

They're statistically defined so that we really know the variability and the precision of the assay. Next paragraph- slide.

The template that we're acquiring is that we produce monoclonal antibodies or the aptamers, we develop the assay procedure. We'll do that within the lab. We qualify the assay to determine its performing characteristics, and this is with our statisticians. We transfer the technology to the manufacturers, the reagents. The validation protocols, we work with them and help them to achieve the same performance characteristics that we've been able to demonstrate, and troubleshoot when that doesn't happen. When all of this is done, we publish the assay, we adopt the assay, and we inform stakeholders that these allergen extracts are now standardized. Next slide, please.

Before I summarize, I just want to acknowledge all the people that have contributed to the work that I've presented today. Those in our LIB reference reagent lab, Aaron Chen, who does a great deal of the work. Mona Febus. Ekaterina Dobrovolskaia has retired. We miss you, Katia. Your replacement will never live up to your legacy. And Robert Zagroski who is our quality manager and really keeps things at a high level of performance in the lab. Those within the division above me. Dr. Slater, my friend and colleague who retired last summer. Dr. Tennant who is now the acting director. Jennifer Bridgewater and Leslie Wagner, who are regulatory coordinators and policy experts. Leslie is really the person who is advising me as to how to do the validation and really working with us. She's invaluable. Jennifer has always been invaluable. And Dr. Robert Hamilton helped us set up the mouse assay- the cat assay originally. And then the people from IgGenix who worked with us and were so kind to license the antibodies to us. Next slide, please.

So, again, what I've shown you is that the replacement of the RID is progressing. We've got aptamers in the works. We have a Fel d 1 ELISA that we think will provide a template for standardization of currently non-standardized extracts. Standardizing the food allergen extracts is a priority for us. The similarity of major allergens may provide templates for standardizing multiple allergen extracts with one hit, and assays for standardization will undergo rigorous validation. Next slide.

And the final slide is to bring to you the voting questions that are relevant to the information that I've shared to you up till now. Question one, scientific soundness of mass concentration measurements. Does the measurement of mass concentrations by ELISA of their major allergens provide a scientifically sound approach for expressing and reporting potencies of cat hair and pelt allergen extracts, and of short ragweed pollen allergen extracts? Question two, appropriateness of revised assays for CBER's allergenic standardization program. Are the revised assays for cat hair/pelt and ragweed pollen allergen extracts scientifically appropriate templates for expanding CBER's allergenic standardization program to include major food allergens and environmental allergens? And with that, I have finished this first presentation and I'm open to any questions.

Dr. El Sahly: Thank you, Dr. Rabin. We need to circle back to Dr. Davis. Dr. Davis, would you please unmute, put your camera on and introduce yourself to the public and the other Committee members?

Dr. Davis: Thank you. Yes. My name is Dr. Carla Davis. I am the Chair of Pediatrics and Child Health at Howard University. I served as a director of the Food Allergy Program at Texas Children's Hospital, Baylor College of Medicine for 27 years and have a translational research program in food allergy.

Dr. El Sahly: Thank you, Dr. Davis.

Replacement of Radial Immunodiffusion (RID) Assays of Currently Standardized Extracts with ELISA or Aptamer-based Enzymatic Assays - Q&A

Dr. El Sahly: So, on the agenda now is the Q&A portion pertaining to the first presentation by Dr. Rabin. Please use the raise hand function and ask any questions you may have to Dr. Rabin. And I see Dr. Davis. Well, no, just a second. First question from Dr. Rubin.

Dr Rubin: Thanks very much and thanks for that presentation. It's hard to argue-- Unless you're Dr. Ouchterlony, it's hard to argue that better measurements of-- Better and easier, and more accurate and reproducible measurements are a bad thing. I guess the only question is: do we know that these measurements correlate with efficacy as either diagnostic, as opposed to just a better measure? And along with that, what do we know about the minor antigens? Are they important and should we be measuring those as well?

Dr. Rabin: Yeah, that's an interesting question. So the first question is do we know that measuring the major allergens correlates with the actual allergenicity? And the answer is that that seems to be the concept that they do, that they do and that their concentration does reflect the potency. And I think implicit in your question, if I'm understanding it correctly, is it is the way that we're measuring them correlative to their potency. Because that's-- I mean, that's the underlying assumption of course, of a potency measurement really, and I think the answer to that is yes. I think that for native allergens, those that have not been mutated to ablate IgE epitopes or T-cell epitopes for example, the best thing that we can do is we can demonstrate that these proteins are intact. And so we have to do that by sometimes denaturing the protein and showing that

it loses its activity on the ELISA or just from the knowledge that we have. For the ragweed, we can do that. You know, the Amb a 1 is fairly heat-labile, and if these aptamer assays recognize a heat denatured protein, then it's not of use and the pair that's chosen is not the optimal pair. Since it's almost impossible to degrade a Fel d 1 allergen, we don't have that tool at our disposal. But again, everything that's known about it is that these antibodies seem to be-- Do seem to be conformationally dependent. It is possible, and it's something I would love to do, but I haven't really had the time to see whether or not we can get some protein structure studies or something to actually look at these antibodies and see where they bind. From what I understand, and obviously I'm not a structural biologist, for Fel d 1, this is-- Because of some of its properties, it's a very difficult thing to do, but it would be lovely to do that. Did that answer your question?

Dr. Rubin: Yes, thanks. Thank you very much.

Dr. Rabin: Great.

Dr. El Sahly: Dr. Portnoy.

Dr. Portnoy: Great. Thank you. Gosh, I was on the Allergenic Extracts Committee back in the 1990s and this conversation sounds exactly the same as it was back then, and I'm really glad that we're finally moving forward with new technologies, but we were discussing the option of using this ELISA-based approach back then. I'm just curious to know why we suddenly decided to just go ahead and go with it as opposed to just waiting the last 20 or 30 years like we have. One of the discussions back then as it was just brought up is that there are more than one major allergen, perhaps Fel d 1. A lot of people are allergic to that, but I have patients who are not allergic to Fel d 1, they're allergic to some of the other cat allergens. I was just wondering if you'd had maybe

some consideration to measuring several of the major allergens rather than a single one and maybe labeling the extracts [Indiscernible - 4:07:14] multiple ones.

Dr. Rabin: Yeah, thank you. That was the second part of the previous question I forgot to ask. And reviewing the literature-- And actually Tom is on the line because one of-- Some of the literature is Tom's literature in a recently published paper of his, and Tom, you can remind me, I think Fel d 4 is one of them, but there are a few other allergens from cats that are turning out to have some importance to a subgroup of patients. I think that that is something that ideally would be great to do with these assays and we can-- Actually, the way to do them might be to apply the mass spec, the technology that we'll be talking about in my second talk to them. I think it's not a high priority. We have to pick our battles, you know? That what we have right now with ragweed and with cat are sort of good enough to keep them going and move them forward. And there are things that I really want us that are higher priority, like the foods, to push forward, but I think that you are really correct. It's something that I've begun to appreciate mostly, and I would say in the past month and a half in preparation for this meeting, that some of these so-called minor allergens in cat and perhaps even in ragweed are not so minor after all.

Dr. Portnoy: Well, particularly like with peanuts because when you look at it in vitro assays, we can measure components now and actually figure out which allergens the patient is allergic to. Are there any thoughts given to maybe comparing the potency of these extracts with the results of in vitro tests? Because it would be nice if they correlated more effectively.

Dr. Rabin: Well, that's the dream. Yeah. Yeah. So, that's kind of the dream and it's more relevant, and I think how we would do that is-- If you'll hold that question for the

next talk, you'll see how we're thinking about it in terms of that, and we're thinking about it more in terms of these really complex allergen extracts, like house dust mite and *Alternaria*, but the idea of circling back and applying them to something like cat is certainly a very good one. But I would appreciate it if you just hold that thought and sort of allow-- So that we can just focus on the first two questions here. Okay?

Dr. Portnoy: My last question is how do you plan to calibrate these results? Are you going to use purified allergen or recombinant allergen, or what are you planning to use?

Dr. Rabin: Yes, the reference reagent will be purified Fel d 1. There will always be an extract that will serve as a positive control and a quality control, a reference extract for which the results will have to be within some window of potency that will either validate or invalidate that particular assay, right? So, you have a quality control, but that is how we intend to do it for these major allergen assays. Yes.

Dr. Portnoy: Right. Thank you.

Dr. Rabin: You're welcome.

Dr. El Sahly: Dr. Paul Greenberger.

Dr. Rabin: I think you're still muted, Dr. Greenberger.

Dr. El Sahly: Yeah, please unmute.

Dr. Greenberger: Pardon me.

Dr. Rabin: Okay, now we can hear you, sir.

Dr. Greenberger: Thank you, Ron. I think-- I just want to make a first comment. Priority is going to be so important. You used the word to limit the array of testing and possibilities until the methodology is confidently proved. And the one that could comment regarding what Dr. Rubin brought up about what I interpret as heterogeneity of the IgE responses, that's certainly known for ragweed, for example.

Dr. Rabin: Yes, sir.

Dr. Greenberger: Not everybody with clinical symptoms reacts to the major allergens. So, that's crucially important, and I think that would extend virtually all the allergens.

Dr. Rabin: I think you're right, and I think we really began to appreciate that now, but I think-- Yeah. I mean, yes, yes, I agree with you.

Dr. Greenberger: My question was, are you able to share with us what ELISA assays you've got operational or what's your experience?

Dr. Rabin: Well, right now we really have the cat. We haven't moved out anything further. We don't have the reagents for the aptamers yet, so the cat and the ragweed, that's the first thing. We got to replace those so that we can discard the RID. Once we're doing that, and particularly once we replace the biologists who retired and we'll have somebody whose attention is turned to this, then the question is tackling the next sets of allergens. But these licensing deals are not pocket change. Obviously, they cost some money. So, we really only approach companies about working with the reagents and licensing them when we're ready to move forward with it. So we haven't gone further until we get this cat assay down to really what it needs to be.

Dr. Greenberger: Do you need help to set up your assay? It sounds like it.

Dr. Rabin: No. We can certainly-- Well, we need a full compliment of people in the lab. We need that. That's what we need right now. But as far as intellectually or operationally, otherwise, we have the tools. We have the people who know how to do this. We have access to a great support from Dr. Hamilton at Hopkins who is a fountain of advice, and really anyone in the community, particularly the food allergy community, I mean, they're always available for advice. So, it's really-- It's just really people, a person whose job will be to move these assays through to make us into a little assay factory. That's the plan. And we are fully capable of doing that.

Dr. Greenberger: Thank you.

Dr. Rabin: You're welcome. Thank you.

Dr. El Sahly: Dr. Omer.

Dr. Omer: First of all, I think the overall approach makes a lot of sense, but I do have a couple of questions. As you described, the pathway for qualification validation and technology transfer, and the qualification step is pretty important in that pathway. Could you elaborate a little bit more on the statistical approaches that you allude to? Like, the specifics of that because that is kind of important, especially for these kinds of approaches that you're proposing. So, if you have that information-

Dr. Rabin: -Unfortunately--

Dr. Omer: Go ahead.

Dr. Rabin: No, no. I have statisticians who have that information, and I know they know their stuff because they work with us when we evaluate INDs and BLAs, and BLA supplements, when manufacturers are doing assays. I know that the relevant document is ICH chart 2(R2) I believe, but I think I would be well outside my lane to start commenting on how the actual statistical approach is done, I'm sorry.

Dr. Omer: That's okay.

Dr. Rabin: If you want to shoot me an email at ronald.rabin@fda.hhs.gov, I can forward it to my statistical colleagues and they can give me a couple of paragraphs to send you back. I'm happy to do that, but I don't want to comment here.

Dr. Omer: Okay. The second question is-- Of course, given the relatively limited experience with the DNA aptamers, are there any safeguards? What are the specific safeguards in place or will be in place to ensure reproducibility and stability of these aptamer-based assays over time and across different entities?

Dr. Rabin: Well, I think that we have to develop a stability protocol for that reagent like we developed for any other reagent. I have to say I haven't given [much thought to that given that] a single strand of DNA is an extraordinarily stable molecule, but obviously we can have it made with some PCR primer end and we can do something like take it out of the -80 and make sure that a PCR is up appropriately and focuses on a gel. And, I mean, those sorts of things I think that we do-- I mean, we do that with all our reference reagents and reference reagents of course have to be checked for stability like everything else. So, I haven't given that a great deal of specific thought, but I don't think that that's an issue that is going to be difficult to deal with. I mean, my experience with these reagents are-- Even monoclonals. My background is on flow cytometry. I

used to do a lot of flow. I mean just tons and tons of flow. And I remember getting vials of lyophilized monoclonal antibodies that were literally 20, 25 years old that I would put into solution and they would work absolutely perfectly on flow. It's kind of remarkable, and I don't think it'd be any different for DNA. So, next question, please.

Dr. El Sahly: Yes. Dr. Meyer.

CAPT Meyer: Thank you for that interesting talk. I know you're really focused on the biological appropriateness of these methods for the voting questions, but for the completeness of the discussion, I was wondering if you could comment on any impacts that there would be on the consumer, like the allergists using these products. If there was a transition made, would there be any supply chain issues? Would there be any changes in costs that might affect people's ability to use these products, those types of more implementation aspects?

Dr. Rabin: Sure. Let's just answer for the ragweed and the ELISA. For the ragweed and the cat, what I would anticipate is that we would bridge the-- As we have the new assay, we would bridge with the old unitage-- With the new unitage and drop the old over time. That's what I sort of imagine. I don't see that as being any increase in cost or any affecting cost. The manufacturers will be speaking to that, of course, because they're already doing an assay, so now they're just going to do a different assay, and the different assay is easier and less time consuming than the first assay. So, I don't see that as affecting it. As far as the additional extracts and using the technology that I've referred to today that we've spoken about so far, I just want to remind you that we supply-- The major cost is incurred by the taxpayer. It's incurred by us because we purchase these reference reagents, we distribute these reference reagents to the manufacturers at no cost. That means the monoclonals or the aptamers. We've paid for

their design, we've paid for them, we conjugate them, we distribute them. So, their cost in materials is an ELISA plate, a secondary antibody, an enzyme-linked IgE, or in the case of the aptamers would probably be avidin, the top aptamer would be biotinylated maybe, and then a bunch of buffer and these ELISA's-- And I don't perceive that as being a huge issue in human power in hours and time. But, you know-- So, of course, I mean I've been a government employee just about most of my professional life. I've not been in private industry, but I don't perceive it as being a big roadblock myself. I suppose that there might be some lots that would have to be rejected that don't meet-- That don't meet a potency criteria, but it's not as if the source materials are rare. It might mean that you have to start with two bags of peanuts rather than one or something along those lines.

Dr. El Sahly: Okay. Thank you. Dr. Platts-Mills.

Dr. Platts-Mills:-Unmute. Thank you, Ron. You mentioned oak and birch and the comparability between them and we had some very interesting dates-

LCDR Reese: -Excuse me, I'm sorry. We will not be able to have questions or comments from Dr. Platts-Mills because he is, I guess, speaker for this meeting. So--

Dr. Platts-Mills: I beg your pardon. I will hide quickly.

LCDR Reese: Thank you.

Dr. Rabin: Tom, I'm always interested in what you have to say and we can talk later. I'm very interested in what you have to share.

Dr. El Sahly: Okay.

Dr. Rabin: Let's go ahead.

Dr. El Sahly: I think we're allowed to take questions from Dr. Assa'ad. Dr. Assa'ad?

Dr. Assa'ad: Yes. Thank you for your presentation, Dr. Rabin, and thank you for taking my question. I have a few questions. First, as Dr.-- As Jay Portnoy mentioned, ELISA has been on for a long, long time and what we are proposing now is to almost choose to use an old method. But, I mean, you're still using an old method with a little bit of new reagents, which are your monoclonal antibodies and your aptamers, but we are still stuck with the lack of biologic significance of the amount. So, if I understand correctly, you are either going to use your monoclonals or you're going to use your aptamers for various allergens and at the end come up with a quantitative assay. So, it's going to quantify how much there is of this allergen in this milliliter, microliter, whatever.

Dr. Rabin: Yes.

Dr. Assa'ad: That's correct. Okay. But the previous allergenic assays also included how the human person responded, which were those skin tests. intradermal tests. We are not going to do this now again. They seem to be-- But my question is-- So, my question is, because there are newer ways to measure the human response to these allergens like the BAT and the MATs and all of this, can we pair this measurement that you're proposing with other measurements that not only measure quantities but quality and function?

Dr. Rabin: Okay, so let me address the premise of your question first.

Dr. El Sahly: Yes.

Dr. Rabin: Again, for these assays, okay? The ones that I've discussed so far, particularly the cat and the ragweed, they've been assigned the BAU values, but the BAU values were considered as a translation of Amb a 1 units and Fel d 1 units. The data that even quantifies the-- I'm not sure where the Amb a 1 unitage came from. I mean, these were done years ago and the literature-- I mean, where it's discussed is in the textbook, but to my knowledge, the manufacturers don't even use a BAU unitage with Amb a 1 one with the Fel d 1, it was an abstract and reading the abstract, they might've only tested one person. Okay? I'm not really certain how many people they did, and it was really a translation of the number of units of Fel d 1 or Amb a 1.

I don't consider, [after] reviewing the literature and having put a lot of thought into this, that the relationship of BAU to concentrations Fel d 1-- To the allergen potency, the total potency of a cat extract hair or pelt, or a ragweed extract is as tight as it is, say, for the grasses. Okay? For the grasses, it's been done. It seems to be reliable. We've never had any issues with it. I don't think that the relationship between the BAU unitage and those two particular extracts is as tight. Okay? Now, the question of whether or not we would tie these to new assays and how we would integrate them into something like a BAT or so forth, I guess I would have to think about that. I guess I would have to-- I haven't given that much thought. I've sort of-- You can be a lumper or a splitter, and I've been a bit of a splitter here to say that there are certain allergen extracts that have one, two, or maybe even three major allergens, and we can really work on that, you know? That you quantify these one, two, or three major allergens, you pick one as a release criteria because you can't continue to stack release criteria. And then you look at some that are just too complex for that. And for that, we have an alternative approach that we haven't discussed yet.

Dr. Assa'ad: Okay.

Dr. Rabin: That's kind of where my thinking is, but I hear what you're saying and it's-- It's worth some thought, but I'm not going to-- I can't give you that any more than that off the cuff.

Dr. Assa'ad: Well, thank you for considering it. The other question, and I know we are not discussing peanuts, for example, we're not discussing other allergens, but using peanuts-

Dr. Rabin: -We are discussing peanuts. Yeah. We intend to do this for peanuts. Yeah.

Dr. Assa'ad: Okay, perfect. So, peanut allergy is related to the components of peanuts, as you may very well know, but-

Dr. Rabin -Right. Of course.

Dr. Assa'ad: But the allergy changes by age. So, most infants are positive for Ara h 2. Some may be positive to Ara h 1, but most are positive to Ara h 2. When you get to the teenage years, they are positive to Ara h 9. And when we started measuring Ara h 6 or the company made it available, Ara h 6 seems to be highly associated with anaphylaxis. So, how are we going to solve this conundrum of the different-- How are you going to standardize that peanut extract to be of maximum usability of a year old versus a teenager, versus an adult? Yeah, okay. Just creating more problems for you, Dr. Rabin.

Dr. Rabin: So, that is a complex-- That is a tough question, and I've certainly-- I've begun to think about it. I was actually talking with Gideon Lack about it a few days ago, and we were talking about different possibilities. I think that we are aware that that is a tough question and we have to find some middle of the road of something where the

perfect can not be-- The perfect is the enemy of the good, right? We have to come up with something that gives us some sense of a reliable, consistent product without putting too much stringency on it, and that the manufacturers A will not want to release it, or B, that they can't make lots consistently that they can release. And I think that there are different ways of doing that. Again, in the interest of sort of expedience and wanting to get these food allergen extracts standardized to some extent, we're probably just going to have to pick. We're going to have to pick and choose, and I would not do that without consulting with the experts in the field who are available to us to advise us on that. As to addressing the complexity of it, if you want to really consider that peanut is a complex extract, then as soon as we're done with this q and a and we launch into the second part of the presentation, I think you'll find that very interesting and hopefully exciting.

Dr. Assa'ad: Okay. Just one last part to the question here. Are you at some point going to release or other manufacturers going to release an equivalency between what they've already had on the market and what now will be standardized? Because practically speaking for skin tests, for environmental allergens at least, and the use of the allergens, and I know we are not discussing using them as therapies, but for allergy shots or allergen immunotherapy, this becomes a major problem. How to-

Dr. Rabin: -Yes.

Dr. Assa'ad: Yes.

Dr. Rabin: Yes. I wouldn't be surprised if one of the consequences of standardizing, non-standardized extracts is that what we have overall is a higher potency extract than one that has been released in the past. And I think that that will require some education

and communication with the healthcare providers. That when these come out in their standardized forms, as you know better than I do because you take care of patients that when you change from one lot of an extract to another, you should dilute that second extract by at least one to two for safety reasons, because potencies can vary even that much among standardized extracts using the available tools that we have. And it may be that they just need-- They really need to go down to a lot less. It may also be that we could do some sort of in vivo testing to kind of get an idea, because since these really haven't been standardized, nobody really-- What is the best concentration? What's the ideal concentration? If it's too concentrated, as Dr. Lack reminded me, you could end up with false positives of peanut allergy. You don't want that. Obviously. That would have a major impact on somebody's life to errantly inform them that they're peanut allergic when indeed they're not. And so, these are things that we are going to have to deal with. And I am grateful for your questions, and I assure you that we are cognizant that what we are doing here is more than just making an ELISA and saying have at it.

Dr. Assa'ad: Thank you.

Dr. El Sahly: We are running a bit out of-- Behind. If the questions can wait, because Dr. Rabin is going to give another talk right now. What do you think, Dr. Davis and-

Dr. Rabin: I think that our next talk is, I think, going to be a little bit shorter than the time allotted. I would really prefer that we allow doctors Dykewicz and Davis to ask their questions now, if it's all right with you, particularly since we started early.

Dr. El Sahly: Sure. Dr. Davis.

Dr. Davis: Yes. Thank you, Dr. Rabin, for your presentation and all of your work to try and move this forward. Given the fact that we've been using these extracts, as you

mentioned, for a hundred years and now Dr. Portnoy mentioned that we've been talking about ELISA for 20 years, I do believe this-- Any advancement is going to be helpful. I want to comment on the consumer and really in your thinking about how to move this forward because of course you would like for it to benefit the patients and the providers. I believe that validation of potency is going to be a critical issue. And we have standardized products now, but in practical application much of the concentrations aren't really utilized. It's really the potency when immunotherapy-- The extracts are put together for the patient. So, similar to Dr. Assa'ad's point, there's going to need to be some kind of validation of potency or what's happening in the patient, I believe, before the standardization is going to be accepted or used by the consumer. I would just really urge you, as I've heard your other comments, to really keep this in mind and really make sure that this happens. I do believe, as you mentioned, that there are two things that are true. One is that minor allergens and major allergens all play a role, but you have to start somewhere. So, I think that-- I just wanted to state those things. And my question would be, given the fact that it is going to make a difference on the consumer, would you be open to expanding your thoughts regarding this validation of potency as you seek to standardize?

Dr. Rabin: Yeah. Yes. Well, I've heard the message loud and clear. I think I could say that I've heard-- We have come to the Committee for your advice, not simply for your approval if you will, but we've come to you for your advice and we've gotten some very solid advice. And of course, I'm a member of the Standardization Committee in AAAAI. Every year I've gone to the meetings, I've gone to them, and they are a potential source of wisdom for that. Paul Ehrlich, the meetings, the triennial regulatory meetings in Germany has its own Standardization Committee. And so, I think it's very clear that we need to seek some outside advice and we have those people available to

us. You are the president of AAAAI, so I could flip it around and say anything that AAAAI, or the incoming president, if you will, and anything that you can do. We could seek advice from this respected organization and we will incorporate that into our plans.

Dr. Davis: Wonderful. Thank you so much.

Dr. Rabin: You're very welcome.

Dr. El Sahly: And the last question comes from Dr. Dykewicz, and just want us to all be cognizant of the subsequent topics because they may touch upon what the questions are.

Dr. Dykewicz: Thank you very much for the presentation. I'll try to be brief. I'm thinking about-- This is a very logical approach, particularly in terms of being able to identify not just several major allergens, but minor allergens that might be present within commercially available extracts. But what I could also envision is that there might be some challenges where you have an extract that traditionally is viewed for one particular major allergen, but then the other major allergens or minor allergens might vary considerably between different extracts. And I'm trying to think from a clinician standpoint, for instance, if we're talking about using these extracts for treatment with subcutaneous immunotherapy, how we might reconcile that. Do we envision, for instance, that there would be some consistency expected from manufacturers that there would be not only some consistency with the amount of the major allergen, but consistency over time or over release of lots, of what would be, shall we say, secondary allergens?

Dr. Rabin: So, I think the answer is that we have to see-- Since we've not been measuring multiple allergens in an extract at the same time, I don't know what's

realistic, what we can realistically expect. And again, Mark, ideally, I would love that. Ideally, I would like to see for some of these products that you have one, maybe two max proteins that are release criteria and that then in the particular lot, what else is in them is listed, and then that can be matched to a component resolved analysis that somebody- that you get through some of these new chips like the ALEX chip or whatever needs this extract is better than that extract. I think the first thing-- We're going to have to learn a lot as we do this. And as we do this, initially the most important thing that we have that for me to consider is that I do not want the perfect to be the enemy of the good. We got to get something out there where we can say that we have some standard of quality and we have to do it in such a way that we say that now that we have a standard of quality, we also have the tools to learn a lot more about this particular product, and we have every bit of license and authority to improve that standard of quality as we learn more and more information. So, this is not an endpoint, it's a beginning, it's a new thing. We're changing the way we do things, and we're going to change the way that we do things as we continue to change the way to do things to get the best products out there. That's the best I can answer your question.

Dr. Dykewicz: Thank You.

Dr. El Sahly: Alright, thank you everyone for this very engaging discussion.

Use of Tandem LC/MS/MS to Measure Potencies of Complex Extracts with Multiple "Major Allergens," Use of HDM Bodies and Fecal Pellets as Source Materials for HDM Extracts

Dr. El Sahly: The next talk is also by Dr. Rabin, Use of Tandem LC/MS/MS to Measure Potencies of Complex Extracts with Multiple "Major Allergens," Use of HDM Bodies and Fecal Pellets as Source Materials for House Dust Mite Extracts. Dr. Rabin.

Dr. Rabin: Well, thank you very much for staying with us so far, staying awake and we'll move on here. And so the part here that we're going to talk about is related somewhat to what we've already discussed because the question of what the delineation of a complex allergen extract is obviously a bit of a moving target based on the current knowledge today. Next slide, please.

So, the desired outcome here is to talk about an updated methodology to better characterize complex allergen extracts from relevant source materials to improve product quality. Excuse me. The problem statements are, current methods are outdated and do not adequately assess complex allergen extracts, and that source materials may not represent clinically relevant allergen exposure. Next slide, please. So, the voting questions and we'll revolve around and we are going to use-- We're going to try to stick to one term here, but we're talking about what's called tandem mass spectrometry, which has also been called LC/MS/MS, analytics to improve product quality. And then, we were going to talk specifically about house dust mite source material optimization. Next slide, please.

I'm going to remind you, if you will, of the existing standardization method for complex allergen extracts and its limitations. The surrogate extracts again-- The surrogate assays again do not detect compositional differences among extracts for different manufacturers or lots among the same manufacturer. And then we're going to discuss the LC/MS/MS as a transformative platform that enables comprehensive proteomic characterization and how LC/MS/MS or tandem mass spec can reveal potential for improvements in manufacturing methods for complex allergen extracts. And I'm going to point out to you that I'm not going to be giving the bulk of this talk. The bulk of this talk is going to be given by my colleague Brad Strader, who is really an

MS expert. For me it is a black box. So Brad will discuss that part for you. Next slide, please.

Just to remind you, there are 19 standardized extracts, hundreds of non-standardized extracts. Last talk we discussed the extracts on the right side of this and on the upper right quadrant, if you will. And now we're going to address one of the non-standardized extracts, but also the house dust mites. Okay? We're not discussing the grass pollens. So, just the house dust mites. Next slide, please.

Okay. Just to remind you again that this was the IDEAL testing that was used on highly allergic individuals. They would do serial 3-fold dilutions. They would come up with a number, some of the orthogonal diameter of the erythema of the redness. They would arbitrarily-- We would arbitrarily call that 100,000 BAUs. The surrogate assay is a competitive ELISA. The ELISA works, but it doesn't detect compositional differences between extracts, it may vary because of differences amongst donors, and these things rely on two assumptions: extracts from different manufacturers are qualitatively similar, and allergic patients react similarly to the same set of allergens. Next slide, please.

I just want to show you that sometimes IDEAL testing doesn't work. And this was a study that was done a number of years ago, I think about 13 years-- 10 to 11 years ago now by my colleague Jay Slater, and really by the NIAID Inner City Asthma Consortium where they were wanting to standardize cockroach allergen extract. And cockroaches are a serious problem of hypersensitivity in inner cities. And here what we have are eight of these ideal 50 plots from eight different individuals. And on the X axis of each one is the dilution of the extract, and then the on the Y axis is the sum of the orthogonal diameters. And then you see three black lines dotted or solid, or whatever, on each of the plots and those represent three products- One product from each of the three

manufacturers of the extract. And what you could see very clearly is that while the patient on the lower right hand corner might've had a nice ideal 50 kind of response that they would hope to get nobody else really did, and they're all different from each other and there was really no consensus that they could come up with to use this particular method to assign for overall potency. So, it just simply did not work. It has its limitations when these assumptions about equivalence of response or equivalence of qualitative equivalence of products fall through. Next slide, please.

The data that Dr. Strader now is going to take you through-- Is going to address the LC/MS/MS analytics to approve product quality. And so I'm going to step aside.

Dr. Strader: Thanks. Okay, next slide. Okay. Thank you very much, Ron. So, what is mass spectrometry? Well, liquid chromatography tandem mass spectrometry or LC/MS/MS is an analytical method utilized to generate peptide fragmentation spectra that are then used for qualitative and quantitative information. And so, in our lab we use LC/MS/MS, which I'll try to refer to as mass spectrometry, to quantitatively compare the complex mixtures of samples that include, say, source materials or allergen extracts to characterize the proteome. And what I mean is we can do this to quantitatively compare the relative abundance of proteins in different samples, and we could also use this towards characterizing the allergen profiles in these different sample types. That is, what is the relative abundance of allergens in one sample source versus another. And thirdly, this is a method of discovery. It allows us to identify prototypic surrogate peptides that we can then utilize for absolute quantification. And so, what do I mean by "surrogate peptide"? A surrogate peptide is a peptide that's exclusive to, in our case, the allergen of interest and it is reproducibly and reliably seen in all our sources, and also has a good signal and is resistant to modification. So, in our next mass spectrometry strategy, we will be using parallel reaction monitoring for absolute quantification. And

in this strategy, we use these surrogate peptides as standards. We synthesize isotopically labeled surrogate standards, and then we use them by spiking the samples that we're studying, and by that we can then generate calibration curves that allow us to calculate the absolute concentrations of the allergens we're interested in. And because we're measuring absolute concentration, we're measuring potency and therefore we can use this as an assay for promoting the manufacture of extracts that are specific, potent and consistent. Next slide. Next slide. Okay, there.

Okay. So, this is a typical workflow for mass spectrometry. First, you reduce and alkylate your complex mixture, and then you analytically digest it. In our case, we're using trypsin to produce peptides. And so these peptides are then loaded onto a C18 reversed-phase column configured with an HPLC system that is coupled on line to a mass spectrometer. In our case, we're using a Lumos Tribid Orbitrap mass spectrometer. And so, during a mobile phase gradient, when we're performing a data acquisition, peptides are eluted as a function of hydrophobicity and as they're eluted off the column, they are electrosprayed at the front end of the instrument and then they're fragmented in the mass spectrometer to produce tens of thousands of LC/MS/MS spectra. And these LC/MS/MS spectra which give us information, are then searched bioinformatically using proteomicsoftware to generate qualitative and quantitative information. Next slide.

Before I talk about *Alternaria alternata* or the house dust mite data, I just wanted to show you this slide that our lab published in 2017 by my colleague Samuel Mindaye. And here we see a plot of the absolute quantification analysis of five separate German cockroach allergens that was measured over four different days. And what this figure really demonstrates or illustrates is that it shows the precision, the reproducibility,

and the linearity of this technology over a large concentrational rate of six orders of magnitude. Next slide.

Okay, so I'm going to talk about *Alternaria alternata* first. So, *Alternaria alternata* is the most commonly associated mold with seasonal allergies. Now, it's a plant pathogen and so it's mainly found outdoors, but it can be found indoors under poorly ventilated conditions or conditions where we have an excessive amount of moisture. Spores are released late in the summer during the dry part of the end of summer, and there's been shown to be a correlation with the summer release of spores and seasonal allergies. So, here we see a picture of a common tobacco plant infected with *Alternaria alternata*, and we also see here an infestation of *Alternaria alternata* in a room. Next slide. Okay. It's important to remember the proteome is dynamic and so it's dependent upon the developmental stage such as these different lifecycle stages in this-- For mold, as you can see here on the lower right hand panel, it's also dependent upon the structural origins and can be impacted by the growth media. Next slide

So, up until this recent publication in our lab, we didn't really understand the allergen profiles for these individual lifecycle components. We just didn't know which of these cycle components, the allergens, were more prevalent. So today I'm going to talk about this study where we actually used our MS/MS quantitative strategy to compare the proteome and allergen profiles for hyphae, non-germinating spores and spores. - In the study we also did a comparison of three individual allergen extracts from US manufacturers and I just want to say that the information that we obtained from the study can be and should be used towards improving manufacturing quality. Next slide. Okay. So, we used visualization techniques to count spores and to determine the sample integrity and identity of each sample component type. Each lifecycle component source was then pulverized with a mortar and pestle, and then we extracted the proteins using

organic solvents. After the protein extraction was finalized, we then utilized SDS-PAGE to confirm that we had suitable extraction and complexity for downstream mass spectrometry analysis. Next slide.

Okay, so in our study we did two separate studies using a total of six biological replicates. That is three replicates per study. So, here you can see that-- What's important in this slide are the numbers. For study one we found 3,906 proteins, and in the second we found 3,885. And you can also see from the Venn diagrams that there's similar numbers between the distributions of different sample types. So, what's important here is the consistency because the similarity between these numbers show that we were consistent in our sample prep procedure as well as our analytical technique. And this is an important critical confirmation step if we're going to use this information as a regulatory guide or a tool. Next slide.

Okay. As I said, one of our major goals in the study was to compare the allergen profiles for these different lifecycle components. So, here we see two volcano plots. Volcano plots are a two dimensional way of plotting the full change differences for those proteins found in both sample types. And so, for the left volcano plot, we see the comparison between hyphae versus spores, and in the right volcano plot we see the comparison between germinating spores versus spores. And what is apparent from these studies, and these volcano plots representing the first study , is that most known allergens were more prevalent in non-germinating spores. And to our surprise, the major allergen Alt a 1 was largely secreted and found abundant in the media that was used to culture the mold, and it was near the limit of detection in these source materials. I just want to point out that we're actually using this information to identify those surrogate peptides that we plan to use next in our absolute quant method for parallel reaction monitoring. Next slide.

Okay. So, for the commercial allergen extract comparative study, a total of three separate lots were analyzed for each commercial vendor for a total of nine separate samples. Using the same strategy, a total of 672 proteins were found in all three studies. And what we found in the study was most of the known allergens were not detected in ALK or Jubilant HollisterStier samples and only a few of them or a small fraction were found in the Stallergenes Greer samples. Another thing that we noticed was that there was major variation between individual lots for each of the vendors. These high coefficient variation numbers clearly show that a standardization method would improve the manufacturing process. Next slide.

Okay, so now I'm going to talk about some studies we did toward house dust mite source material optimization. Next slide. Okay, so house dust mites are ubiquitous in the Northern Hemisphere, except for cold dry climates. House dust mite allergens are often the first respiratory allergens encountered by infants and are considered initiators of the allergic march from allergic rhinoconjunctivitis to allergic asthma. Now, house dust mites eat epithelial shed from humans and their pets and live in upholstered furniture carpets and bedding. And it's been actually estimated that 10% of the weight of a 2-year-old pillow is made up of mite bodies and feces. Next slide. House dust mites are coprophagic and their fecal pellets are structurally organized and contain digestive enzymes so that food within the ingestive fecal pellets is absorbed for nutritional benefit. Now, the fecal pellets are particularly important because these digestive enzymes are also allergens. And because-- Unlike the bodies, they are small and so when they are inhaled, they actually make their way deep into the lower respiratory tract. So, as Ron pointed out earlier, house dust mites allergen extracts are standardized for overall potency, which implies that they are qualitatively similar. However, the method of cultured house dust mites may differ among manufacturers and thus their

allergen extracts may qualitatively differ. And another issue with house dust mite extracts is the source material. As a consequence, made after a meeting of the Allergenic Products Advisory Committee in 1987, it was decided that house dust mite extracts were derived from mite bodies exclusive of the fecal pellets. However, in the last 35 years or more, it has become evident that the fecal pellets are an important source of house dust mite allergen. Next slide.

So, we used the same mass spectrometric strategy used with *Alternaria*, which was to make extracts from two source material components. In our case, we made extracts from fecal particles and house dust mite bodies. And we also included in the analysis house dust mite extracts from Stallergenes Greer. Okay. So, in this study, where we used three replicas for each sample type, we identified 1,411 proteins. And what's really interesting was that from the feces proteome, 7% of all ion current matched to peptides belong to known house dust mite allergens, which implicates or indicates clearly that these allergens make up a substantial amount of the proteome. They are relatively abundant. And also, this was over twice what we saw in the body's extract and over three times what we saw in the house dust mite extract. When we plot the allergen ion current values, the pie chart shows how the distribution of abundant changes for each sample. So, there's clearly a change in how these appear depending on what samples you look at. Next slide.

What is important is that a relative abundance comparison for these allergens show that the feces extract is enriched for several allergens, including the major allergens Der p 1 and Der p 23. And interestingly, Der p 2 was equally abundant between all samples and there is some evidence that the bodies do enrich for certain allergens. But what we can say about the pie chart and this table is that these data

collectively show that house dose mite extracts can be improved by including the fecal pellets as source materials. Next slide.

And so, before I go over my summary slide, I'd like to make some acknowledgements. I'd like to acknowledge everybody that I work with, of course, Jay and Sam Mindaye, and Kavita. But I also wanted to mention Philippa Hillyer and Andrew Freeman who played a substantial role in the house dust mite project. And I'd like to thank my collaborators from Portugal for their efforts and help in making the source materials we used in the *Alternaria alternata* study. And so, the next slide.

I'd like to summarize by saying that what we've said today is that mass spectrometry is precise, it's accurate and reproducible for qualitative info and identifying surrogate peptides that can be used for absolute quantification. And I mentioned that parallel reaction monitoring with these surrogate peptides would be an ideal way to measure potency and therefore this can be used as a potency assay. Now, when used to improve product quality, CBER's reference reagent lab will transfer technology and reagents to the manufacturers for lot release. And so, I also said that mass spectrometry can guide manufacturing of complex extracts, and we demonstrated that today by showing that you can actually improve *Alternaria alternata* extracts by increasing the ratio of spores. We've also shown that you can improve house dust mite extracts by including fecal pellets. And finally, this is a mature technology and mass spec and parallel reaction monitoring are already used in the industry, and they are now suitable for promoting manufacturing of extracts that are specific, potent and consistent for allergenics. And with that, I'd like to thank you-

Dr. Rabin: -Next slide.

Dr. Strader: Oh, next slide. Sorry. Yeah. Okay. So, now I'd like to read the questions. The questions that we will be discussing is question three. LC/MS/MS analytics to improve product quality. Does LC/MS/MS technology, compared with the currently used analytic technology, provide sufficient fit-for-purpose analytical capability for better characterization of complex allergens extracts to improve product quality? And question four, house dust mite source material optimization. Does the available data support inclusion of both house dust mite bodies and fecal pellets as source materials for HDM allergen extract to more adequately mimic clinically relevant allergen exposure? And now we can take questions.

Use of Tandem LC/MS/MS to Measure Potencies of Complex Extracts with Multiple "Major Allergens," Use of HDM Bodies and Fecal Pellets as Source Materials for HDM Extracts - Q&A

Dr. El Sahly: Thank you so much. Please use the raised hand function for questions you would have for our speakers. Dr. Rubin.

Dr. Rubin: Thanks a lot for that presentation. I'll get [the] video. There we go. I have a technical question and a separate question. The technical question is, is there a variation among the allergens that you're looking at that could affect the peptides that you're measuring? In other words, could the PRM not work because you would have a different peptide, a different peptide mass. The second question is-- You're focusing on quantitating the major allergens, but is there a lost opportunity here? Because you also get for free the quantification of anything else. So, it is an opportunity to measure purity at the same time that you're measuring-- At the same time that you're measuring the amount of the major allergen. Thank you.

Dr. Strader: Well, thank you very much for your question, Dr. Rubin. First of all, that's a really good question. And really the whole success of a MRM or a PRM strategy for absolute quant with mass spectrometry is how you select your proteolytic peptides or your surrogate peptides. And so, one of the things that we try to do is when we look for peptides that are exclusive to our allergen that can serve as a surrogate, is we try to avoid peptides that are amenable to modification such as oxidation or methylation, or any kind of post-translational modification-

Dr. Rabin: -Or an amino acid substitution.

Dr. Strader: Well, yeah. I mean-- Yeah, the idea is that we want to find peptides that we reproducibly identify in multiple samples that actually are exclusive to that protein. And, of course, if there's an amino acid substitution, you miss it, but that would be an isotype. And what was the second question? It's-- You know, the good thing about this technology is that you can quantify multiple isotypes or allergens simultaneously, because you're using a mass spectrometer. You can design PRM or MRM assays to look for what you want to actually quantify and spike into your sample multiple peptides representing different allergens of interest, and then you can use the same data acquisition to quantify those allergens to determine their absolute quantification. So this is one of the reasons why people are moving towards this type of technology.

Dr. Rabin: It is our intent-

Dr. Strader: -That's our intent.

Dr. Rabin: It is our intent to measure multiple allergens. That is the beauty of it.

Dr. Strader: Absolutely.

Dr. Rubin: Thank you.

Dr. Strader: No problem. Yeah. Next question.

Dr. El Sahly: Dr. Davis.

Dr. Davis: Thank you. Yeah, thank you for that presentation that I think has just a great deal of promise in, as you mentioned, detecting several allergens and doing it in a more standardized way. I have two questions. The first is that I noticed in the mold extract evaluation for *Alternaria* that even though HollisterStier and ALK had a better quantification of Amb a-- Or Alt a 1, the major allergen, they did not have these minor allergens that Greer extract had. And I wanted to understand what you think is the reason for that, if it's actually the spore versus hyphae, and if you think that this is going to have implications for manufacturing. I think you alluded to this and what those implications are. And then my second question is with regard to cost. So, ELISA typically per sample is $5 to $20. LC/MS/MS probably more like $40 to $200. Do you think that they're-- Is it feasible financially? And do you think there'll be any pushback from manufacturers to this kind of assay?

Dr. Rabin: You take the question.

Dr. Strader: Okay. Yeah. About the commercial allergen extracts. When we did this source material analysis and we saw that the allergens were enriched in non-germinating spores, it became clear to us that that's where you want to go. And I think one of the things that's problematic is that there's no standardization of these extracts. And so, while they might be-- They're just adding spore and hyphae without any emphasis on-

Dr. Rabin: -To our knowledge.

Dr. Strader: To our knowledge, yeah, I just heard you whispering. To our knowledge. We don't know exactly how much the ratio of spore hyphae is, but I think it's clear that they would benefit substantially if they increased their ratio of spores. And so that's one of the things that might be done to improve allergen content-- Do you want me to try to answer the second question?

Dr. Rabin: No, I'll answer the second one. I think you're going to hear from the manufacturers and yeah, it is-- There are two different approaches. Ironically, I would say that the reason-- How it came to be that we brought this technology into our lab and considered it as a regulatory tool actually happened-- It was probably 12, 13 years ago when I attended a meeting in Cuba of all places, and one of the manufacturers presented data in which they were using this technology as quality control or to assess their allergen extracts. I mean, that's how I learned of it, and that's how it was brought to our lab. But yeah, the technology costs a lot of money and it would cost a lot of money. And you're right, maybe it would cost two or $400 to contract it out to have somebody else do it. I don't know how much that would cost over a lot of-- How much that would increase the cost over a lot of extracts. Again, here you do have a substantial investment in equipment and somebody who knows how to use it if you're going to do it in-house. So, that's a big deal. The heavy labeled peptides are not a cheap item, and again, we supply the reagents. But yeah, I think you might hear from-- When you hear from the manufacturers, you might hear some pushback about that with regard to price, but that's-- They'll be getting their talk, so you can ask them directly.

Dr. Strader: Can I add one more thing?

Dr. Rabin: Yeah, go ahead.

Dr. Strader: Everything he said is relevant. I just want to say that a majority of the cost probably would be upfront, getting the instrumentation or deciding whether or not you wanted to work with a contract company that specializes in this. But the payoff is once you've developed your PRM assays, the efficiency is really good, and so you can get a turnaround quickly.

Dr. Davis: Yeah, thank you for answering those questions. I think that there's an opportunity there for potentially one provider of LC/MS/MS to really service the few manufacturers of this worldwide.

Dr. Strader: I would think so too. Next question.

Dr. El Sahly: Thank you. Dr. Omer.

Dr. Omer: Yeah, so I little bit, I want to know a little bit about the global alignment aspects of this. So, are there any precedents from WHO or European reference labs that support the dual source HDM extracts and equally importantly, how would FDA's adoption interact with these standards?

Dr. Rabin: So, to my knowledge, the answer is no. What I can tell you is that when we took this project on with the eventual goal of incorporating it into the regulatory atmosphere, we did so as a collaboration with Paul Ehrlich Institute in Germany. In fact, one of their fellows was here with us for a few years. Unfortunately, they've sort of dropped the project for reasons that we're not certain, but everybody's got to make those choices. This is something-- As far as the global aspect of it, what I can tell you is that that is addressed-- You may not be familiar, there's a triennial meeting called the Paul Ehrlich Seminars in Germany. It's a very unique meeting that is really centered around the regulatory aspects of allergen extract manufacture and distribution. And so,

regulators from all over Europe are there. Of course, we've traditionally gone to those meetings and participated in it. I'm on the scientific board of it and so forth, and then there are some academicians as well. And there is always a meeting of a standardization subcommittee. And the last meeting that we had was heavily, heavily discussed about mass spec. So, there's an interest in this, but it appears right now probably that we are going to be the pacesetters on this, which I'm actually quite proud of. And my guess is that when we do these things, the Europeans will follow us. And that's okay with me.

Dr. Strader: Next question.

Dr. El Sahly: Dr. Assa'ad. Dr. Assa'ad?

Dr. Assa'ad: Yes, thank you. Thanks for the presentation. My question is, since all this is done for the purpose of standardization, at the end of the day, once you do your MS and find the percentages of different allergens in different parts of *Alternaria* and in different preparations or extracts from different companies, how are you going to determine the optimal?

Dr. Rabin: Yeah, yeah. Well that gets back to the conversation that we had previously. I mean, I think we really have to consider the optimum-- What the optimal quantities are. As we solve one problem of deciding what the minimal requirements of a particular extract would be, minimal release criteria will be, we're going to have other problems as to what it actually means to standardize these. Are we standardizing them or are we characterizing them? If you're accepting a certain level of heterogeneity, you can't really call it standardization. We might have to come up with a different word for some of these things. I think these are problems that we anticipate. I've anticipated them before we had these conversations today. I've certainly-- They are a lot brighter on my

radar screen after these two conversations that we have today. And I think that we're very fortunate that we have experts on the Committee here, we have experts within the academy and European colleagues, and we're going to solve these problems as we move along.

Dr. Assa'ad: So, the other second part of the question relates to the population that is exposed to these allergens. I mean, we know that for dust mites, *pteronyssinus* and *farinae* have different distribution by geographic area. We also know that some populations are more reactive to some allergens, like African-Americans are more reactive to cockroach allergen and to dust mite allergen than Caucasians and other races. So, again-- I mean, that all needs to factor in when you-- Like the discussion we had before about age, different populations are different. And so, what's utmost characterized and standardized for a population may be different from another, and you'll have to come up with something that covers everybody and every age. And that's not a small task.

Dr. Rabin: Nope, I agree with you. I agree with you on that. And the only thing to really say to it is that the first solution that we may come up with may not be the ideal one. I think Dr. Assa'ad that I would hate to see us 90% there with a given extract and then stalled for three years because we're trying to solve some of the issues that you brought up. Okay? I want to get something in place for some of these extracts that will improve things or reach some endpoint. And then we, the FDA, and the community will be there to remind us that the job is not done. That the first iteration is not necessarily the last iteration. I think that that's the best way that we can incorporate your comments and your concerns, your plural, after all, because your colleagues on the Committee are agreeing, have stated the same concerns in a way that will be efficient and it won't

become just another thing that we plan on doing but haven't really done. That's my biggest concern. My biggest concern is that. Okay?

Dr. Assa'ad: Okay. So, I'm not sure if you're going to be answering us any more questions, but if you are not, I have one more question. I don't know if you have more presentations or what's the agenda.

Dr. Rabin: No, no. Well, there are more presentations. There's a presentation from Dr Platts-Mills and from APMA.

Dr. Assa'ad: Yeah, but from you? From both of you.

Dr. Rabin: No, from us this is it. So, please go ahead.

Dr. Assa'ad: Okay. So, my question is: The status of things is that at the end of the day, as users of these extracts, particularly in diagnostics, we are always faced with the question, and particularly in food allergy, I know we didn't discuss a lot of food allergy here, but there is a major discrepancy, major discrepancy between the skin test responses and the serum IgE, specific IgEs to the foods. If we do for environmental allergens, the range for specific IgE for a certain allergen is different. It's very high for grasses, it's much lower for *Alternaria*, middle for dust mites. I published a paper years ago on that. So, we left with this reconciling the results of skin tests with serum IgEs and we also left with trying to reconcile or even to interpret all of that. What does a positive skin test of this size mean versus that size? These are all the things that in practical terms, and I know that may not be your issue, but once you approve something, you approve it and standardize it, and when you have this much Alt a allergen. Does this mean you expect this allergen that is standardized to produce a positive skin test? What's the positive predictive-- How big is the skin test? What's the

positive predictive value of this skin test versus a different skin test? So, all these become more steps and things that practically affect the use. I mean, to the point that I, for example, in my practice, very rarely do skin anymore for foods. I just use the serum IgEs. They seem to correlate, give me more information and components. CM IgEs and components. So, the skin test just muddy up the water.

Dr. Rabin: So, let me ask you then, what do you think accounts for that? And in an ideal world with unlimited resources, how would you reconcile those things? I'm curious to hear what your thoughts are.

Dr. Assa'ad: Yeah, yeah. I mean, this is where translational research comes in. You bring those highly allergic patients, use that standardized extract that you have decided and show these curves of positive skin tests. You can also bring patients who are not highly allergic. Again, in food allergy, it's a spectrum. And in these patients, those skin tests of this size correlate with such. I mean, you said you talked to Gideon Lack. After a lot of discussions with Gideon Lack on the NIH Committee for peanut prevention and looking at those studies with the Australian studies with other studies, the number of seven millimeters became-- Yes, that's what predicts you're going to be peanut allergic or not allergic. But that is not actually universal too. I think--

Dr. Rabin: Well, yeah. Especially since you don't know what that means in terms of how much allergen is in the extract because it's not a standardized extract.

Dr. Assa'ad: Well, that's true. If we end up with standardized extract, then we need to almost repeat-

Dr. Rabin: -That number may be more meaningful.

Dr. Assa'ad: We would almost need to repeat those epidemiologic studies to say where is the cutoff? And I think that's part and parcel of what you are going to do.

Dr. Rabin: Okay.

Dr. Assa'ad: Yes.

Dr. Rabin: Okay. Fair enough. Dr. Greenberg.

Dr. El Sahly: Dr. Greenberger.

Dr. Greenberger: I would like to thank you for your presentations. I have one quick comment and then a question. The comment is I'd like to see the United States and FDA be leaders in standardization of allergenic extracts, and I'm glad we have the-- This was commented by Dr. Rabin and I hope that this can be the case. A question is, what would be a satisfactory fit-for-purpose, let's say in a year or two, for let's say ragweed? What do you see under your criteria meeting fit-for-purpose?

Dr. Rabin: I think fit-for-purpose-- Well, fit-for-purpose for the ragweed assay would simply mean that we have the linearity of the range of measurement of Amb a 1 that covers-- That well brackets the range of the concentration of Amb a 1 in the allergenic extracts. I mean, in a limited sense that that's what that means. If you're talking about, say, taking the mass spec test and applying it to Amb a 1 or for that matter, any other-- Ragweed or for that matter, any other extract, I think fit for purpose is demonstrating a range at which you're measuring these allergens and ensuring that you are measuring intact allergens, which-- Because that's the one big drawback of that. People like Dr. Strader know very well and make absolutely sure that, you know, you could be starting off with a bunch of digestive peptides and then you're measuring a

bunch of digestive peptides and it's garbage in and garbage out. But fit-for-purpose sort of by definition is accuracy, precision within the range of-- That brackets the range of concentrations that what you're measuring, that you intend to find. We don't need a hypersensitive assay. We're not looking for contamination of wheat flour by a little bit of peanut protein, what's necessary in the food industry. We're looking at extracts. Does that answer your question?

Dr. Greenberger: It helps, but I would like to hear that the-- I would like to hear how the mass spec data could help us look at heterogeneity or at least say what would be the major allergens with these measurements, say of the mass spec?

Dr. Rabin: Well, a major allergen is defined by what people react to. So, I don't think that mass spec addresses that. I mean, what mass spec addresses is what comprises the major concentrations. And the idea, I think, of applying it to the clinical-- The dream is to be able to put it together with component monitoring and individualized patients for therapy for those who would benefit from it. Did you have something else to add?

Dr. Strader: Well, yeah. So, with mass spectrometry, we can get the relative abundance or absolute quant for a number of allergens simultaneously in the sample. We can also compare how these differ from one, say, batch to another batch. So, we can definitely address heterogeneity just by looking at changes from one sample to the next, and what we're hoping to see is that if there is a standardization in process, is that these differences would narrow and they would become less. In other words, your coefficient variation for identified peptides would decrease and it'd be tighter and smaller. Say, ideally a great number would be below 20% instead of 40 or 50% like what you were seeing in the data slide I showed, which is outrageously high.

Dr. Rabin: Okay. So, I think that's the best answer that we can-- I hope that answers your question the best that we can answer.

Dr. Greenberger: Well, I-

Dr. Rabin: -Not quite.

Dr. Greenberger: Before I turn off, I'd like to see a meeting of this Committee in one year to get a progress report and we could continue to help. Is that feasible?

Dr. Rabin: All right. That's not my decision.

Dr. Kaslow: Taken under advisement.

Dr. Rabin: Taken under advisement, I have been told by the upper management. Okay?

Dr. Greenberger: All right. Thank you.

Dr. El Sahly: Okay. And one final point, you may have clarified this, including the fecal pellet and the extract improves the diagnostic sensitivity?

Dr. Rabin: I could only imagine that it would. I would hope so.

Dr. El Sahly: Do we know that it's false negative because we don't have the fecal pellet?

Dr. Rabin: I don't know that's-- I don't know that that would be known. I don't know. You know, Dr. Platts-Mills, that is a great question for Dr. Platts-Mills.

Dr. El Sahly: Alright, that is a great segue for the next presentation. And I want to remind all the Committee members that we do have a designated two hours for discussions, Q&A, and all the presenters usually are available to clarify points and answer questions.

Approaches to Allergen Standardization Related to Dust Mites

Dr. El Sahly: Next on the agenda is Dr. Thomas Platts-Mills. He's a professor of Medicine, Division of Asthma, Allergy and Immunology, Department of Medicine at the University of Virginia School of Medicine, Charlottesville, Virginia. He will be discussing approaches to allergen standardization related to dust mites. Dr. Platts-Mills.

Dr. Platts-Mills: Thank you very much. It's a real pleasure to be here. Some people think that inviting me to talk to the FDA was rather inviting the fox into the henhouse, but it's a pleasure. And I need to say that I have a conflict, which is that we have received support from Phadia, Thermo Fisher for doing assays, but they don't control anything that we publish or talk about. And I'm grateful for that. I'm grateful to the NIH for their support and continuing support at this time. The dust mite-- When we first started here-- You'll notice that I use the term we in a royal sense. When we started in 82, they were thought to be upwards of 5 million people on treatment with house dust extract. And the house dust extract was collected in vacuum cleaner bags, and so it was standardized in vacuum cleaner bags per liter, which was not satisfactory. Over that period, cultured dust mites appeared firstly from Voorhoost in Amsterdam who developed the technique which was taken on by Bencard in the UK and then Hollister in Spokane. And now of course there are many people growing dust mites and we've had this issue already brought up. Should we use isolated mite body's, whole culture or mite feces? And I'll address that.

To provide a suitable method for comparing batches of our product to establish standard, we've heard about skin testing by end-point titration, and I will talk about that in vitro methods of measuring potency of an extract and immunoassays for Der p 1, Der f 1, Der p 2 and f 2. Maybe I should spell out the skin testing. The ideal technique had many problems. Firstly, it was much too demanding on the subjects and Harold Nelson actually said, "I refuse to recruit anyone to do that skin test titration." And if you've ever had intradermal skin tests on your back, you will understand why. In vitro methods of measuring potency of an extract-- Yes, Dr. Rabin has discussed those and there are methods, but obviously skin tests tell you that they really react or BAT can do that, but they are all demanding in terms of routine use. And their immunoassays for the allergens. And as we've heard, there are several forms of mass spectroscopy, but there is also the original thing developed by ALK, which was Cross-Radial Immunoelectrophoresis or CRIE, which can give you a wide range of proteins that are seen and was established by Henning Løwenstein in the 70s. And finally, proteomics, which I will mention. If I can have the next slide. Do I have control over the slides? I don't know.

When we talk about the sources, we really come to the data that came with a purification of dust mites in Der p 1. And I want to stress that the first allergens purified, which were Lol p 1, Amb a 1, Fel d 1 and Der p 1 were all purified before cloning or monoclonal antibodies, so they actually focused on identifying a protein peak, which is once you start cloning you don't do. But in keeping with that, Der p 1 we can measure in the air quite easily and Der p 2 we can measure, but none of the other allergens in dust mite can be measured accurately in the air at the moment. And Der p 23 is actually-- - Which is an important allergen, is actually difficult to measure in dust extracts or mite extracts. And then I'll talk about Dr. Tovey in a minute, but we purified-- Martin

Chapman and I purified Der p 1 in 1980 and together with Dr. Heyman and Dr. Aalberse, we purified the Group II allergens and published those in 89. Next slide.

This is-- Euan Tovey turned up in London in 1979 or 80 and he took a job as a publican pulling pints of beer and he started talking about mite feces. and we heard about it and it turned out he'd started doing a PhD in Sydney, Australia where the plenty of dust mites, and he said that it's feces. We had developed a technique to measure antigen p1 with rapid antibodies to Der p 1. And with that, we've presented good evidence that the quantity of Der p 1 created in a culture was largely in the form of the feces. I doubt that this percentage is correct, that is 99% and I don't intend to do the experiment again, but he also showed in a very elegant experiment with double-sided sticky tape inside a Pasteur pipette that you could actually not measure Der p 1 coming out of live mites, but you could rapidly out of mite feces. It came out within a minute. And the concern was, is there paratrophic membrane on the outside of these fecal pellets? And Euan in the next slide went into houses, if I can move-- Can I move the slides? No. Next slide, please.

He went into houses and proved pretty convincingly that there was no airborne measurement in undisturbed houses. These were actually some disturbed ones, but in undisturbed houses we could never measure airborne allergens. But in the-- Under disturbed conditions, we got quite significant levels in nanograms of what we thought was Der p 1 at that time. What was Der p 1 at that time. And really interestingly, the size of these particles is on average 15 microns and often greater than 20. And at that time, it was widely thought that particles had to be less than five microns to get into the lungs, which simply isn't true. And there was a very good scientist in Sweden called Svante Gran [sp?] who proved that a proportion of large particles do enter the lungs, not into the distal lungs but into the lungs airways which are involved in asthma and that

included fecal particles. Here is a picture of the first stage of the impactor and there are actually three fecal particles stuck together there, but you can see an immunodiffusion ring very nicely with a rabbit antibody to Der p 1. Next slide, please.

Then we come to the question, what should we use for extracts? This is ALK's work from the early on. This is a paper published in 2016 where they described their details-- Well, they started this long before that, and they started sieving extract and created a multiple panel sieving program to separate particles rich in whole bodies and rich in fecal particles. Next slide. This is their picture. So, if you have a sieve that has a micron particle openings of 350 microns, then 90 and then 50, you can get a fraction that is very rich in whole bodies and a fraction rich in feces. And the reason we're focusing, we knew that you could sieve to get feces from Euan's work, but these fractions are then killed by freezing and dried to below-- Killed by freezing at -20 and dried to below 15% moisture content before sieving. It is very difficult to sieve if there's any moisture in the particles because everything sticks together. But with this they've got an extract of feces which was rich in Der p 1, an extract of bodies which was richer in Der p 2 and they worked out how to mix them together to create extracts of the proportions that they wanted. Next slide.

This is a study published by ALK or supported by ALK, and they describe their extract as having 15 micrograms of Group I allergens, Der p 1 and Der f 1 combined, as well as 15 micrograms of Group II allergens, Der p 2 and Der f 2 combined. But in addition, they stated that the tablets contained the broadest possible spectrum of major and minor allergens from these mite species. Now, that-- they didn't state how they knew that, but they already understood CRIE and I suspect by 2016 they were definitely using mass spectroscopy already. So, there's a model here of a company that knows how to balance Group I and Group II allergens, but a ratio of one-to-one. And I'm not

suggesting that we know that one-to-one is correct, but it's certainly not far outside the correct zone and they've had good data with sublingual immunotherapy. And, of course, allergens are being used widely now for oral exposure, for subcutaneous exposure, and for sublingual exposure, and it's actually the development of tablets that has pushed the accuracy of components in relation to the companies. Next slide, please.

This is a separate, completely different company. This is HAL, which is a company in the Netherlands which has never attempted to come to the United States, but this is Claus Bachert who tragically died last year and a large group of European investigators including Ronald van Ree and Oliver Pfaar, and they used different ranges of doses, but they pretty well knew that they thought there should be 15 micrograms and 13 micrograms of the *pteronyssinus* allergens Der p 1 and Der p 2 in a dose. So, they knew that already and they did something else which was to actually measure IgG4 responses to Der p 1 and IgG4 to Der p 2. And if I have the next slide-- I'm including this data for a very specific reason, but here is IgG4 to Der p 2 and with the maximum dose of allergen-- This is used in allergoid kit subcutaneous immunotherapy. The G4 response to Der p 2 is modest. By contrast, the G4 response to Der p 1 is not the same as that with dust mite whole extract, but is up much greater four or five fold greater than that to Der p 2. And I'm mentioning this because we have data much more recently suggesting that this is a real difference and important. Next slide.

Just a word about allergoids. There's a long history of allergoids in the United States. David Marsh, who trained in England and actually may have known people who in the main company making toxoids in the United States- In UK, that is tetanus toxoid and diphtheria toxoid, and developed the technique of glutaraldehyde-modified allergens at that time. Unfortunately, this allergen never got approved in the United States. Harold Bayer was very opposed to allergoids because he said you can't measure

what's in them, but we're well used to them in toxoids for other purposes. And Roy Patterson developed polyethylene glycol extracts, which many people thought were very, very effective but never got approved. Can you measure allergens in allergoids with mass spec? And the answer is almost certainly yes and that you could measure them. Of course, you can't prove that the whole object of allergoiding is to make them not react with IgE antibodies and therefore you can't prove that they're allergenicly active, that you can only do by control trials, which appeared to show that. Next slide. I'm moving fast probably because I can't see the faces of the audience. The faces of the audience are something that normally control you when you are speaking, but--

This is another preparation. This is funded by Stallergens Greer France, and of course Greer is in North Carolina. and they give potency in the index of reactivity, but they have clearly stated that the quantities here are 14 to 17 micrograms of Der p 1 and 53 to 68 micrograms of Der p 2. That's strongly biased to Der p 2, but I'm not saying that that's wrong. I think the important thing is that you can state these ranges, and they said in addition that the extract includes the following allergens, including Der p 23 and Der p 36, which I think are definitely allergens. But most of these are discovered by cloning. And when you clone an allergen, you provide no evidence that it's actually-- You can get the definition of a protein with no evidence whether it's produced by the environment or whether it can be measured. And as we know, Der p 23 is very difficult to measure and we believe that many of the others are, Der p 11 may be. It's a larger protein and actually associated with atopic dermatitis in its sensitization. This was a large study, primarily of allergic rhinitis- And it's all sublingual, but they had a good effect on the asthmatics as well. Next slide.

This is the major study published in the New England Journal by-- The first author was Brian Vickery, Andrea Vereda, Casale, Kirsten Beyer. A lot of extremely

distinguished people were on that paper. And it absolutely showed that oral immunotherapy for peanut allergy in children is effective. This data was impressive and led to the licensing of Palforzia. And remember that we know the dosage, we know what is measured in that, in terms of Group I, Group II and a wide variety of other allergens. Next slide.

I'm not showing pictures of mass spec, but I do want to say a few words about proteomics. This is a really impressive paper on the proteomics of Dermatophagoides pteronyssinus, but this is the figure, one of the figures in the paper, and you'll see that there are multiple allergens identified here and you could get information about them through proteomics as you could from mass spec. And what I want to discuss is how much could an allergist in practice deal with data like this. If you knew that there were 20 allergens that had been already identified by cloning or by purification, and you could get some estimate of the quantity in an extract or samples made from mite bodies, which I think most of the data in this paper was, can you get data that your individual patient is sensitized, which of these allergens your patient is sensitized to, and then choose an extract accordingly? And my own view on that is the answer is no. No allergist has time or effort to do that. And getting accurate measurements of IgE antibodies to specific proteins is expensive and difficult to deal with, and matching them up with a picture as complex as this would be impossible.

So, I'm really not convinced that we want to know all the components of an extract. And I think that it's worth knowing that there's some models that a company has of what their extract produces and that they follow that so that if there was a major change in their extract growth conditions, which could easily happen, or the strain of mite that was growing in it, yeah, they could know, and that would be helpful, and it could be done with mass spec or proteomics or crossed radioimmunoelectrophoresis in-

house. Making standardized maps of either proteomics or mass spec and saying that a company had to match up to it might really increase the cost of manufacture. I made my point, I hope. Next slide.

Some years ago, it became clear from data coming from Sweden that in some studies, children living in a house with a cat were actually less likely to be sensitized to cats. And there are other studies where that's not found, but there's certainly a study in New Zealand, where it was found quite clearly, and actually it appeared in that study that the highest levels of mite did not decrease sensitization, but Euan Tovey-- Again, the same Euan Tovey who first proved that mite feces were an important source of sensitization, published data on nonlinear relationship of mite allergen exposure to mite sensitization. So, we published a paper called "High risk of asthma among early teens associated with quantitative differences in mite and cat allergen specific IgE and IgG4", and I need to mention Dr. Keshavarz and Dr. Wilson who played a major role in that and which was published in EbioMedicine, which is a part of Lancet science in 2025. Next slide.

So, this is the data from Sydney with Euan; Catarina Almqvist, who did elegant studies on cat allergen in Stockholm; and Guy Marks who has taken over that group since the tragic death of Euan Tovey. But what you'll see is that allergy to house dust mites is lower in the highest exposure group. The wheezing is lower, but above all, asthma is highly significantly lower in the group with the highest exposure, and these exposure levels are very high. That is the highest group with all greater than 23.4 micrograms of Der p 1 per gram of dust. Very interestingly, our own data suggests that exposure can change the relative importance of allergens.

So, for the next slide, this is data-- Some of which comes from Indoor Biotech from Martin Chapman who's an author on our paper. And you'll see Fel d 1 we can

measure in dust easily, we can measure it airborne and have done for years, Fel d 1. Der p 1, we can measure airborne during exposure, as I've made clear, and we can easily measure it in floor dust. Der p 2 levels in our hands are lower, but that's not always true. And Der p 23 is too low to measure in the air, we've never succeeded-- No one's succeeded in measuring it, and is very low in floor dust, much lower than the others. So, you'll see the next slide.

This is dust mite data from the paper in EbioMedicine, and here you'll see the blue is non-asthmatics and the orange is asthmatics. So, here's IgE to dust mite, IgE to Der p 1, IgE to Der P 2, which is actually higher and slightly more common than IgE to Der p 1. And finally, Der p 23. You'll see there is a good population of subjects who have IgE above asthmatics who have IgE to Der p 23. If we now-- And this is all the subjects with asthma. This is dust mite patients, all the subjects with asthma, and 199 subjects without current asthma randomly chosen. And so, this is IgE to Der p 23, but if I can have the next slide. Here we're looking at specific IgG4 to dust mite, Der p 1, Der p 2, and Der p 23. And you'll see that there's a much lower number-- Percentage of positives, and a higher number of unmeasurable G4s to Der p 2, and Der p 2 seems to be more strongly associated with asthma, but much less G4 and Der p 23. Among the asthmatic, there were 26 asthmatics associated with Der p 23, only one had detectable G4 and that was very low. And so, we have seen with what I believe is due to high exposure, you get good levels of G4 with dust mite, a good prevalence with Der p 1, but 80% of the asthmatics were positive to G4, only 24% of the Der p 2 positives-- IgE positives had detectable G4, but Der p 23 effectively 1 out of 26. Next slide.

We had followed up the Swedish data and in a large school study in England and in Virginia-- Well, actually, this is a study done between Los Alamos and rural Virginia and the city of Charlottesville. And among those patients who had all the houses

measured, these are the levels of Fel d 1 in micrograms, Fel d 1 per gram of dust. And you'll see that the highest level of exposure had lower prevalence of sensitization and much higher prevalence of significant IgG. And we had other evidence at that time. This is in 2001, we had evidence that these were IgG4 antibodies. But let me show you the data, the new data. Next slide.

When we look at IgG4 to IgE ratios for cat dander-- So, this is IgG4 in nanograms per mL. This is cat dander IgE in nanograms per mL, and we're getting in comparable units. And some of you may know that there's been real trouble with the units of IgG4 in nanograms because some papers were published with nanograms per liter and then milligrams per mL. And the difference between nanograms per liter and milligrams per mL is enormous. What we see here is that if the ratio is less than 50 to 1 of G4 to E, there's a highly significant increase in asthmatics, and the red dots with a black circle around them were moderately severe. That is that they had two or more acute episodes in the past year and had a diagnosis of asthma and were using inhalers. By contrast, if they had greater than 51, only 2 out of 20 had asthma. And there are a large number of subjects who make G4. These are all 135 subjects living in a house with a cat, approximate age 13 in the Viva cohort in Boston, which is Emily Oken and Diane Gold's, and Sheryl L. Rifas-Shiman is the main statistician. Next slide.

I want to just apologize for the complexity of this slide, but these are IgG4 to IgE ratios for asthmatics, and this is cat dander data. And here you'll see that the mean ratio is under 20 here. And here's-- So this is 20, 30, 40, 50 up here, and that's the data I've already shown you. For Fel d 1, this data is actually slightly more impressive. There are a large number here with very low ratios of G4 to E, and these are asthmatics in the blue circles. And for Fel d 4, looking at the same issue, there are only three where we had G4 and E among non asthmatics. So, the statistics don't work, but the level of

the ratios to G4 to E to Fel d 4, six out of eight were below 10. And it looks as though this ratio of G4 to E to Fel d 4. So, there's two minor allergens, we're looking at Der p 23 and Fel d 4 where the allergen appears to be strongly associated with asthma and makes poor or no IgG4. So, next slide.

In conclusion, I want to make a point about intra- and inter-molecular epitope spreading. And I apologize about these terms because they get confused very often. If we use the term intra-molecular epitope spreading means that you can have one antibody binding to Der p 1 and no-- Only IgE antibodies to only one site. Or you can have IgE antibodies to four or five sites on Der p 1 and that seems to be associated with much more ability to induce histamine release and symptoms. The alternative is epitopes spreading from one protein to other proteins from the same source. And this may be equally important, but it's inter-molecular epitope spreading. And what we appear to be looking at here is that major differences in inter-molecular epitope spreading occur between IgE and IgG4 antibodies to cat or mite components. That is that the low or absent G4 responses to some protein allergens, Der p 2, Der p 23, and Fel d 4, could be the reason why some of these allergens have a more significant role on symptoms including asthma.

But what matters here is that the relative importance of the minor allergens such as Der p 23 and Fel d 4 may be different if you're in an environment with very high exposure to mite allergens or cat allergens compared to an environment where the level of exposure is much lower, that means that effort to make a decision about what level of allergens you need in an extract could be extremely complicated and unlikely to be fruitful. And that the position taken by some of the manufacturers appears to be that you should measure Group I and Group II allergens, make a decision about the ratio and state what that decision is, and keep to that ratio within certain range in your statement

of the extract, and to monitor either with CRIE or with proteomics or with mass spec. But if the FDA takes a position that people should use mass spec for their intermittent monitoring, that may be fine, but making a decision that you've got to have this, that and the other allergens, multiple different allergens, would be exceedingly difficult. Next slide.

So, in summary, the ability to separate fractions of dust mite cultures has been available for many years. Over the last 20 years has been a progressive move to defined extracts, particularly in the production of tablets used for oral sublingual. Assays of Group I and Group II mite allergens have been possible or available for at least 30 years. However, there's no simple basis for defining a ratio apart from one to one because the relative importance of these or other mite allergens can change with different levels of exposure. By selecting or sieving different sources of mite, it is possible to enrich whole bodies of feces and to create extracts with defined quantities of Group I and Group II allergens. It is-- It would be difficult to extend-- I apologize. It would be difficult to extend this to other allergens because their quantities in extracts or the environment are much lower or much lower. Next slide.

In conclusion, in the process of standardizing mite allergen extracts, skin tests are essential. Skin tests at some level are essential to be sure that the product is clinically active, but no form of skin testing can define the strength of an extract because it depends on the choice of the subjects tested. That is there are patients who differ in their sensitivity by logs and if the company can choose the subjects, they can decide what the strength of their extract is. Assays for Group I and Group II mite allergens to assess the consistency of products should be used to relate batches to local, national or international standards. And I think that applies to Der p 1, Der p 2, Fel d 1, Bla g 2 and Bla-- Cockroaches are probably Bla g 2 and Bla g 5.

In relation-- To answer to Dr. Assa'ad, I don't believe that African-Americans are more sensitive to those cockroach allergens. I think many African-American communities in the United States do not like having cats in the house, and cats are major consumers of cockroaches and the increased levels of cockroach allergen in houses of African-Americans, in part, or in major part, reflect the lack of cats. The assessment of the quality of extracts by either CRIE or mass spec or proteomics should be encouraged. But I think the FDA has made a very strong case for using mass spec as a monitoring system, but not as a definition of standardization. With a focus on standardization, what will be necessary is a standardization of the mass spec techniques used. If I can have the final slide, thank you.

Thank you very much for your audience, but I'm showing you this utterly beautiful picture to remind you that I don't believe that an allergen practice can handle thinking about more than 10 or more allergens. They can't think about it and they can't make decisions on the basis of it. Thank you.

Dr. El Sahly: Thank you, Dr. Thomas Platts-Mills. I do know that the Committee has many questions and in the interest of time, though, we will move to the next presenter, and Dr. Platts-Mills and the other presenters will be available for at least an hour's worth of Q&A after the next presentation.

Dr. Platts-Mills: Thank you.

Industry Perspective from the Allergen Products Manufacturers' Association (APMA)

Dr. El Sahly: Thank you. So, now I would like to welcome Ms. Trenna Repp. She will give us the perspective from the Allergenic Products Manufacturers of America. Ms. Trenna Repp?

Ms. Repp: Hi, thank you. Yes, I'm presenting on behalf of the manufacturers. So, on the behalf of the Allergen Products Manufacturers Association, this slide just shows who we are. We are a nonprofit association of the businesses listed. I'm the current president, so I'll be presenting on our behalf, and I am employed by ALK, one of the manufacturers. Next slide.

So, we did want to thank Dr. Rabin for inviting us to present in this discussion. And I think as was already mentioned, allergen products have been around since the early 1900s. Since then, there's been a lot of knowledge about allergy and treatments that have grown and the technology is greatly advanced. We do agree it's important to continue to innovate and approve our products and to provide the best treatments to the allergic patient. We also want to thank Dr. Rabin and his colleagues. We did notice some of our questions within our presentation were addressed during their presentation, so we do want to thank them for that. We are still going to present it, but they may have already been addressed. Next slide. So, we created this presentation before seeing the briefing book. We did have a preview of what the topics were going to be, so we did want to include what we believed the topics were. We don't see-- We believe this aligns with what was presented today, so we don't need to do much more about this slide. Next slide.

So, the first thing we do want to talk about is the change from the allergen units which are related to the BAUs for cat and ragweed. We do-- I'm not going to explain the ID50, that was very nicely explained by other presenters. But we do want to emphasize our position that we do want to maintain that clear link of the current units and the BAUs when changing to Cel d 1 and Amb a 1 units. It does maintain that link to the original skin testing. I know there's some issues with that method; however, that's how it's been linked previously and we also want to link it to that to also provide a

conversion for allergists when they're adopting the new units in the clinical setting. We do want to say the Agency has kept us informed on the new ELISA development and we do support the change, we've been involved. We did want to mention at this time for the cat ELISA, we're not sure we're convinced yet that the currently published data shows a strong correlation between the current Fel d 1 units and the future Fel d 1 milligrams or micrograms per mL. We do feel additional data is needed to make that conclusion, but we also know there's been some additional work since that publication. Next slide.

There is one consideration we would like to put out there when referring to the BAUs or the major allergen units to the micrograms per mL, and that is the labeling. Currently when we label in the BAUs on our products, for example for the cats, we will say it's a 10,000 BAU per mL cat extract. When we do the actual testing for the standardization, we have an acceptable range of 10 to 20 Fel d 1 units per mL.

We would like to propose or to-- It's our perspective and our stance that we would like to maintain this labeling in the future, so essentially not reporting the exact rate results of micrograms per mL on the label, but essentially saying, "If it's within this range, it is an X microgram per mL extract." So, for example, if you take this 10,000 BAU and the current range and you multiply it by approximately four Fel d 1 units per gram, or micrograms per Fel d 1 unit, any extract that has a result of 40 to 80 Fel d 1 units per mL would be labeled as a 60 microgram per mL of Fel d 1 protein and not, say, a 45 microgram per mL protein extract. This really counts for the method variability. It would be consistent with the BAU labeling and it would also discourage some unnecessary discrimination in batch selection at the clinic. As manufacturers, we are a little concerned because it wasn't clear to us what the labeling expectations would be that if we were to put on there the exact results from the assay that we might start

getting requests for specific batches because it's perceived as being a higher potency than one that would be within the normal range of the method variability. Next slide.

So, let's talk about the mass spec, as a very interesting presentation. So I think I will acknowledge that we do want to thank Dr. Rabin for clarifying at the beginning of his presentation that these standardizations are for essentially the traditional aqueous extracts, not necessarily the newer products like the SLIT tablets or the OIT.

So, you will see some of the new products in our presentations just because when we heard standardization, we weren't sure if it was going to apply to these traditional products or to all products. So, you will still see it, but the points we're trying to make are still relevant even though we have included some of this data in here, or information in here. So, what we wanted to highlight with respect to the mass spec, currently we do have multiple house dust mites and we are using the mites as the example, but this could apply for all of our products.

We have house dust mites, we have the three manufacturers, we have different processes, we have different source material. All of these products are approved, they've got real world use, they're considered safe and effective, but they may be different. So, next slide. So, we have also had some updates over the years about mass spec. These slides are actually from a presentation given at an APMA meeting that we had with the FDA, and this is from Dr. Spiric in 2016, I believe. And the point of this is that you may have an extract that has the same potency, but the actual composition may be very different. We expect that if you were to look at all of our extracts, they will be different even though they have a similar potency in the current standardization.

Our concern is that all of these products have a history of safe and effective use. When I say some are supported by clinical trials, that's really the SLIT tablets. But what

our concern is that if we create a single composition, a specific composition that these extracts need to conform to, that might not be applicable to all of the products. We also have some concerns with the cost. So MS mass spec equipment is expensive and also there's a lot of batch to batch variability within both the source material and the extracts. When you're looking at a small selection of extracts, I mean, as we can see in the data presented, some of the CVs when they did three batches was high. When you look across 20 batches or a hundred batches, you can really see the variability, the natural variability in the product, which can affect these types of assays. Next slide.

So, when you're considering these factors for the mass spec, it's kind of our position that when we're talking mass spec-- And I do want to say within the industry there's some minimum-- There isn't a lot of experience. I know some of the companies have this implemented, as Dr. Rabin said, one of the first presentations he saw was from a manufacturer. So, there are a couple of manufacturers that have this technology. I think maybe one might be using it for product on the markets, but a lot of it is used in research and development.

And from our perspective, we're not against using this type of technology, but from our perspective, it's our position that it shouldn't be used as a release test. It's very useful in characterizing a product during development or to even evaluate an impact of a process change during lifecycle management. But if you were to use it as a release test, you need to have robust specifications that need to be set, and it should also consider the acceptance criteria at the end of shelf life. This can be very complicated considering the inherent variability of the source material and across the manufacturers. We all have different processes and our processes can very much impact a composition in this type of analysis. I mean, it sounds like it is a crude extract, they're all very similar in how they've been done for a hundred years. You take a source material, you extract it, you

purify it, you sterile filter it, but they're still not the same because differences in the source material and small differences in the manufacturing can result in differences in the extract.

Mass spec is also performed on degraded proteins and only measures the presence of the allergens, but not necessarily the potency. And it also does not measure stability. So, we do want to question what the stated link between the quantitative measurement and the biological potency really is because presence doesn't necessarily mean potency. So, it is our position that if it is implemented, we do agree it could be very useful in characterizing our products, either retrospectively for the current products, I think I will not mention any of the development products or future products since that's not in the scope, but to use immunochemistry methods as the batch release to continue using those, either the current-- Whether it's the current relative potency ELISAs or or new major allergen ELISAs, we do think it's very important to maintain that link with the potency combined with the characterization. And yes, I think that's the main point for this slide, that if it's to characterize presence, it's one thing, but it's different to use it as a release test for composition. Next slide.

So, the addition of the house mite fecal to the extracts. So, it was our perspective, and the data is very interesting that's been presented, but that the current extracts do contain fecal, essentially the major allergens related to the fecal. Particles, there are some present in the purified bodies, it's not just there is going to be some gut/fecal. It is made available for extraction by grinding the bodies prior to extraction. And then also there is the house dust mite SLIT tablet that's already on the market. It contains both the body and fecal extracts with the defined ratios of the major allergens.

We did want to point out as an industry there is a patent for that process. So, Dr. Platts-Mills had presented one of the ALK's papers with the picture of the sieve. That is

a patented process. So, if this is an expectation for all manufacturers, it just needs to be considered that there's not an infringement on the patent held by ALK, and that patent does cover from the source material to the drug product. And I do want to state as somebody who was involved in that process development, the manufacturing of the source material and the processing of the source material to get those defined ratios can be very complicated to achieve it consistently. Next slide. Oh, next slide. I am still seeing the current slide. Thank you.

So, in general, on standardization of new products, this refers to both the potential new ELISAs, the potential implementation of mass spec. We do support innovation and modernization of the allergen products in ways that benefit the patient. There are just some things we want to be aware of. Some of these have already been touched upon. There are some global standardization initiatives, specifically in Europe. They've recently standardized two products, the Phleum pratense and the Betula verrucosa. A lot of us are manufacturing products that are approved in markets in addition to the US, and some of those markets have their own standard standardization initiatives. We are concerned that if we start getting multiple standards created worldwide for the same allergen, it'll be very costly for us to demonstrate conformance to all of the standards, and in some cases it may not be possible because when you create these assays, the reagents are very important on what exactly they're detecting. So, we just want to keep that-- I mean, it sounds like everybody's aware, but just when we're doing that, we're doing the standardization initiatives, and it's very important to consider the global standardization initiatives as well.

We also want to talk about the cost considerations for the clinics and patients. So, standardized products are more costly to produce. And I wanted to touch on this. It's very true that the reagents are provided by the FDA and we really appreciate that, we do

transfer the methods, but we still have to do some sort of validation internally of these methods, specifically in our labs with our individual products, there is cost associated with that. If we add additional standardized products, then we're going to have to consider an increase in the number of analysts in our labs and quality personnel for reviewing and approving and releasing those products. And some of these initiatives say, for example, the fecal addition to the mite extracts, and even potentially looking at different ways to grow the molds, that might have to result in some process development activities to meet the standards, which will also contribute to the cost of the standardized extracts.

So, on the surface, it does look like just implementing a new method with reagents provided by the Agency. However, there's a lot of backend work that does need to be done by the manufacturers that will increase the cost of the standardized products that are essentially going to be handed over to the prescribers and the patients in the end. So, we did want to point that out. And with that, are the prescribers willing to accept these additional costs? And the cost benefit for the patient, has that been determined? I think it's come up several times. We're a little concerned on how these standards are being selected, especially with the mass spec and how it ties to clinical relevance. And that part there is-- If it's arbitrary, is it considering--? And I don't think it is fully arbitrary, but is it--? There needs to be a link for the patient and to benefit the patient if it's going to be implemented for all the manufacturers. And I think this last point was already addressed. We did have the question on if alternate methods would be accepted. For example, if we had already met a standard in the EU and we're using that method or had an internal method that could be demonstrated as appropriate, but they accept the method and it sounds like yes, they will. So we do appreciate that already being addressed. Next slide.

So, we do have a slide specifically on the foods. I'm going to comment again, a lot of this was-- We were concerned that these standards would be applied to all product lines. It's been clarified that for the newer technology, this will not be applied to, but I think we still want to emphasize that these new products-- We agree it should not be applied to the new products. They're all either currently backed, already backed by clinical trials or they're in clinical programs, so they're going to be standardized. There is-- Or, I guess, they won't be standardized, but they'll be backed by clinical data and they have a very high level of characterization.

So, we now understand this will only be applied to the current extracts, which are only diagnostics. So, I think that's important. Those are diagnostics, they're not the therapeutic treatments. For now, I guess, I think we aren't against standardizing for the foods, the diagnostics, considering the recent recalls and issues with that product type. Our main concern was also for the new products related to this slide. Next slide.

And then we just had some general considerations. This is going to be a lot of new methods. We're going to be applying it to products already on the market. And just-- I think you did touch on this as well, Dr. Rabin touched on some of this as well, just to make sure we're considering some of the-- We do have some guidelines for analytical procedure developments and lifecycle management and validation. We have some ICH guidelines, Q14 has some very nice guidance for when you're applying new methods or standards to current products.

So, just making sure that these will be considered as these are being developed. We do want to just make a note that to remember, not every product matrix will work with every standardized method. This was more directed towards some of the newer products. We have some manufacturers with experience when the EU introduced their new standardizations that didn't actually work with the matrix of certain products. And

then the last thing is to consider ring trials with manufacturers before implementation. I know we've been collaborating quite a bit on these and that we will be testing them, but doing actually a formal ring trial from a manufacturing perspective, we would prefer to see that so that we can also show it's working in our lab. Every time you send a new method out to a different lab, especially if it's a brand new method, you're adding additional variables. You have different people, you might have slightly different equipment, just to show that it's going to work everywhere. So, we would like to request ring trials before implementation. Next slide.

And then one of the other important things for us as manufacturers is there is an impact to our current BLAs. This will result in a lot of updates to our BLAs. We're going to have a lot of changes to labeling. We might have to have some labeling system changes. We'll have PI updates, SPL submissions, NDC/GTIN updates. This is a lot of updates. And depending on how many products this will apply to, I mean, it could be several, it could be quite a lot of work for us and for you as reviewers. So, we would like the Agency to consider a defined path for these changes that are directly resulted from the standardization initiatives to better utilize our time and your time and resources. Possibly it could be an expedited review period for prior approval supplements, for example. We also would like to request that if this is approved, that we are able to see a roadmap for implementation of these methods, and so what the framework will be and the interaction of industry for these methods. Next slide.

And just lastly, we do want to just remind everyone the APMA and CBER have been collaborating since we were incorporated in 1988. That timing is not a coincidence. This was essentially created, this association was created when the standardization program started for allergen products. And part of our purpose, if you look at the-- I'm not going to read all of them, but one of them is to develop and assist

in the development of industry standards. And we have been working with CBER for over 35 years. We worked on the original collaborations and we've had continuous collaboration with different initiatives. And we do want to say that we look forward to the continued collaboration with CBER on these new initiatives. And next slide. That was the conclusion of my presentation, and thank you.

Dr. El Sahly: Thank you, Ms. Repp, for enlightening us on the industry perspective here. I would like to inform the Committee members that we will break for 10 minutes now. There are questions for Ms. Repp, questions for Dr. Platts-Mills, with which we will begin the Q&A portion or the discussion portion of the meeting. And these questions would need to be pointed to the actual presentations and then it would be more of a general discussion. Right now it is 3:06 Eastern time, so let's reconvene at 3:16 Eastern time. I'm sorry-- Yes. Eastern time.

Question & Answer

Dr. El Sahly: Welcome back to the Question & Answer portion of the meeting. We will begin by specific questions to Dr. Platts-Mills and Ms. Trenna Repp pertaining to their respective presentations. So please use the raise your hand function in the zoom so we can begin the conversation. Dr. Eric Rubin?

Dr. Rubin: I have a question for Ms. Repp, actually, about the proposal to limit labeling to ranges rather than specific concentrations. That's not the way we label drugs that we-- Actually, we measure concentrations. If you take ibuprofen and it says 400 milligrams, you assume that there's not between 300 and 500 milligrams, it's just 400. And of course, if you were given a dose, what a manufacturer of a drug would do is it just the, you know, the concentration so that it fit whatever the requirement was? So, I'm curious what the justification is for not giving exact doses.

Ms. Repp: So, the justification is more around the variability in immunoassays, they can be very wide. The precision of the assays are not-- I think a lot of the assays can be around 25% variability. Also, currently the BAUs are measuring Fel d 1 units, which is in a way measuring Fel d 1, but we're not using the exact label, we're using a range. So, it's exactly how the BAUs are labeled now, is to use a range. And that is to consider-- At least as far as I understand, I was not here when it was created, but looking at some of the past discussions, it looks like it was selected that way because these methods have such a high range of variability.

Dr. Rubin: But the new methods we're talking about don't have that, they don't have that error, they're much more precise. LC/MS is extremely precise.

Ms. Repp: That's the LC/MS. This is specifically for the new ELISAs that we're speaking about for the cat and the ragweed.

Dr. Rubin: So, if you were to use LC/MS, would you feel like that would be-- It would make sense to give a precise measure?

Ms. Repp: I think the issue with the LC/MS is that it is measuring a presence of an allergen, not necessarily potency. So, the immunoassays are actually measuring what's binding to the antibodies. The mass spec is just saying, "Yeah, this protein is here," but we don't know. It could be degraded, it could not be active. So, for the mass spec, I think it's a little bit more difficult to say that this is the potency because it's being detected by mass spec. Does that make sense?

Dr. Rubin: Yeah, it makes some sense. I mean, that assumes, though, that the ELISA-- That the antibody binding and ELISA is a stand-in for the biological effect. No that's true-- There's evidence for that. You're using an artificial IgG or monoclonal to measure the amount, and that's not the same as the IgE that is mediating the allergic

effect. So, I'm not sure that that is any more predictive of response than the absolute value, the absolute concentration of protein. The skin test is different because it's a biological output, ELISA is just another way of measuring, though. Measuring the same thing.

Ms. Repp: Yeah, but there still is some binding and I know that there are cloned antibodies, but they're still based off of human sera. So, I think it's still more linked to biological response than a mass spec would be.

Dr. Rubin: Yeah. Can I just say something?

LCDR Reese: No, I'm sorry. We can only allow you to take questions and answer questions from the Committee members.

Dr. Rubin: I'm answering the question.

LCDR Reese: Oh, okay. If you want to answer--

Dr. Platts-Mills: Well, Dr. Rubin? Dr. Rubin, most of your molecules are small molecules which are absolutely ideally dealt with by mass spec and you've got a purity of product. We are dealing with measuring something in the middle of a very complex mixture of biological molecules, and that's different.

Dr. Rubin: I completely understand that. I take your point. But it seems like the solution to that is to measure more of the molecules than to just say, "It's about 300." The whole concept here is to produce a reproducible reagent and measuring something, or measuring two things, or measuring three things, seems better than saying, "It's roughly right."

Ms. Repp: But I guess the other side of that is if you're measuring the proteins in the mass spec, you're saying, "We're measuring it, they're precisely there," but what if

they've degraded and you're not detecting degradation? So, maybe there's actually no activity in the extract without the complementary immunoassay.

Dr. Rubin: My last point, and you're welcome to respond, but it seems like-- We want to get to better levels of precision. And I understand the limitations, these are crude reagents, but even for crude reagents, it seems like the more things you measure and the more precisely you measure them, the more you get to standardization. And that's the whole point, I think. But feel free to respond.

Ms. Repp: I think one of the, I guess, complications with that is currently, as you said, they're crude, I would say they're complex extracts from natural products. We don't know what the variations are. We don't know the differences between manufacturers. So, if we're talking about a mass spec standardization, we really need to understand where our products are now. At least for the previous products, I know there's a question of-- I mean, the current products, we don't know what kind of variation we currently have, what their compositions are. You know, the mites have been on the market for years, they've been shown to be safe and effective. Yes, there's probably some differences, clinically, but what that ties to in a mass spec is not clear. So, I do think in theory it sounds fantastic. I think when we start-- I think we need a lot more data before we can say it's an absolute way to determine potency.

Dr. Rubin: Thank you.

Dr. El Sahly: Dr. Portnoy, is your question specific about those two presentations or is it more about the general topic and the questions at hand?

Dr. Portnoy: I guess I basically just had a question about how standardized do we need these extracts to be? Allergists right now are currently using extracts and they're going to throw a fit when we change standard, the nomenclature and the standardization.

They're not going to know what to do. They're not going to like this at all. And I was really taken by the comment about other countries already having standards for their extracts, and this is a different standard perhaps, and I'd be curious to know what the standardization process is from the other countries and maybe we should just adopt one of those that have already been used and shown to be effective--

Dr. El Sahly: I'm going to take it that your question is more general and we will begin with this question. So, it has to do mostly with the overall exercise today, which is what is the thrust behind the standardization? Where do other countries stand and what can be adapted? So, this is a question really to Dr. Rabin and the FDA. Dr. Portnoy, you can stay so they can answer your question. I think we lost your audio and video.

Dr. Portnoy: So, do you want me to ask it again or just wait until--?

Dr. El Sahly: No, I think-- Let me see if Dr. Rabin can answer it.

Dr. Portnoy: Oh, okay.

Dr. Rabin: Yeah, I don't know. I don't know what's done in Europe other than what I explained to you, that I don't believe that the countries-- I don't believe that it's harmonized. But certainly we can find out, we are not looking to reinvent any wheels. And so if somebody already has something in place that works, that would be fine. But it was not my understanding, it is not my understanding that that's the case. And if things are manufacturer-specific, I can't think of a reason that a manufacturer would be interested in sharing their reagents on a large scale with the United States of America. So, I think it makes sense on the question, but I think implementing the answer is a lot more complex than you might imagine.

Dr. Portnoy: I can imagine.

Dr. El Sahly: Ms. Repp, are you trying to address the question from Dr. Portnoy?

Ms. Repp: Yes.

Dr. El Sahly: Okay. Please, go ahead.

Ms. Repp: Yeah, so I think that in my presentation I had mentioned there are standardization efforts across different markets. Specifically, I mentioned the EU. There has been a recent standardization for essentially two species, I'm going to say species, not products, because it's supplied to the products that contain those species. And that's Phleum pratense, and specifically a standard for Fel d 1. And Betula verrucosa has-- There is now a standard for Bet v 1 and they have been implemented as part of the European Pharmacopoeia. They are actually pharmacopoeial reference standards. And the manufacturers that have relevant products are now testing-- Using those standards to determine the Bet v 1 content using the same method and Fel d 1 content using this method for the relevant products.

And I believe that they are not being reported as the label strength, but still being either put on the label or in the insert as additional information. The product strength is still being reported as the strength determined by the individual companies because in Europe the standardization is done by the-- I mean, the strength is determined by the individual companies compared to an in-house reference that has a whole approval process through their registration process. So, I don't know if that answers your question, but our concern is if we have a Bet v 1 standard that we're testing to in Europe, for example, and we get a new standard in the US that is-- I like the idea of this standard that covers birch, oak and all of the related allergens, but having to test to both on the same product is [Indiscernible 07:01:45.] I hope that helped.

Dr. Platts-Mills: Can I say something about that?

Ms. Repp: Yes. Oh, sorry.

Dr. Platts-Mills: Yes. Yeah. What I tried to do in my talk a little was to point out that three companies that are active in Europe have taken the situation with dust mite and said, “We know how to measure Group I and Group II allergens, and can define a range that we think would be appropriate without being particularly strict about what the ratios are.” And then each of them has stated at some point that they’re actually looking at other allergens and know that they’re present. And that seems to me a rational approach to the dust mite at the moment. We’re not ready to define the quantity of Der p 23 in extracts, and it’s not clear that many allergists in practice would know what Der p 23 meant. So, I think that’s what I see as the way that the European market is likely to go. And if-- Dr. Repp, if you have a feeling that it’s different since you represent two companies that are really active in Europe--?

Dr. El Sahly: Okay. Question from Dr. Assa’ad.

Dr. Assa’ad: Yes. My question is for Dr. Repp. I do have a problem with a lot of things you said, but the major problem is your declaration that these products are safe and effective. Basically, the major impediment to us knowing safety and efficacy is that we don’t even know what’s in there. The safety is not a hundred percent. There’s been-- We don’t know the positive and negative predictive value of the skin tests, which we use these products for [Indiscernible 07:04:21.] And they’re not equivalent among different manufacturers and different products. The safety is not a hundred percent. There are lots of reports over the years of anaphylaxis to the allergen immunotherapy, the subcutaneous allergen immunotherapy. So, I’m not really sure about what you’re saying. That’s one comment or question.

The second one is, I mean, from the point of view of a manufacturer acquiring a machine, or a person to own that machine, or finding a way to get the assay done will be just the first step because a lot of these other allergens are going to hopefully also become standardized and the FDA will move on from one allergen to the next, hopefully. So, it's not like all the cost is going to go to dust mites if you have to acquire a machine or do something else. So, I'll be eager to see what you have to say.

Ms. Repp: So, thank you. Thank you for the comments. Regarding the safety and efficacy, I guess when I say that, I mean within the constraints of the licensing and the labeling. There are black box warnings on all of our products and that is to acknowledge, you know, there are-- I mean, there are risks with them, but when I say "safe and effective," I mean within the whole construct of the licensing approvals and applications and within our labeling and our package insert information. So, that's, I guess, what I meant by that. So, I guess I don't want to disregard there are some risks with our products. They're pretty well known, and you're very right, we do get adverse reaction reports and we monitor them. But those are, I think, pretty clearly discussed in the information across the products.

For the costs, yes, that is correct, there will be-- The initial investment for the equipment will be high, there is maintenance and continuous qualification of equipments that can also be costly. And depending on how many allergens we are going to have to implement and potentially release on, there is cost for validations. If we have to do process improvements as each allergens are introduced, that will still contribute to the cost and we will have to develop and validate methods for those maybe internally in addition to the ones that are provided by the Agency just to be able to assess our overall product. So, I think it's also hard to assess the actual cost for the implementation of the methods. I think you can either overly simplify it or you can overly complicate it. The

truth is probably somewhere in the middle, but there will be additional costs. I hope it was okay--

Dr. Assa'ad: I understand that, but isn't that the cost of doing business? Like, you are creating a product and it has to be measured one way or another. Your package inserts don't usually say how much there is. I mean, it goes by weight per volume in some things. Some things would do say the BAUs or the AUs, but that doesn't end up being anything that's of major use other than us trying to utilize guidelines of how to prescribe allergen immunotherapy for example, based on the previously standardized AUs and BAUs, but nothing-- The rest is weight per volume, which doesn't mean very much. And you've seen the data there that when they actually look at different products or different batches from the same product, they are not similar. They're not the same.

Ms. Repp: Yeah, and I fully acknowledge that they aren't the same. But, I guess, when it comes to the new standardizations is also-- I think Dr. Rabin said the same thing. There has to be a prioritization. I think we have over 200 species in the US, maybe more. I don't remember the exact number. And to create-- To characterize and define each and every one of those might not be a practical task, at least-- Unless we want to take 20, 30 years to do it. It's not that different in other markets. There are weight per volume products in Europe, for example. So, I do absolutely understand your point of view. We're not against standardizing more products, it's just we want it to be tied to something, like clinical relevance, just measuring an allergen, what is it tied to, I guess. We of course want our products to be potent. We do understand the issue, especially with the recent diagnostics of the foods that we would love to see resolved as well. So, it's acknowledged, but also understand from a manufacturing standpoint, there are a lot of practical aspects for us as well. But--

Dr. Assa'ad: But are you comfortable--?

Ms. Repp: I'm sorry?

Dr. Assa'ad: Are you comfortable, as a consumer, not as a representative of the manufacturing, that you might be given one day an allergy shot with a little of something, and then the next day you are given an allergy shot with a lot more, and the third day you're given it with much less? Because all of this is not equivalent and is not standardized, and you change from analogies to another, then you have to start from the beginning again. And it's all unknown. It's really unknown, and I think at the end of the day, it really damages the reputation of allergists and the practice of allergy in general.

Ms. Repp: Yeah, I would say that's a fair statement. I don't-- I'm presenting here on behalf of the industry, so I'm not the consumer representative, so I might have some personal response to that, but I'm going to avoid saying that just because that's not my purpose here today. The other-- I mean, there are some costs associated with it and also-- Yeah, we want to benefit the patient, so there does need to be a cost-benefit for the patient as well. And I don't know what that is because as you said, we don't know a lot about these extracts. I would say how currently they are kind of consistent within a manufacturer's through the process. We have a very consistent process, and these are biological products and sometimes there's things we can't control.

So, even if we did know the exact composition-- For example, I've done development within the source material world, when there's a drought, we will see differences in how the pollen itself looks and what it's expressing. So, we do also have to deal with nature. And so we are a little concerned of overcharacterizing a product in a way that we might even have shortages because of some of this natural variation and we won't be able to supply anything or any sort of treatment, which for us, we see better than no treatment.

Dr. Assa'ad: I understand that, but I mean, when you look at the cost for the patients, they're paying for something they don't know what it is. There is no measure of what's in there except for a few allergens. So, it's like, is it a gimmick that we are giving them the stuff we don't know what's in it? Or are we doing skin tests with stuff that we don't know how much the potency is? I mean, that is a real problem. And I think, I personally think that if the FDA has to move to try and standardize-- To start this standardization process on a sound basis is a good move. Anyway--

Committee Discussion, Recommendations, and Voting

Dr. El Sahly: I want to-- It's a great place to move on from this particular-- It's enlightening to hear both of you discuss this, but we've got to move to the four questions on hand. Cicely and AV, do you mind putting up the four questions we'll be deliberating?

LCDR Reese: Yes, here we go. Thank you.

Dr. El Sahly: Okay, so I'm going to go through the four questions. I'll be reading them. Please prepare your questions that you want to deliberate amongst the Committee members or by asking the FDA representatives in order to get informed voting, hopefully, around these four questions. Question one pertains to mass concentration measurements. Does measurement of mass concentrations by ELISA of their major allergens provide a scientifically sound approach for expressing and reporting potencies of cat hair and pelt allergen extracts, and of short ragweed pollen allergen extracts? Next questions. Where's question two? Or did it jump--?

LCDR Reese: That's question four.

Dr. El Sahly: Okay. Do the available data support inclusion of both house dust mite bodies and fecal pellets as source materials for house dust mite allergen extracts to more adequately mimic--?

LCDR Reese: We'll just go back, we'll do two and then go right through to four. I think they pulled up four by mistake.

Dr. El Sahly: Okay. Question two is CBER's Allergenic Standardization Program. Are the revised assays for cat hair/pelt and ragweed pollen allergen extracts scientifically appropriate templates for expanding CBER's allergenic standardization program to include major food allergens and environmental allergens? Next, does LC/MS/MS technology, compared with the currently used analytic technology, provide sufficient fit-for-purpose capability for better characterization of complex allergen extracts to improve product quality? Question four, do the available data support inclusion of both house dust mite bodies and fecal pellets as source material for the HDM allergen extracts to more adequately mimic clinically relevant allergen exposure?

So, I know there were at least a couple people with raised hands. Please, use the raised hand function again so we can begin discussing these questions and deliberating around them. I can begin by indicating a common thread that sort of went through these four questions. The uncertainty of the clinical output of the endeavor, definitely having more precise measures, more modern measures of extracting and purifying and measuring these allergens would be welcome and would diversify the tools we have. But there are many questions pertaining to the improvement in diagnostics and therapeutics that would come out as a result of this particular project. And in a sense, it opens the horizon for many research questions that can be answered by the scientific community to help enlighten a lot of the issues that were raised today. Dr. Davis?

Dr. Davis: Thank you. Yes, I just had some comments and one question. I do think that this is a step in the right direction, but agree with you that there has to be this connection with the outcome of the test and the clinical relevance. And that speaks to so much of the discussion today. I have actually two questions surrounding these questions. One is for Tom Platts-Mills, and that is the use of IgG4 to IgE ratio and if that might be one of these markers of clinical relevance. Just wondering since there was an outcome of an atopic disease, like asthma, to this particular test. It's not likely as functional as the BAT or the mast cell activation test, but could that be also another outcome besides the skin testing? The other question I have is for Dr. Repp. How easy there's a process to isolate the fecal pellets and also the bodies. How easy is it? I know something would have to be done, but it seemed to me like it might be fairly easy to just change the ratio and increase the fecal pellet protein amount. But I'd like to know just from a manufacturing standpoint how easy or difficult that might be. And then a corollary to that is how easy or difficult it might be to change the spore-to-hyphae ratio for mold production? I know it's complex [Indiscernible 07:20:23] question.

Dr. Platts-Mills: Can I answer-- Address the IgG4 question? Clearly, doing IgG4 assays, as far as we know, has to be done with immunoCAP and it's clearly not inexpensive. It certainly has a cost to it. Doing IgG4 on ISAC is tricky because these are all recombinant allergens on the ISAC and you get different answers. But I think IgG4 ratios is a very interesting research question. I don't see it as part of standardization at this point.

Dr. Davis: Okay. Thank you.

Ms. Repp: So, I will take the questions on how easy it is to change the mite processing and also the mold. So, for the mites, yeah, essentially it's a separation of particles by size. It's a size particle separation. It sounds simple. There are some

complications. The amount of allergens in the fecal pellets and in the mites themselves aren't consistent. There's actually quite a bit of variability from batch to batch. I'm not going to be able to share data and I'm not going to be able to share too much. But we do trend that, and we do see quite a bit of variability. So, if you essentially are purifying your mites to remove the food and then to a certain amount of-- Like, if you're trying to aim for a specification of the amount of fecal within the body extract to have a mix there and you just extract off of that, you're going to have some variability.

The idea of sieving or separating somehow the bodies from the fecal and then adding in a specific amount, that's where we're getting into maybe the patent from ALK, where they've gotten that. They have a process defined and described in that patent. So, there could be some issues there. I've worked with mites for quite a long time on a production commercial scale, and there's still a lot of things we don't know about their biology, but we're always amazed at how complicated it actually is. But it is doable. I mean, it's not impossible, but there's some complications with it. The molds-- Actually, the data on the mass spec was extremely interesting.

One of the first things I did in my career was grow mold, and you can-- You're essentially growing mold pelts. You're starting out with a small culture and then you're going to pelt. Probably-- We'd have to do some development on that, but probably you could stop it at a point of pelt development of where there's more of one type of spore versus a hyphae. Also, I was reading the briefing book. There's some allergens that are expressed into the media. I believe some of the source material removes the media, maybe some doesn't. So, there probably could be some adjustments made to include those, but it would be some development work. And when we're talking about multiple species, you do have to do that across all the species, and then it's development on the source material and then through the extract just to make sure that it's all getting

through to the final product. So, I don't know, does that answer your question or did I just make it more confusing?

Dr. Davis: No, that's fine. Thank you very much.

Dr. El Sahly: Dr. Dykewicz?

Dr. Dykewicz: Yes, thank you. This is a question of clarification for Dr. Repp. And in your presentation you said that one might consider ring trials before implementation. Could you elaborate on everything that would be involved with doing ring trials?

Ms. Repp: Sorry. Yeah, so the ring trials essentially would include a protocol that covers all manufacturers, like a universal protocol. You would send out the reagents from the test method to each manufacturer that would have a relevant product and essentially have them test it within their labs with their people to make sure that they're getting consistent results and expected results using that method with similar precision and repeatability across all the manufacturers and their products. Because there could be some differences in the product matrices that might be interfering with it that maybe wasn't seen in the development in a research lab, for example, but that you would see in a production environment, and then you would essentially take all of that data and evaluate it for the appropriateness of is it ready to be used as a standardized test for the industry or not.

Dr. Dykewicz: So, the point that you would have is that until everything had been really definitely clarified as being reproducible across manufacturers, that there would not be any regulatory requirement for such labeling?

Ms. Repp: Yes, I think that is essentially what we're proposing. We want to make sure it's appropriate for the intended use of the products that are currently on the market. You wouldn't want to release a standard that we find out after it's been implemented

that it doesn't work with one of our products and now we can't release it, for example, or at least work as intended.

Dr. Dykewicz: I mean, my other alternative thought of this would be that you would set some standardized requirements for release, but then as part of characterization, make the, if you will, the mandate that there be assessment of the content of these extracts using these other techniques. And after the fact study, if there were any issues with reproducibility or comparability, I'm kind of viewing the idea of requiring ring trials before implementation as obstruction to moving forward with admittedly the need that as we implement new standards, there have to be studies of the relevance and the applicability of these. Thank you.

Ms. Repp: Sorry, was there a specific question?

Dr. El Sahly: No, I think it was more of a comment.

Ms. Repp: Okay. I just wanted to make sure.

Dr. El Sahly: Dr. Greenberger?

Dr. Greenberger: Thank you. Thank you for the presentations. I have to say that I think when the new methodology should be applied, they should be applied to currently standardized products such as ragweed or cat and to see if the technology, say ELISAs, used in a modified way, could be useful to try to have some release criteria put into place. And I am viewing these questions sort of as compared to this current situation, how much research is needed to get there because I think it would be helpful to try to get the release criteria as a target. So, it's more of a statement than a question.

Dr. El Sahly: But I can take-- Because again, this is a recurring theme since the beginning of the talk, and I would like to ask Dr. Rabin and the colleagues within his

lab, are there plans to compare the standardized methods, modern methods with a particular clinical readout?

Dr. Rabin: Well, there weren't particularly for the ragweed and the cat because we were simply revising one assay to another assay. It might be-- I mean, we've had this discussion and I think I've said a number of times that, you know, I'm hearing that, and so I'm taking it under advisement. It might be useful, it might be a useful thing to try to do with cat and ragweed sort of as a precedent to see what comes out before we venture into the other uncharted territories. But it's not going to stop us from adopting the new assays for ragweed and cat. So, I think that's the best answer I can give you, given the fact that this is the first time that I've heard this argument being very vociferously and passionately made, and it's very rational. But obviously I'm not going to make any decisions about our strategy on the fly on the day of an Advisory Committee meeting. The Committee will have to trust that we're taking their advice very seriously and we know who to consult to move forward, and we'll do that.

Dr. El Sahly: Okay, thank you. Dr. Dykewicz, is that a new question or from before? Your hand was raised.

Dr. Dykewicz: Oh, sorry. I should have lowered my hand. Thank you.

Dr. El Sahly: All right. Dr. Portnoy?

Dr. Portnoy: Hello. So, as the consumer representative, I'm really concerned about the transition from the current status to the new standardized extracts. I think that's going to be a real talk of mess when allergists are told, "Here's the new extract, you have to start using it. It's going to cost more." It's going to be very confusing. So, that's just one comment that I have that gives me a lot of pause about this. On the other hand, I really

do think that we need to move forward into the 21st century with new technologies because the old way is really very outdated.

My question is, when we vote on these four issues, I assume that this is just giving the lab permission to study these as a way of possibly standardizing these extracts. I don't see any wording that says that it will then become mandatory that they must do this. And if it's something that I'm voting for that says, "This is how it's going to be and it's mandatory," I'm probably going to vote "No" on these things, because I don't think that the data's there and that it's ready for prime time. On the other hand, if we're giving the lab permission to move forward with studying these approaches to see if they would be a valid way of standardizing these extracts, and perhaps will the extracts be better when they're standardized, then I would be more inclined to vote "Yes." I'm just kind of asking for clarification on what exactly it is that I'm voting for.

Dr. Rabin: So, with regard to your first question, I would remind you that once upon a time, none of the extracts were standardized, and then a bunch of [Indiscernible 07:32:37] extracts were standardized and the medical community seemed to adopt to that standardization just fine. So I see it--

Dr. Portnoy: It was a chocolate mess, I remember it, but they eventually came around.

Dr. Rabin: Okay, well, then it'll be another chocolate mess and then we'll eventually come around or maybe we'll have learned-- Or maybe we can figure out with consultation with the quad AI [AAAAI] and the college as to how we can minimize the chocolate in the mess. But no, let me state very clearly that it is our intention to move forward with this, okay? Particularly with the foods, I really feel, and we really feel, I think I'm speaking for upper management here, that we've had some failures that have caused harm, and that as a regulatory agency that has the authority to take corrective

action so that this harm is not caused again, I think we would be remiss not to standardize these food allergens. I feel very strongly about that and I believe I have support from upper management on that. So, yeah, you're voting on action, okay? We didn't come-- Let me make it very clear, sir. We did not come to the Advisory Committee to ask you if we can think about doing something. We came to the Advisory Committee to ask you that-- We intend to tell you that we intend to do something and to seek concurrence on that.

Dr. Portnoy: Okay. Thank you,

Dr. El Sahly: Dr. Monto?

Dr. Monto: This is very far away from my area of expertise, but I heard something which Dr. Rabin can give a specific answer to, and that is the issue of material in the mass spec assay being denatured and not active, which will show up in that assay. And is that the case?

Dr. Rabin: Well, I'm going to let Dr. Strader answer that and then if there's anything he left out, I'll--

Dr. Strader: Yeah, so first of all, this is a good time for me to make a clarification to an earlier comment. You can calculate absolute concentration using mass spectrometry. You absolutely can. You can also calculate relative abundances, the targeted approach, the PRM method that we're describing, would be the next step where you would calculate absolute concentration and therefore potency. That's one thing. Another thing too is there is this possibility that the peptide that gives you the quant doesn't tell you anything about the intact form. And we realize that that's why you would do this orthogonally with methods where you look for the epitope.

Dr. Monto: Yeah, yeah.

Dr. Strader: Yeah, so I mean, we're not saying replace. We're saying, "Use this new 21st century technology orthogonally to better characterize your potency."

Dr. Rabin: Obviously if there's a drawback to a particular technology and there's a way to address that drawback, then we would do that.

Dr. Strader: And one more thing. There's a cost to it, but remember, you can measure multiple allergens in an assay using the single data acquisition. Once you know what your allergens are, you can find your peptides, provided you do that, right? You can calculate the quantity of several allergens in one assay.

Dr. Rabin: Okay. I think we've answered that.

Dr. Strader: Yeah, I think we have.

Dr. Monto: I'm very sympathetic to the issue of when you start having false positives to a peanut allergy that you've got to do something.

Dr. Strader: Yeah. Thank you.

Dr. Monto: False negatives. Excuse me.

Dr. Platts-Mills: Can I say something?

Dr. El Sahly: Dr. Platts-Mills, unfortunately, you can only answer particular questions to your presentation. That's the rule here. Dr. Greenberger, did you have a question or comment?

Dr. Greenberger: My comment is that we have to keep the safety of the patient, and stability in the physician office is very important for all of us to keep in mind when we make changes. I've seen modifications in what we bought as extracts when there have been shortages, but that was with venom and we had standardized product to make the

change. And that went, at least in Northwestern, I can speak of my own patients, it went smoothly. But then we had standardized extract measured on the two enzymes that are used. So, that's why I've talked earlier about using-- Having assays to make sure we have standardized products that we can agree on. And I think the changes can be made with the help of our major organizations. But I still seem to think we would need the data before we can make any major changes. So, I asked that earlier of Dr. Rabin about moving forward with research.

Dr. Rabin: Yeah, of course. I mean, I would remind you that we made it very clear in that part of-- We showed you that whole template. You might remember, it was a number of hours ago now, but it was on an arrow. And on the arrow we had the different stages. And one of the stages is, "We publish the data before the assay is adopted." So yes, of course you would see the data.

Dr. Greenberger: Great. Great.

Dr. El Sahly: Dr. Portnoy, you have another question?

Dr. Portnoy: Actually, I just have a question for Dr. Platts-Mills and that is what were you going to tell us that you thought was really important? I'd like to hear your answer, and since you can't ask a question, I'm asking you to answer it, please. Hope you're muted.

Dr. Platts-Mills: I apologize. Dr. Rabin brought up the issue of the peanut situation where a peanut extract appeared on the market that had very little peanut in it, and that would've been solved if there had been an assay of Ara h 1 and Ara h 2 in that extract, it would've been picked up immediately. And you didn't need to have assays of 8, 9, 10. You just-- Those two major allergens would've certainly prevented that problem.

Dr. Portnoy: And by the way, I'm concerned about both false positive and false negative skin tests with peanut because if somebody's not allergic to peanut, but the extracts are so strong that it elicits a non-specific response that could be just as harmful.

Dr. Rabin: Yes, we're aware of that. We're aware of that. In my conversations with Dr. Lack, we discussed that and yeah, that's right. I mean, it makes it no different than any other pharmaceutical. There's a range, there's a dosage range in which is optimal.

Dr. El Sahly: Dr. Assa'ad?

Dr. Assa'ad: Yes, I have a question for Dr. Rabin. Your last slide, or one of your last slides in the first presentation showed that the assay is still not-- Or you said that it wasn't tight enough. Did I understand correctly that you're still working on it?

Dr. Rabin: The assay, the Fel d 1 assay, as we published it, was not tight enough for regulatory purposes. We are improving it. We've improved it already. We've been improving it. We tightened it up. I showed you some preliminary data showing-- And we basically tightened up the CV from 25% to 6% or something like that. Preliminarily. I mean, we've tightened it up a great deal.

Dr. Assa'ad: Okay. Very good.

Dr. Rabin: And we're not done, okay-

Dr. Assa'ad: -Okay, great. Yes-

Dr. Rabin: -You know, obviously-- I mean, that is why I spent those four slides speaking about the steps that we have to go through for validation. We take that very seriously.

Dr. Assa'ad: Very nice, thank you. The question I have though is after 20 years of doing this, or 30 years, and thinking a lot about it, is what you're proposing as doing the

ELISA and also maybe with or without-- I'm not clear where the ELISA will stand versus the mass spectrometry. Are these the best options, and if at some point they do not work like any grant or any proposal, what's the alternative?

Dr. Rabin: I guess I haven't given that much thought. I guess I have confidence in ELISAs and I have confidence in the technology, but if there are alternatives, I'm sure they're not secrets and I'm sure we can find them.

Dr. El Sahly: Okay. Thank you. Dr. Carla Davis?

Dr. Davis: Thank you. I wanted to make a couple of comments and maybe a question at the end. So, one comment that I would like to address is the concept that better-- My major and minor allergen detection is going to be better, I believe, for characterization, but in having some standards for release or [Indiscernible 07:44:10] standardization, we've talked a lot about using one major allergen or two of the major allergens. I just want to bring up the potential downside of having too many allergens for standardization or release that would cause batches to be discarded. And in the case of peanut, if there are too many batches that do not have all of the required stent allergens, right? We want to avoid-- So, I really do favor this concept of a simplified release, but then characterization on the back--

Dr. Rabin: I hear you. Yeah, we get that. I include that in my comments about perfect being the enemy of the good, we have to do what works, okay? So yeah, we're not going to ask people to do things that are unrealistic or that cause you to throw out a lot of lots because then you end up with shortages because the company-- You know. So, we don't want to shoot ourselves in the foot.

Dr. Davis: And that just hadn't been discussed. I wanted to make sure it was. The other comment I would say, or clarification, would be this word "potency." So, the

potency could be utilized as concentration, which means presence or more presence, but it doesn't necessarily indicate function. And so, I think we've clarified this, but I just wanted to find out if you agree with using the word potency for a concentration of a protein, which does indicate presence but not necessarily function or clinical relevance.

Dr. Rabin: I think potency sort of means strength in a regulatory sense. And in terms of unmodified allergens, it is implicit that potency is tied to biological activity. That is the ability of the intact molecule to bind to IgE, which is of course the bottom line of what allergic disease is about. So in that context, it is, and sometimes as is the case with the Amb a-- You know, with a lot of-- I mean, the potency of the venoms is the enzymatic activity of two venom allergens and the enzymatic activity may have something to do with the allergic response. We know that enzymes can do that, but that's obviously a proxy for an intact molecule that IgE can recognize. So, that's sort of how we think of it with a broad brush. Does that answer your question?

Dr. Davis: Yeah, so it's a proxy. I just wanted to clarify that it is a proxy.

Dr. Rabin: Yeah, sometimes it's going to be a proxy. Yeah. Because, well, because we can't do ID50s on everybody and we are not going to ask the manufacturers to do basophil activation tests and so on. I mean, we have to-- For your first comment, you have to do what's realistic and you have to--

Dr. Davis: Yeah. No, that sounds great. And I do agree that the major allergy organizations would be key in really making a smooth implementation. Thank you so much.

Dr. Rabin: Absolutely. You're very welcome.

Dr. El Sahly: Can you hear me okay? I have a minor question that I asked earlier, and this would be addressed to Dr. Rabin and Dr. Platts-Mills. Is the inclusion of a defined

component from the fecal pellet going to improve on our ability to either manufacture the extract, diagnose the allergy, or treat the allergy?

Dr. Rabin: It is our belief. I can't speak to manufacturing because I don't do that, but it is most definitely our belief that it will improve the reagent as a diagnostic and a therapeutic reagent.

Dr. Platts-Mills: Yeah, I would add that I think that unequivocally the dust mite fecal pellets are the thing that we really know becomes airborne and is most likely to become inhaled, and it retains large quantities of Der p 1, and that marketing and extract that is dominantly Der p 2 and has much less Der p 1 seems almost certainly wrong and that it is better to have a mixture of Der p 1 and Der p 2. The reason for not going further than that is the practical reasons that many people have proposed, and just the inevitability that allergists are not going to be aware of all those things. The amount of immunology that allergists are now expected to learn is overwhelming, and asking them to know ten different allergens for every source is absolutely unreasonable.

Dr. El Sahly: Thank you.

Dr. Platts-Mills: Not sure that answered the question.

Dr. El Sahly: Okay. So I see there are no more raised hands, so we're going to go through the questions for this afternoon. Cicely, do you want to put up the slides for the questions?

LCDR Reese: Yeah, so we should run through those questions one more time like we did before. Just bring them up one by one and have you, Dr. El Sahly, read each one.

Dr. El Sahly: Okay. Question one, Mass Concentration Measurements. Does measurement of mass concentrations by ELISA of their major allergens provide a

scientifically sound approach for expressing and reporting potencies of cat hair and pelt allergen extracts, and of short ragweed pollen allergen extracts? Question two, are the revised assays for cat hair/pelt and ragweed pollen allergen extract scientifically appropriate templates for expanding CBER's allergenic standardization program to include major food allergens and environmental allergens? Question three, does LC/MS/MS technology compared with the currently used analytic technology, provide sufficient fit-for-purpose analytic capability for better characterization of complex allergen extracts to improve product quality? And question four, do the available data support inclusion of both house dust mite bodies and fecal pellets as source materials for the house dust mite allergen extracts to more adequately mimic clinically relevant allergen exposure?

LCDR Reese: Thank you, Dr. El Sahly. So, because we had the general discussion of the four questions at the same time, we're going to hold voting of the four questions in one Voting Session. So, I'm going to give the instructions for voting and then we'll move into the breakout room while-- Those Non-voting Members will be moved into the breakout room while we conduct the vote. Excuse me. So, a voting window will appear. This is for the members where you can submit your vote. There will be no discussion during the vote session. You should select the button in the window that corresponds to your vote, which will be "yes, no, or abstain." Please note that once you click the submit button, you will not be able to change your vote. Once all Voting Members have selected their vote, I will announce that the voting is closed. Please note there will be a momentary pause and it may be a little longer than usual as we tally the votes results and return the Non-voting Members into the meeting room. Next, the vote results will be displayed on the screen. The four vote results will be displayed on the screen, and I will read the results from each one as Dr. El Sahly handles questions one,

two, three, and four in sequential order. I will start out by reading the poll results and she will go into the round robin with rationale from each member for the vote. So, are there any questions before we begin? Okay, I'm not seeing any questions. All right, so we'll go into voting. Thank you.

Okay. Voting has closed and is now complete for the four questions. The voting results will be displayed. Okay.

Dr. El Sahly: What are we--? We're seeing question one now. Looks like two questions.

LCDR Reese: Yeah. Okay. This is question one. So, for question one, we have 12 "Yeses" and one "Abstain." And Dr. El Sahly, you can-- The question was, does measurement of mass concentration by ELISA of their major allergens provide a scientifically sound approach for-- Can we move that? Let me pull it up myself. Let's see. Question one was, does measurement of mass concentrations by ELISA of their major allergens provide a scientifically sound approach for expressing and reporting potencies of cat hair and pelt allergen extracts, and of short ragweed pollen allergen extracts? And so we had 13 yeses and one abstain. Thank you.

Dr. El Sahly: So, what we're going to do now is ask each member to give a rationale for their voting, beginning with Dr. Greenberger.

Dr. Greenberger: I voted "Yes" because it is scientifically sound to use this approach and gather the data. And I would urge the priority of the FDA to focus on cat and ragweed in the beginning.

Dr. El Sahly: Okay. Thank you. Dr. Monto?

Dr. Monto: I agree. I think that ELISA is now and has been for a number of years a standard and relying on something like radial diffusion is really not as accurate or precise. So, I voted "Yes."

Dr. El Sahly: Thank you. Dr. Meyer?

CAPT Meyer: Hello. So, I voted to abstain on this question as well as subsequent questions. I felt like there was great evidence presented about the need for standardization, the need for strengthening the standardization program, for modernizing it. I thought all of the evidence supported voting "Yes" for this question, but I felt like the questions, they're very technical, requiring a certain area of expertise. I think allergy is a new area to the VRBPAC Committee, and I did not feel in a position to vote one way or the other today.

Dr. El Sahly: Thank you. Dr. Perlman?

Dr. Perlman: Yes. I thought that changing to an ELISA was an improvement. I think just looking at that radial diffusion apparatus, I don't think there's really a choice because that looks like it [Indiscernible 08:08:05.]

Dr. El Sahly: Thank you. Dr. Davis?

Dr. Davis: I also feel that the ELISA is a standard and the current way of measuring was really antiquated, and so I'm glad that FDA is moving forward. I believe starting with the major allergens, especially those who've been very well characterized, is a great approach.

Dr. El Sahly: Dr. Assa'ad?

Dr. Assa'ad: Yes. I voted "Yes" because I have studied allergy back in the 80s, and radioimmunodiffusion was the thing to be done back then. So, it's pretty antiquated, and

Dr. Rabin has presented very convincing evidence of the work with ELISA being scientifically sound and productive.

Dr. El Sahly: Thank you. Dr. Dykewicz?

Dr. Dykewicz: I also voted "Yes." I think ELISA is a very well established procedure. Radial immunodeficiency or diffusion rather is-- Well, it's antiquated. I think, again, the data that was presented by the FDA by Dr. Rabin was compelling. It was demonstrating consistency of results, and I think it is going to be ready for prime time. It should be a standard.

Dr. El Sahly: Thank you. I also voted "Yes." Modernizing the methods of measuring the specific allergen is a good start as presented by the FDA labs and especially when paired with their willingness to take some of the advisement given during the meeting today. Dr. Rubin?

Dr. Rubin: I'm going to answer for actually all the questions, because I think that measuring things better and not by 1970s methods is always good. We should know what's in these things. I hear the concerns brought up by some that we don't know what to do with the information, and I think that's fair. We don't know about-- We don't know how well the measurement of the major allergen is going to predict the biological effect that you want to either measure or get, and that's fair, but you should start with good measurements before you even worry about that.

Dr. El Sahly: Thank you. Dr. Durbin?

Dr. Durbin: Thank you. I voted "Yes." I don't have a lot to add to what others have said, just to say that I thought the data were very sound and scientifically supported the move. Thank you.

Dr. El Sahly: Dr. Portnoy?

Dr. Portnoy: I also voted "Yes," and it's about time that we moved allergen standardization into the 21st century. I've used ELISA in the lab for many years. It's a very well established technique that's widely used in healthcare. When I get labs drawn, a lot of times the tests that are done are done by ELISA, so it's well established. There's no reason why in allergy we can't use this proven technique.

Dr. El Sahly: Dr. Omer?

Dr. Omer: So, I voted "Yes" as well, and I think it's high time we moved from RID-based assays to something more advanced or relatively more contemporary. So, I think it makes a lot of sense to do that. The case was made pretty convincingly. But I will note something that is relevant to both question one and two, which is that it would be helpful not only to present biological evidence in these kinds of deliberation, especially when it's a new approach being recommended not only biological evidence, but also quantitative evidence supporting it or the approach that is proposed. So, as I noted in my question as well-- And it doesn't have to be any extensive detail of statistical methods, but at least either in the background material, that may suffice in a lot of cases, if not in the presentation. So, the full range of science should be presented in these kinds of situations. It may or may not be relevant to other situations, but I felt for both question one and two, that was something that would have been appropriate to have been included.

Dr. El Sahly: Okay. Thank you. Dr. Bernstein?

Dr. Bernstein: Yes. I voted "Yes" as well. A lot's already been said. The need for standardization is there and this would be a notable improvement, so that's why I voted "Yes."

Dr. El Sahly: Thank you. So, to summarize, the first question got 12 "Yes" and one "Abstained." So, mostly "Yes" precisely because everyone agreed that modernization of allergen extract measurement is needed and adopting newer methods would be welcome. The abstention had to do mostly with not feeling comfortable with the expertise around this question that is highly specific. Of note, the need to provide additional statistical analysis around the assay performance was a noted deficiency that should be provided with additional or with subsequent VRBPAC or questions revolving around assay development. Next?

LCDR Reese: Okay, we can move to question two results. Okay. So, we had 11 yeses, one no, and one abstain for the record. And the question that people responded to was, are the revised assays for cat hair/pelt and ragweed pollen allergen extract scientifically appropriate templates for expanding CBER's allergenic standardization program to include major food allergens and environmental allergens?

Dr. El Sahly: Okay, great. Dr. Greenberger?

Dr. Greenberger: I voted "Yes." Similar thinking as question one, and I would feel that there should be input in interactions with industry along with interested academics. Regarding environmental allergens, I've seen how, with my own eyes, how modifications in how Aspergillus fumigatus is cultured either cause lack of reactivity, IgE binding or not, and lack of T-cell stimulation or not. So, there needs to be a lot of input with interested parties.

Dr. El Sahly: Thank you. Dr. Monto?

Dr. Monto: I'm in favor of expanding the standardization. Many things that are of importance from what we've heard are not well standardized, and that's the reason I voted "Yes."

Dr. El Sahly: Dr. Meyer, I know you indicated that your answer--

CAPT Meyer: Yes. It's a consistent reason across all of my abstention votes.

Dr. El Sahly: Understood. Dr. Perlman?

Dr. Perlman: Yes. So, I voted "Yes" for the reasons that have been mentioned already. I think that expanding the program to include food allergens and environmental allergens is a good idea.

Dr. El Sahly: Dr. Assa'ad?

Dr. Assa'ad: Yes, I voted "Yes" because there is a need to extend the more modern standardization to major food allergens and environmental allergens. Still, with two issues that I would like to see addressed. When we say major food allergens, does it include some of the components or just the general overall food allergy? And also encouraging to move on to clinical significance by various means.

Dr. El Sahly: Thank you. Dr. Davis?

Dr. Davis: I voted "Yes" because although these assays will start with cat hair, pellet, and ragweed, there are 19 standardized allergens. And then there are also many, many more. And I would hate for there to be any delay in actually using these for the many allergens that are clinically relevant. I agree that there needs to be academic involvement as well as clinical relevance, and I would say some clinical correlation when these assays are moved to other food and environmental allergens. But this is a great step, and I support expansion.

Dr. El Sahly: Thank you, Dr. Dykewicz?

Dr. Dykewicz: Well, of course I echo the comments made by my proceeding colleagues. I think the need to move forward, particularly for-- As an example, the peanut allergen

characterization, standardization, that's one of the most compelling issues that I think the Committee has been presented with. And I think moving forward with characterizing the peanut extract and other food extracts is really essential to move forward with assuring clinical reliability of extract. So, very much in favor of this.

Dr. El Sahly: Thank you. I voted "Yes." I wanted to vote "Yes, but…" However, there is no such option. The issue here is that from moving from question one to question two, I think that particular space is where the issue of clinical utility and implementation questions would need to be addressed before expanding to the full on portfolio of allergens, at least beginning that work. That was not an option as a vote, but I voted "Yes" with this particular caveat. I do know that the division of allergy and immunology and transplant within the NIH is always updating their priorities of research, just like any other division, and I think the discussion we've had today and the direction with which the FDA is going sort of opens the door for a full on research program to partner on. Dr. Rubin?

Dr. Rubin: I agree entirely with what you said. There is no reason we shouldn't better characterize what we're putting into people, but what you do with the information you get, I think is a question. So, we should measure well, and then we should figure out what we do with those measurements.

Dr. El Sahly: Yeah, it's just that I would hate that we characterized all 19 and then realized, "But could have found out earlier that something needs to be tweaked?" But yeah. Dr. Durbin?

Dr. Durbin: Yeah, I voted "Yes," and I echo my colleagues' comments. And just to say that I think it's critically important the standardization for these allergens, including

food allergens, so that we don't mischaracterize people, which could lead to serious consequences. Thank you.

Dr. El Sahly: Dr. Portnoy?

Dr. Portnoy: And I also voted "Yes." If it works for cat and for ragweed, it probably will work for all of the other allergens. We might as well get it done, but as every medical paper ends with the following statement, "Further research is needed." Thank you.

Dr. El Sahly: Dr. Omer.

Dr. Omer: I voted "Yes," and I don't have anything additional to add beyond what my colleagues have stated.

Dr. El Sahly: Thank you. And Dr. Bernstein?

Dr. Bernstein: Yes. So, I voted "No." I was-- Like you, Dr. El Sahly, I was teetering. It ended up being 51-49 in my mind. I think the expansion is appropriate and important, but I had concern for the possible degree of imprecision and how that would translate into clinical relevance. So, that's why I said no.

Dr. El Sahly: And I will raise the issue pertaining here to what you just mentioned is that when we vote sometimes on vaccine and there are uncertainties, there are always post-approval research requirements versus commitments. And I think here something along those lines is maybe adequate. So, to summarize, this particular question received 11 "Yes," one "Abstain" and one "No." The Committee members wanted at this stage of the standardization project within the FDA to see more industry and academia engagement. The issue of food allergy, especially peanuts, is a pressing question for which standardization of product and linking to clinical relevance is needed. And then

the issue of partnering with other organizations to set the research priority as these expansions or these expansions take place. Next?

LCDR Reese: Thank you. Moving on to the third question posed to the Committee. Does LC/MS/MS technology compared with the currently used analytic technology, provide sufficient fit-for-purpose analytic capability for better characterization of complex allergen extracts to improve product quality? Okay, we have eight--

Dr. El Sahly: Ok, so--

LCDR Reese: Oh, sorry.

Dr. El Sahly: No, go ahead.

LCDR Reese: Just for the record. Eight "Yes," three "Abstain" and one "No."

Dr. El Sahly: Two "No."

LCDR Reese: Two "No." Oh, two "No," sorry. Two nos. Eight "Yes," two "Nos" and three "Abstain." Thank you.

Dr. El Sahly: Dr. Greenberger?

Dr. Greenberger: I voted "No" because in reading the question, I looked at sufficient fit-for-purpose, which I asked Dr. Rabin about, and I don't think I have information that I can confidently answer the question positively. I'd like to come back in a year and be asked the same question and see some data and then vote "Yes."

Dr. El Sahly: Dr. Monto?

Dr. Monto: I voted "Yes" because of the response to my question about mass spectrometry that a number of different procedures will be used and there will not be total reliance on one if there are any questions.

Dr. El Sahly: Okay. Thank you. Dr. Meyer?

CAPT Meyer: Nothing further to add from my prior--

Dr. El Sahly: Okay. Thank you. Dr. Perlman?

Dr. Perlman: Yeah, so I abstained on this one because I thought that using LC/MS/MS-- I could step forward, but I wasn't convinced that the data was going to be processed appropriately or not appropriately, but we were going to be able to use it in a meaningful way. So, that's why I just ended up at upstate.

Dr. El Sahly: Thank you. Dr. Assa'ad?

Dr. Assa'ad: Yes. I voted "Yes" because of, well, the data presented as well as the issue of complex allergen extracts because many of the allogenic extracts are complex, and the ELISA by itself, as it was proposed to just address the major allergens, is not going to solve the issue of the complexity of the allogenic extracts. And I heard that the LC/MS/MS will solve that question.

Dr. El Sahly: Dr. Davis?

Dr. Davis: Yes, I voted "Yes." I do have some LC/MS/MS experience in research, and I was pretty confident that there is a way to use that technology to identify specific allergens and specific major and minor allergens in an extract. And I felt that the data presented was adequate to say that yes, this could be sufficient for characterization purposes, and as long as it is connected with some other simpler test that would be utilized for release rather than this particular one. But I do believe it is important to characterize all of the allergens, and I don't think ELISA or the other methods would adequately do that, but this would be effective, that's why I voted "Yes."

Dr. El Sahly: Okay. Thank you. Dr. Dykewicz?

Dr. Dykewicz: I voted "Yes," and to echo Dr. Assa'ad's comments, I think particularly when we're looking at trying to characterize complex allergen extracts that have many different components, some which may be relevant to some may not. But in terms of best characterizing complex allergen extracts, I think this approach is important to use. Now, I am mindful in terms of the caveat that the statements were made that as part of the preparation process of samples, that could be denaturation of materials that wouldn't necessarily equate when they were detected with allergenic importance. But overall, I think the evidence is quite sufficient to move forward with this.

Dr. El Sahly: Okay, thank you. I voted "Abstain" because the data presented did not sort of lend itself to a fit-for-purpose, assuming the purpose is improving diagnostics and therapeutic approaches. I think it still is very intriguing and worth researching, but probably not enough data to convince that it is ready for prime time and wide usage as proposed. Dr. Rubin?

Dr. Rubin: I'd say that the immunodiffusion tests are 1970s or 1960s, and the ELISA is 1970s, and this is 2020s now technology, it's something we use in the lab all the time, including LC/MS and PRM, and it has the ability to measure very, very precisely many, many things simultaneously. So, especially for complex mixtures, this is the way to go. So, technically it's the right test. I think that the question that Dr. Monto asked is a good one, though. For conformationally specific allergens, it will not be able to detect the difference because the proteins have to be natured to and digested before they're put onto the mass spec. So, we will not identify those, and other methods will have to be used if we know about that issue.

Dr. El Sahly: Dr. Durbin?

Dr. Durbin: Yeah, I voted "Yes" because I think the data did support this, particularly for complex antigens, mass spec is known to have-- As Dr. Rubin said, it's incredibly precise and useful for that, so I think that it really is the needed step forward. Thank you,

Dr. El Sahly: Dr. Portnoy?

Dr. Portnoy: Actually, I voted "No." ELISA is a great way of measuring allergens. Mass spec is a fantastic tool for characterizing complex allergens. However, I'm not sure that that's really something that's needed for standardization of extracts. A lot of the companies, I was told, don't necessarily have it and if this is approved, this could become a required thing. These companies are going to have to do it, and what are they going to do with the results? I just haven't seen any information that suggests that knowing what's in the extract is going to improve the quality of the extract. So, I think it's a great research tool, but it's not quite ready for something to be mandated that extract companies must do it for their extracts. And so for that reason, I voted "No."

Dr. El Sahly: Okay. Dr. Omer?

Dr. Omer: Yeah. So I voted "Yes" for the reason that it's a scientifically sound and validated approach, and more precise than legacy assays, but more importantly, it's capable of detecting clinically meaningful compositional differences, and it is directly applicable to regulatory lot release testing. So, that's a practical consideration there. And it is essential for producing allergen extracts that are not only accurate, potent, but also are consistent with modern analytical standards. And so it is for both specific reasons and big picture reasons that I voted "Yes" on this question.

Dr. El Sahly: Okay. Thank you. And Dr. Bernstein?

Dr. Bernstein: Yes. So I voted "Yes." Certainly the precision is excellent. I was a bit concerned about the expense of using it, but that didn't deter me from voting "Yes."

Dr. El Sahly: Right. Thank you all. So, in summary, on this particular topic, the Committee had a bit more questions. Nonetheless, the vote passed, we should say. There were-- The pros of the approach, of course, is it's modern, novel, precise, can detect smaller components, which is useful for complex allergens and maybe helpful for lot release. On the flip side of it, the issue of expense, whether or not it'll be mandated, and if so, is it needed? Which sort of leads to the question, is it really fit-for-purpose or proven to be fit-for-purpose? Because it might be, it's just that we didn't see the data that it is. The issue of denaturing the proteins that will be measured was brought up earlier in the discussion today, and again now, which is a concern with the use of this technology. So, the older technologies or complementary technologies will continue to be used for complex allergens because of that. Next question?

LCDR Reese: Thank you. Question four, do the available data support inclusion of both house dust mite bodies and fecal pellets as source materials for the HDM allergen extracts to more adequately mimic clinically relevant allergen exposure? We have 11 "Yes," one "No," and one "Abstained."

Dr. El Sahly: Dr. Greenberger?

Dr. Greenberger: I voted "Yes." And as I've said, you don't have a good skin testing reagent or in vitro assay without good starting material. And to get clinically relevant information, you need the relevant allergens presence. So I voted "Yes."

Dr. El Sahly: Dr. Monto?

Dr. Monto: I think this was a relatively easy one because from what we've heard, the fecal component is really maybe the most important and should be included.

Dr. El Sahly: Dr. Meyer?

CAPT Meyer: No further input from a prior response.

Dr. El Sahly: Dr. Perlman?

Dr. Perlman: Yeah. I also thought that the data showing that the fecal pellets and much of the allergen was super compelling. And for me, it was an easy "Yes" vote.

Dr. El Sahly: Dr. Assa'ad.

Dr. Assa'ad: Yes, I voted "Yes" because Dr. Platts-Mills has ingrained in us that it's the dust mite feces that has the allergen, so it doesn't make any sense to not have the dust mite feces in the dust mite extract.

Dr. El Sahly: Okay. Dr. Davis?

Dr. Davis: Yes, it is textbook. The major allergen is in the feces. And so the data were compelling and it did not seem as if it would be that difficult to make it more prominent in the extract. So I said "Yes."

Dr. El Sahly: Thank you, Dr. Dykewicz?

Dr. Dykewicz: Yes. Again, I think this was an easy question. The fecal particles are very important as the allergenic source and should be included.

Dr. El Sahly: I voted "Yes," in agreement with the data we saw today and the pointed questions I asked the experts also, which affirmed that this should be the way to go. Dr. Rubin?

Dr. Rubin: I'm just glad we got to talk about real feces this time. And I agree with everyone else.

Dr. El Sahly: Dr. Durbin?

Dr. Durbin: Yes, I voted "Yes" for the reasons others have already stated. I think it was well justified. Thank you.

Dr. El Sahly: Dr. Portnoy?

Dr. Portnoy: Yeah, we saw data suggesting that we should include it. So, I voted "Yes" because the data supports that.

Dr. El Sahly: Okay. Dr. Omer?

Dr. Omer: Yeah, I voted "Yes" based on the data presented. Nothing further to add.

Dr. El Sahly: Thank you. Dr. Bernstein?

Dr. Bernstein: So, I voted "No." This was a close one for me. Again, I thought it more adequately mimicked relevance, but I was concerned again about the-- I wondered its clinical precision. So I voted "No."

Dr. El Sahly: Thank you. So, in summary, the Committee voted mostly "Yes" on this because the allergen-- The allergic components are more represented in the feces than the body, although they do exist in the body as well of the mite, and potentially including the fecal pellet will improve the utility of these extracts. However, I think it sort of goes back to the main point that and main thread we've had today that needs to be confirmed in epidemiologic or other research design studies. I think we answered all four questions.

LCDR Reese: Yes. And if we can invite Dr. Kaslow for some closing remarks.

Dr. El Sahly: I have a question to Dr. Kaslow. We still use RID for flu.

Dr. Kaslow: Yes, we [Indiscernible 08:40:30.]

Dr. El Sahly: We kept talking about how--

Dr. Kaslow: Yes, we know. We can talk about that maybe at a future VRBPAC meeting in general. So, thank you all--

Dr. Monto: I was thinking about that same thing.

Dr. El Sahly: I know.

Dr. Kaslow: All right. All right. So then on behalf of OVRR, I'll start by first thanking Dr. Weir and Kondor. If you remember their presentation from earlier today and VRBPAC members for today's Topic I on the 2026 Southern Hemisphere formula for seasonal influenza vaccines. So, we duly noted today's discussion on the contribution of neuraminidase, the emerging and potential challenges and opportunities of considering more than one H3N2 strain in seasonal influenza vaccines, and the input of keeping a close eye on the influenza B/Austria-- Austria like virus and seasonal influenza vaccines. It hasn't changed for a number of years.

Next, I'd like to thank the presenters, VRBPAC, and our four temporary Voting Members for discussion and recommendations on Topic II. And that is CBERs effort to modernize our allergen extract standardization program. So, with respect to major allergen standardizations, we heard clearly that beyond analytic qualification and validation, there's a need to consider clinical validation and a learning agenda to enhance public trust in these allergenic products. With respect to the complex extracts with multiple major allergens, we heard about the complexities associated with standardization of these complex extracts and the need to recognize that we're on an iterative journey to continuous improvement in product quality while recognizing the incremental costs and implementation complexities associated with those improvements.

So finally, let me thank all for another productive convening of VRBPAC. I'd like to specifically thank our CBER Advisory Committee staff, the FDA Advisory Committee staff, our AV staff, and my colleagues in OVRR who willed into existence another flawless VRBPAC meeting on short timelines, and in the midst of the ambiguities associated with lapse in appropriations. So back to you, Chair, with sincere thanks to you as well.

Adjournment

Dr. El Sahly: Thank you so much. I want to thank all my colleagues on the Committee for a long yet productive meeting. And the meeting is adjourned.

LCDR Reese: Thank you. It is 5:10 PM.

Dr. El Sahly: Thanks, everyone.

Dr. Kaslow: Thank you