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IMMUNE GLOBULIN POTENCY IN THE 21st CENTURY

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PARTICIPANTS:

Welcome and Announcements

SESSION III: Immune Globulin Potency Testing -- The Future:

Proposed Chairs:

JOHN BOYLE
Immune Deficiency Foundation

WILLIAM SHEARER
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Introduction:

DOROTHY SCOTT
Food and Drug Administration

Infections in PI Patients

A. Epidemiology of Infections in Primary Immune Deficiency Patients:

PAUL MAGLIONE
Mount Sinai, New York

Potential Candidate Potency Specificities and Tests

B. Antiviral Antibody Levels Across IG Products:

THOMAS KREIL
Shire

C. Vaccine-Induced Antibodies in IG Products:

MEL BERGER
CSL Behring

PARTICIPANTS (CONT'D):

D. S. pneumoniae Antibodies in IG Products and in IG recipients, Test Methods, Relevance to Primary Immune Deficiency Patients, and Lot-to-Lot Variability:

RICARDO SORENSEN
LA State University

E. Questioning the Accuracy of Currently Available Pneumococcal Titer Testing:

JOUD HAJJAR
Baylor College of Medicine

F. Opsonophagocytosis and Other Methods to Detect Functional Antibodies Against Encapsulated Bacteria (ex: S. pneumoniae, H. influenza):

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P R O C E E D I N G S

(8:35 a.m.)

DR. SCOTT: I want to welcome you back this morning. I'm looking forward to a very interesting discussion. Today we are going to talk about: Immune Globulin Potency Testing, The Future.

The disclaimer, I also want to mention that we are going to have a slightly different agenda this morning. We are just changing the order of things. Dr. Shearer and Dr. Hajjar will be speaking first, and then we'll proceed along with the rest of the agenda in the order that you have.

We'll go back to the definition of potency, the long one, that potency is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory test, or by adequately controlled clinical data obtained through the administration of the product, in the manner intended, to effect a given results.

We decided this morning to revisit the potency testing for immune globulin, especially

in light of the discussions about polio and about measles in our quandaries with regard to two out of three of the potency tests that we have. I'll remind you that the third potency test for immune globulins is for diphtheria antibodies, and although we haven't discussed it here today, the relevance of having that as a potency text is compared with some other tests that may be possible, could be questioned.

Well, our potency specifications, depending on which one we are talking about, are 56 to 64 years old, now I like that kind of age, I think it's a good age and, you know, an age of wisdom (laughter) and great experience, but perhaps in the case of potency tests, it is worth rethinking. Potency tests should reflect ideally: diseases of concern in the general population, or they did reflect -- I'm sorry -- diseases of concern in the general population in the pre and early vaccinations era.

And they also reflect the availability of certain assays at that time, to look at potency. Our products are changing, because of the epidemiology of antibodies specificities and

donors is changing due to vaccinations, herd immunity, elimination of certain diseases and other factors.

For the infection risk for primary immune deficient patients, our diphtheria, polio and measles potency assay is still relevant to infection risks in those folks, and what are the current most important pathogens for primary immune deficient patients now? These are the questions that we are going to ask?

We also need to think about what's a wish list for potency tests? What do they need to have to be good potency tests for immune globulin? But they should be relevant and predictive of the desire by logical effect, which is prevention of infections. They need to be feasible, they have to be able to be validated, the reagents need to be available to use those tests and they need to be stable themselves, and this should be specific, sensitive quantitative and reproducible.

Reference standards need to be available because our potency specifications are that they are specifications, and they have a

cut-off value, and you need to know that you are using the same cut-off value every time, and that is a reason that you need reference standards.

They should measure antibody functions, so these would be neutralization tests, optimization tests, they can be in vivo tests, but usually they are not for obvious reasons. The specificity selected and their specification should be related to the effectiveness of the product, and the capacity of manufacturing, and extreme lot-to-lot variation should be fairly unlikely because these tests also should demonstrate some aspect of lot-to-lot consistency.

This is a quote from an article written about 17 years ago, "We should not be afraid of selecting a potency test if the choice is based on the best science available, and common sense." I thought those were good words to start out with today.

The questions for discussion will be: Which antibody specificity is irrelevant for primary mean deficient patients, and among these, please comment on the test method availability

and robustness.

The second question; and you'll be seeing a lot of data relevant to this: Which antibody specificities are likely to be highly consistent across products? And the third, which test for relevant antibodies may warrant further studies in IG products? So, I want to make it clear, we are not going to select a test and say this is it, and then go out and tell all the firms that they've got to do it.

Usually any kinds of specification tests undergo some collaborative studies in labs to figure out the specification, the method and work with the reference standard. So, it wouldn't be and instantaneous decision and it wouldn't be an instantaneous change.

We are fortunate to have a lot of terrific speakers, it will make the morning crowded, and we'll try to keep to time. We've changed the order of the talks, so we'll first be hearing Dr. Shearer on the accuracy of currently available pneumococcal antibody testing; and Dr. Hajjar, they will be speaking to this.

Then we'll go back and hear about

epidemiology of infections in PI patients, by Dr. Maglione; antiviral antibody levels across IG products from Dr. Kreil; vaccine-induced antibodies in immune globulin products from Mel Berger. Pneumococcal infections, immunity assessment, and recommendations for IG product evaluation, Dr. Sorensen; and finally from Dr. Nahm, opsonophagocytosis and other methods to detect functional antibodies against encapsulated bacteria.

So, thank you to all the speakers. Thank you to all the audience for returning, and we looking forward to useful and helpful information and discussion.

I'd now like to introduce Dr. Shearer, and his talk will be followed on by a talk by Dr. Hajjar, also from Baylor. But I want to say something special about Dr. Shearer, he is as people who are treaters realize, he has made immense contributions to progress and transplantation for SCID patients, and in taking care of and helping advanced treatments for primary immune deficiency diseases.

And a list of his accomplishments is

very long. I'm just going to mention a few. He directed the David Center for transplantation and care of children with severe combined immunodeficiency. He is also famous because he cared for David Vetter who was also known as the "Bubble Boy" at the time; and made many efforts to try to improve his life, and learned a lot, we all learned a lot from that experience.

He is also very well-known in HIV research, in pathogenesis and treatment of pediatric AIDS. He also, interestingly, did some space immunology, looking at the impact of space travel on immunological parameters. He has won numerous teaching awards, he is clearly a superlative teacher, an advocate for patients, a researcher who has made great contributions to the field of primary immunodeficiency, who has supported and initiated a lot of fruitful research, and has been, as I said, a superlative teacher, an advocate for patients and a humanitarian. So we are very pleased to have him. Thank you for making it here, Dr Shearer.

(Applause)

DR. SHEARER: I'm not sure what I

should say after that beautiful introduction. I hope I'm the person you are talking about. (Laughter) Today we are going handle a two-part presentation, and I'm not the main show. I'm the introducer to the topic.

Dr. Joud Hajjar is the main show, and she is a rising star to Baylor College of Medicine, and I'm sure you will enjoy her presentation.

Why are we doing this? Well, primarily because of John Boyle who contacted me about setting up some kind of various testing program, because the state of art of diagnosing immune deficiency critically depends upon an accurate antibody function. Do we have that commercially available? No, we don't. And Dr. Hajjar's presentation will convince you of that fact, I'm pretty sure.

But we need a test that is reproducible, and should be within healing distance in another laboratory. And again, we don't have that. And the data that Dr. Hajjar will present will convince you, I believe. So, what we'll want to do is to strike out immune interaction.

This will take some time for a clinical immunologist to work through the exact methods, and hopefully some

(inaudible) setting tests are a revision of an old test will be in the offering. And again, we test the function of antibodies.

This started a new thought, which started several months ago, and the Baylor College of Medicine drew blood from approximately 50 immune deficient patients, and inspected samples half went to -- testing (inaudible) Lab A, and 50 went to testing Lab B, and each other labs, so there was complete testing of all specimens.

Now, what direction should we take? Immune Deficiency Foundation wants to work with the FDA in providing clinically immunologist with a test they can count on. It's been very frustrating to have the lab results come back, and then the exact opposite of what the Doctor predicted. And then to find out, as we did, that there's no period of agreement between labs is very disconcerting.

So, you are about to hear her presentation, a very brilliant, young researcher, and I'm very pleased to be associated with her. Dr. Hajjar, would you come up?

DR. SCOTT: Thank you, Dr. Shearer.

DR. HAJJAR: Thank you, Dr. Shearer, for introducing our study. Good morning, everybody. I'd just like to take a moment and thank the organizers for having us, and for allowing us the opportunity to project this work. Dr. Shearer has set the grounds for this presentation, so I'm just going to go ahead and take you directly into the study that we did. So, as Dr. Shearer mentioned, our aim from the study was to compare 14 Streptococcus pneumonia antibodies that were performed simultaneously in two different commercially- available labs.

And what we wanted as well; is to assess the clinical management of those patients that was based, at least in part, on the interpretation of the providers to those lab results. So, our protocol was approved by the Institutional Review Board, at Baylor College of Medicine. And the blood samples were collected between September

and December of 2015, and this allowed us two years of follow up of those patients.

So, the procedure was that the providers on a clinical basis have ordered Streptococcus pneumonia antibodies to be sent to Lab B, and then in the Pathology Department the blood was collected and split, and simultaneously was shipped to Lab B and Lab A for comparison.

And later on we accessed the medical records to obtain the clinical history of those patients, the immunological phenotype when available, and we specifically looked at the office visit at which the providers receive those results, and interpreted them, and then what was done, for those patients was based on the lab results from Lab B, and then we followed those patients for two years, and we saw what happened to those patients.

So, in our study we included 14 serotypes, and the ones that I highlighted in white are the serotypes that are shared between the Prevnar 13, and the Pneumovax 23, and the three serotypes that are highlighted in purple are the ones unique to the Pneumovax 23.

The reason that we chose 14 serotypes, is as I'm going to show you the next slide, we have a younger cohort of patients, so we thought it might be more relevant to have shared serotypes between Prevnar 13, which is administered for younger children, and Pneumovax 23.

Both commercial labs report 1.3 microgram per ml, as a protective value, and based on the lab's recommendations we went ahead and used that point as a cutoff point to consider a value to be protective versus non-protective. So, we started with 48 subjects, and we had to exclude three subjects from the study. Two patients have hemolyzed blood samples, and one patient had missing data from Lab A, so we ended with a cohort of 45 subjects.

And as I mentioned we have the young cohorts, so the median age of our patients with seven years, ranging all the way from three months to 24 years. Our cohort was evenly distributed between females and males, but they were heterogeneous in the background in the clinical history. So, more than half of our patients were being evaluated for history of recurrent

infections, but we had a handful of patients who were status post hematopoetic stem cell transplant either for hematological malignancy, or PID.

In our cohort we had one subject with severe combined immune deficiency who received a hematopoetic stem cell transplant, and another with Chronic Granulomatous Disease.

We had four patients who were evaluated for heart transplant per protocol in our Transplant Center at Texas Children's Hospital, a couple of patients with chronic lung disease, refractory ulcerative colitis, and a patient with a history of recurrent rash.

As you would expect, because those patients had different clinical phenotypes their immunological phenotypes varied. But as a group the median value of their immunoglobulins IgG, IgA and IgM were within normal, and they had protective titers as a group again, for both tetanus and diphtheria.

So, what did we find? Across all 45 patients, when we looked at their Streptococcus pneumonia antibody value, using a cutoff point of

1.3 microgram per ml, Lab A, showed that there were two out of the 14 serotypes that were consistently above 1.3 microgram per ml cut-off point. But Lab B showed 11 out of those 14 serotypes to be above that cut-off point.

We looked at the specific serotypes that were both statistically significant and were on one side of that cut-off point versus the other. So, this figure summarizes those serotypes, the X-axis represents the different serotypes, and the Y-axis represents the numerical value in microgram per ml units.

Lab A is represented with the blue columns, and Lab B is represented in red, and the dash-green line represents the 1.3 microgram per ml cut-off point. And as you can see numerically, those values deferred and probably most profound in serotype 5, when the median value in Lab A across the 45 patients was 0.6, and was 9.43 in Lab B.

So that was very interesting, but what did this mean to the individual patient? From that point on, we went in and reviewed the medical charts of those 45 patients, and we looked for

discordant results, and what we defined as discordant findings is those patients who have 50 to 70 percent of their 14 serotypes to be above 1.3 microgram per ml in one lab, and 50 or 70 percent of those serotypes, less than 1.3 microgram per ml in the other lab.

And we found 14 patients, so about 30 percent of the patients in our cohort had discordant findings. To remind you that the providers receive the results of Lab B because this is what they asked for, and we have the results from Lab A, as research value.

So, as you see, and as I presented earlier that Lab B, across the board showed higher values of this Streptococcus pneumonia antibodies; and based on those results, and the clinical phenotype of those patients, the providers did not diagnose any of those patients to have an antibody deficiency disorder. None of those patients was started on immunoglobulin replacement therapy, and none of the patients was started on prophylactic antibiotics.

Now, on your left you will see the prophylactic antibiotic column showing three

patients who are on prophylactic antibiotics. Those patients were started per protocol, and it happened that all three patients are status post hematopoetic stem cell transplant were lymphopenic, and were started on prophylactic antibiotics per protocol for lymphopenia.

We were fortunate that we had time follow up those patients clinically. So, after two years of follow up, those patients are all still alive, they followed the natural course of their underlying disease, so some patients with hematopoietic stem cell transplant had some issues with graft versus host disease. None of them required to be on IV antibody or was hospitalized for severe infections. And none of them was treated with immunoglobulin replacement therapy later on because of the change in the clinical history.

There were two patients who continue to have recurrent infections, and those are subject to anyone, who was a little girl who continued to have otitis media, and required maybe two to three course of antibiotics per year.

This patient was never evaluated by an

allergist immunologist, because this study was based off the Pathology Department, and not all of them were allergy immunology patients. So, continued to have antibiotics, although stayed alive, never needed to be in the emergency room, just kind of outpatient management.

Subject 46 is another little girl with trisomy 21, who continued to have symptoms of upper respiratory infections, mixed between viral and bacterial infections, and the provider thought that other contributing factors, given her underlying trisomy 21 diagnosis, could contribute it to the history of recurrent infections.

Maybe worth highlighting, subject 36, that I noted that had a history of 13 infections before the study was done. This was a little girl who had recurrent otitis media, and probably the 13 infections were one long chronic otitis media that ended up in the mastoiditis. The patient underwent surgery, and after the surgery was evaluated by allergy immunology, and this is when the Streptococcus pneumonia antibodies were sent.

After the surgery the child did very well. Did not require any more antibiotics, continued to grow, and so her growth curve was normal, and was discharged out of the allergy immunology clinic at the end of the two years follow-up period.

So, this is kind of in a nutshell, those patients that had the discordant results. We'll also take it one step further, and try to correlate between the clinical and immunological phenotype. So we said, let's only focus on the patients who have a history of recurrent infections, and so I showed you that some of our patients did not have any infections.

There were 27 patients with history of recurrent infections in our cohort, and similar to our general cohort, Lab B continued to show higher Streptococcus pneumonia antibody values compared to Lab A. What was statistically significant and the values where numerically different on each side of the 1.3 cut-off points, the serotypes were 1, 4, 5, 14, 26, 56 and 68.

We decided to zoom in a little bit more and look at patients who only had a history of

pneumonia that was defined as a physician-diagnosed pneumonia proven by chest X-ray, we had 10 patients whom had this definition, and again, Lab B, showed higher Streptococcus pneumonia antibody values that were statistically significant in serotypes 5, 51, 56 and 68.

Our study has limitations that we need to acknowledge. This cut off point of 1.3 microgram per ml, was determined by the labs, and certainly is a controversial point, that I'm sure the discussion will come up to talk about it further during the morning session. Both labs used Luminex-based assay to measure the Streptococcus pneumonia antibodies with slightly different techniques, so this limits the way of the comparison.

However, it's important to know that those are two commercial available labs that the clinical immunologist and other providers do send their patients for evaluation, and sometimes because of other limitations the providers might send one set of Streptococcus pneumonia antibodies to one lab, vaccinate, and then send

the other post vaccination antibody values to another lab.

So, I think we highlight an important discordant in the results of two commercially-available labs that potentially could lead to different diagnoses of those patients and potentially management. But this just stresses the need for improvement of the available testing techniques that we have right now, and I'm sure that we are going to hear about this more this morning.

With that, there are so many people that we need to acknowledge. Of course Dr. Shearer, who is here with us, has been instrumental in advancing every part of this project. Of course Dr. Jordan Orange, Dr. James Dunn from our Pathology Department at Texas Children's Hospital; Dr. Al-Kaabi, our Fellow and our Biostatistician; and of course the David Fund, and the Jeffrey Modell Foundation for their support, and our patients and their families. And I thank you very much.

(Applause)

SPEAKER: We'll take questions

afterwards.

DR. BOYLE: Thank you so much, Dr. Hajjar, and of course Dr. Shearer. And just a little bit of housekeeping here. We, as was mentioned by Doctor here, we have a very packed schedule, so what we are going to do is beg your indulgence and try to move through all of the presentations, write down your questions, that's what we do at our events at IDF, so that we can get a chance to get everything and then have adequate time for questions, discussion as we deal with these presentations, and look at them in conjunction with the questions that we have to answer later this morning.

So, I thank you for working with us on that, and also for the alteration in the schedule here, which I think actually worked out very, very well, because as Dr. Hajjar was mentioning, the difference in the testing can have a major impact on the management and, of course, you know, the question that we are all posed with today revolves around immunoglobulin.

So, some of the discussions we were having yesterday about antibody-related, so

mediated issues here, are somewhat underpinned by these diagnoses, and whether immunoglobulin is proper for some of these patients, and what are the responses if some of these testings that were done early on are discordant, as was said. So, being able to start with that, and have that serve as a centering point from yesterday, and as we go in today, I think is great.

So, without further ado, would like to call up PJ, Paul, by his mother if she's angry with him, Maglione of Mount Sinai. And PJ will be talking about the epidemiology of infections in primary immunodeficiency patients.

DR. MAGLIONE: Well, thanks a lot, John, and thanks a lot to the organizers for having me speak. I've been tasked with giving some background to the types of infections that the primary immunodeficiency patients get, and in the hopes to shape some of the discussion to come.

I have some disclosures to mention, they are listed here. And then what I wanted to do was to start with the case, which I think illustrates a bit of the difficulty in sort of the question we are facing, in terms of trying to

understand the types of infections that patients have, and how viral they might be.

So, here's an example of a 50-year-old woman, had a history of shingles recently and was referred for some otherwise vague symptoms, and this is kind of case that we see somewhat frequently. They may have had an uneventful medical history during childhood or teenage years from what they can recall. In this case the woman had -- began to have sinus issues in their 20s that were frequently treated with antibiotics and ultimately did improve with surgery.

However, the sinus issues returned requiring antibiotics again. There were no major infections, no pneumonias here. And this is sometimes a clinical, you know, challenge to figure out is this a truly immune deficient patient, and sometimes there's other clues that go beyond the infections that help us, at least clinically, and in this case the patient had three episodes of ITP.

And one of the things that we knew from patients from our experience at Mount Sinai, and other places obviously, that one of the most

common symptomatic immune deficiencies, CVID, presents in many cases with autoimmunity that most-commonly will manifest as cytopenias, and that kind of is a clue which starts to make us think we are going to this case seriously.

So, I think sometimes the infections can be, you know, difficult to interpret is, I guess, the point I'm trying to make here. And this is the data that we can get, and this is from an idea of patient survey which really offers some of the larger sources of data on the epidemiology of these patients. And you'll see that, you know, sinus infections which are a common type of infection whether you have immune deficiency or not remains, you know, among the most common conditions that occur in these patients before diagnosis.

Pneumonia is there, and it is something that should trigger this diagnosis on the differential, but again in 51 percent, so about half the patients maybe not having pneumonia, and it's a challenge. Bronchitis is quite high as well, ear infection. So, these all can be theoretically different pathogens and, again,

raising the challenge of, you know, how we interpret this.

Another piece, to the complexity is, what we are dealing with is age, so there seems to be maybe two peaks of presentation or, you know, diagnoses in our primary immune deficiency, certainly there are a lot of immune deficiencies that present in childhood, and this is, you know, a lot of what -- sort of the textbook cases that we review in early stages of medical school, and so forth.

But we do appreciate that there are some diagnoses that occur, quite significant numbers of them that occur in adulthood. A lot of these might be primary antibody deficiencies like CVID, but of course this is going to make the complexity because older, you know, adults versus infants might have a whole different group of pathogens that you're thinking about.

And then, of course, when we are thinking about the types of infections, a lot matters in what is the type of immune deficiency that these patients have. You know, here we are discussing immune globulin, so obviously that's

going to affect patients that have antibody deficiencies, so defects in the B-cell compartment of their immune system.

However, immune defects affecting other elements of immunity can certainly have a domino effect in affecting antibody production. For example, defects in T-cells, T-cells are important immune cells to providing help the B cells. And in the absence of T cells you are open to a whole another group of pathogens that, you know, are going to be affecting these patients.

And some defects of innate immunity might also affect the ability to make certain types of antibodies, such as anti-carbohydrate or anti-pneumococcal that we are hearing about. So, there certainly is -- elements to, and trying to understand what the immune problem is, and that's going to shape the types of infection.

And here it's sort of just the reminder of that. You know, we can't really -- although sometimes you might see figures that thinking about antibodies and B cells in isolation, and important for things like extracellular bacteria, certainly there's interaction with

helper T cells, and a role for these in -- you know, these patients can be suffering from, you know, quite broad infections, theoretically.

Far and away, the most common type of immune deficiency we are going to encounter clinically, are antibody deficiencies, so about two-thirds or so, 65 percent. But you count also the other patients that have combined cellular and antibody deficiencies that will likely need immune globulin replacement, and that's a very large fraction of these patients that we are seeing. So, again, it's going to be a broad spectrum of the immune deficient patients that we are thinking about.

And so what are the key factors? What can I summarize about what I've been showing? I mentioned that the type of immune deficiency is going to be important, it's just something that's affecting the immune globulin production antibodies alone, maybe certain specific antibodies. Or are there a broader immune defect that's compromising T cell function as well, and that will shape the types of infections that are there.

The age of patients, we talked about, you know, children might be facing with different infections and might, you know, someone who has been diagnosed in their 30s. And then chronic complications, this is an area that we are appreciating about primary immune deficiency, these are immune disregulation disorders, and it goes beyond there just being infections as the problem. They might have chronic inflammatory conditions that will cause structural damage, something like bronchiectasis, for example, can emerge and that might shape the types of infections that these patients become -- you know, are likely to get.

So, going back to the case, and these are the kinds of way that we think about the patient in terms of the types of laboratory testing the evaluation we do, we might do, you know, a CBC with differential, and in this case, not particularly informative. This is a patient with generally a normal CBC and differential, routine testing liver, chemistries, kidney function within normal limits.

But the piece that did stand out was

profoundly decreased immune globulins, reduced levels of IgG and IgA, and really no evidence of losing protein in the urine so, you know, a key thing is to think about ruling out, you know, secondary causes of this, and these results coupled with this history of ITP in infections really began to shape our diagnosis.

But in many ways we are really faced with some of the same questions that we are going to discuss in terms of evaluating IgG replacement products when we are testing our patients, because in the end you are interested about part of the diagnosis of CVID specifically as showing impaired antibody function.

So, how do you do that? You know, and I think some of the issues that was just brought up, you know, trickled down to the diagnosis that how can we trust these assays. So, we have a lot of the same discussions.

And so in this case, what we do is we do functional antibody testing, measuring antibody responses to numerous vaccines, and in this case, in this patient showing absence against diphtheria, tetanus (inaudible)

vaccination, and all of the 14 serotypes of pneumococcus. So, this, you know, together gives us some strength of evidence.

You can also look at other forms of data to help, you know, shore up your diagnosis. We expect to see impairment of the cell maturation in these patients, and often severely reduced plasma cells. So, you might see that if there's biopsy available, having the biopsies of the GI tract of these patients, the GI complaints or the bone marrow, if there was another reason.

We don't do this specifically to make the diagnosis, but if it's already been done, you might want to go review those slides, that can help, and then we look sometimes, if it's available, looking at isotype-switched memory B cell levels.

But I just wanted to highlight this specific diagnosis, this is -- so we diagnosed a condition with common viable immune deficiency, this is the most common symptomatic primary immune deficiency, occurring about 1 to 2,500; IgA deficiency more common, but usually asymptomatic. Typically, diagnosed between 20

and 50 with a significant diagnostic delay, so again, this is going to shape maybe some of the infections you are going to think about, and it's defined by a marked reduction of IgG as well as IgA, and often IgM; and it must have documented impairment of antibody response to vaccination which, you know, again raises some of the questions we are discussing.

And then the other features that I just mentioned about other components of their B cell maturation, and importantly about half the cases are complicated by non- infectious complications, which could affect, again, the type of infections that these patients might get if there's structural damage to the lungs, or other organs, they might become more susceptible to certain types of infections.

And the other piece to thinking about how you evaluate products, so what's needed for patients is, you know, what stage of B cell development is the immunodeficiency occurring. You have some disorders like X-linked agammaglobulinemia, that essentially will wipe out all sickle in B cells, in this case there is

no -- there is impaired signaling through the pre-B cell receptor due to the absence of BTK, and you are not going to have as many B cells in circulation will be profoundly reduced, and then consequentially a profoundly-reduced antibody response.

And you can think of these patients really needing all their immune globulin replaced. And you really find along the spectrum some defects that are much further along, and some patients might have, you know, really defects in B cell activation, or specific responses to certain type of pathogens, like patients with specific antibody deficiencies that might be concluded that they need to be placed on IgG replacement.

And so, you know, their questions on what needs to be in the product for them; might be slightly different. And we think CVID, the example I gave is somewhere up here, where it's a defective B cell activation and maturation that can have, you know, circulating B cells at normal levels, or near normal, but what they are lacking are isotype-switched plasma cells and

isotype-switched memory cells.

So, the big question, right, of what I was tasked with. So, what are the infections? Well, you know, you can come up with a list like this, and I'm sure everyone in the audience can come up with exceptions in the cases that they know about that are not really included, but we tend to think about with antibody deficiency, or bacteria, particularly encapsulated bacteria that's a, you know, classic teaching point.

We do appreciate that there are other types of infections that sometimes come up particularly in CVID patients, things like mycoplasma that can have typical presentations, viruses as well. We are appreciating that perhaps more with time that this is important as well.

And then certain parasites that, again, might be overlooked when thinking about the mere evaluation for these patients, but they certainly have had issues with those as well.

Now, whether those are related to the IgG defect that you are replacing, or whether they have -- that's due to their absence of IgA at the

mucosal services, that you wouldn't necessarily be replacing with the currently-available products; you know, is another question. And again, that might shape the types of things you are going to evaluate in an IgG replacement product, is what the G is targeting for our patients.

So this I thought was interesting for the scope of the talk is really, you know, what are the infections? So, there is a nice study, you know, now it's quite a few years ago, but there really are few that really focus so much on infections within an antibody deficient population like this, with a lot of patients, so with more than 250.

And what these patients were presented with, are things like bronchitis, most commonly would be bronchitis, sinusitis, pneumonia, sort of what I was hinting at, presumed in many cases to be bacteria, but a limitation to really try and understand this, is that we don't often know the pathogens, they are not often identified, right.

So, this paper was nice, and that it does identify in many cases, what the pneumonia

was due to, and the most commonly identified pathogens were Streptococcus pneumonia and H-flu. So, these are things that, of course, we are going to be talking about today, and already have been talked about, so things that have been on the radar, and things that are likely really important to consider when evaluating IgG replacement products, if you are looking within the context of what infections the patients get.

And then GI tract infections are very important, and they are listed here, but again, is this related to -- these are going to really directly related to the IgG replacement, the IgA absence that are -- you know, I think that remains to be fully understood.

Now, other patients with immune deficiency that will benefit from IgG replacement include those with more of a broad immune defect. So, those with severe combined immune deficiency, or SCID, often are prescribed IgG replacement as a part of their clinical care. We now know with the implementation of newborn screening in most of the states of the U.S., we have a really strong idea of what the incidence of this is, and it's

now, we think, around 1 to 58,000 live births in the U.S.

This is a disorder that is typically diagnosed in infancy, historically it was something that was diagnosed due to severe infections very early on in life, now we have really the exciting change has occurred, in that newborn screening is now adopted for 45 states, and in the District of -- in Washington, D.C., that allows for the diagnosis of SCID right after birth before infections, and allowing for treatment to occur very early.

And obviously the hope for this is to improve patient outcome. These patients are defined by having profoundly low levels of T cells, and really no, or very little T cell function in assays, usually when, you know, assimilated with a mitogen.

These patients, depending on the gen-etiology of their SCID may also have profound reduction of B cells and in K cells, however, immunoglobulin replacement therapy is really a mainstay in the treatment regardless of the etiology even if there B cells present, because

when you have a profound T cell defect that T cell help is absent, and that really is believed to be required for adequate antibody immediate immunity.

So, these are patients that really will benefit from IgG replacement, at least in the initial stages. Now, so as I mentioned IgG replacement therapy is really a part of their care along with pneumocystis prophylaxis prior to a definitive treatment like bone marrow stem cell transplant, or gene therapy, but even after one of these definitive treatments in many cases the patients need to remain on IgG replacement, and that can be particularly about half --

Well, in general we say about the half the patients, but that percentage is even higher in certain types of etiologies of SCID about, for example, about 80 percent of patients with RAG 1 or RAG 2 deficiency who continue to require IgG replacement. So, this is a whole different phenotype of patients, a whole different type of patient that is on this therapy that might have slightly different concerns about the infections that they get.

So what are the infections that SCID patients will have? So, a very nice paper and is recently coming out in Blood, that is illustrating this and show some of the data. From this, it's a little hard to convert the table to a slide, but basically there are a couple of -- there's a few different groups here that we are talking about, so these are SCID patients that we are thinking before definitive treatment, before stem cell transplant, and after.

And then groups being divided by those that are being identified on newborn screen or through family history, so before they start to get infections, you know, the earlier diagnosis, versus those who are diagnosed based on clinical science, so before the implementation of newborn screening.

And you see some interesting differences when you compare with the patients that are more strictly antibody deficient, you see this emergence of gram negative bacterial infections that become a major piece, but maybe we can be, you know, excited about the fact that though it is occurring in 41 percent of these

patients before stem cell transplant, these are the clinical science group; we see this markedly reduced in the patients that have been identified through newborn screening, and through family history.

So, perhaps this kind of question may become less of an issue with time, at least that's the hope, and the state is exciting in that. But it's important to recognize that there are things here, Klebsiella, Pseudomonas, E. coli, Enterobacter, things that we haven't talked so much about thus far in the epidemiology of the other patients that were a factor here, at least in this group that is being identified through clinical science and not through newborn screening.

One piece that's still there is staph infections that, you know, is pretty consistent. This is a tricky pathogen to deal with. We know that it's a major pathogen even those who are, you know, competent, so I think it's difficult to interpret this infection data within the context of immune globulin replacement, but interesting to note that data as well.

And then there's a broad list of other pathogens, that are affiliated here, what we saw though again, with other bacterial infections, so not clear on, you know, that's a broad group here, but much, much more improved with earlier diagnosis through newborn screening or family history, you know, that are there as well.

So bacterial infections are clearly very important, viral infections as well, things like RSV, parainfluenza, and flu (inaudible) were, you know, present and present in fairly significant numbers even though those patients that were diagnosed earlier.

So, we are left with the question that we are going to be discussing about today, whether protective antibodies, you know, should be evaluated in immunoglobulin replacement products. I think you'll see from the epidemiology data that I showed you, really are not showing a lot of data relating to diphtheria, measles and polio, so I think that's really wherein it was already highlighted in the introduction and that's where that's where this discussions is really coming out of, out of other

things.

And I think that clearly the epidemiology data really strengthens that point that, you know, maybe other things should be discussed. You know, I think a lot of the data we are seeing is relating to respiratory pathogens, both bacterial and viral, this is probably a major concern immune deficiency and something that we should think about in terms of epidemiology of the patients.

And what I found is interesting, is you can find, you know, literature even that you can maybe target the evaluation based upon outbreaks of specific pathogens, and maybe these are things that they'll have to be so static and can change over time as well. So, there's some flexibility that we should also consider that, you know, when looking at these things, that you can implement something that maybe would emerge.

And, you know, we've seen there are certain viruses that have emerged in some of our CVID patients with GI disease, you know, and I'll be curious to see how lots of IVIG sort of might, you know, relate to that, and it can answer some

of the questions about maybe more clinically about how IgG is protecting maybe versus pathogens, maybe, but perhaps not others, in the GI world anyway.

And so we talk about, you know, functional assays which will be discussed, and so I won't really mention that much here, but things about, you know, how do the antibodies function in terms of lysine bacteria and phagocytosis. And I just want to end in the last five minutes, because I think this is a really interesting topic, and really happy to be included with some basic data.

So this is sort of in the hopes to spur on maybe some collaborative work. I know there's some very -- there's some, you know, very well established researchers here, that have been studying these kinds of questions for a long time, I'm very junior, I'm very eager to kind of get involved in more stuff, and one of the assays -- one of the things we did, this is a paper now of a few years ago, that we published really looking at, really a different question, but we were beginning to play around with some assays

looking at the function of antibodies.

And one of the things we were looking at, and we were able to -- find that we were able to do is we could be combined looking at function and specificity together on the same assay. And so we've been able to do in the lab, and we used it in this publication to looking at basically the same ELISA.

And basically what you are able to do is measure the specificity, and here we are interested in IgM because of the clinical question we were addressing, but to a specific antigen which was here, which was actually -- which was phosphorylcholine, and we were able to see that there was a correlation between the levels of this IgM and the ability to fix complement, or to (inaudible) complement C3 into its C3A and C3B products.

And so it's raising some questions of whether we can maybe make some assays, or do some research that might combine both functionality and quantity together. And we were doing this in the context of these IRAK-4 and MYD888 deficient patients because they are, you know, a very

interesting patient cohort, they sort of have this unique feature in terms of primary -- they have this, I guess, not totally unique but interesting feature in the context of primary immune deficiency as they improve with age.

There is a high mortality, and there's a lot of infections in the early ages, but as these children grow to become older they do a lot better, and it's led to the idea that adaptive immune system is doing something to compensate. And there's really, in this large study, the largest study of these patients that was published, reported no death after the age of 8, no invasive infection after the age of 14, and patients that were suffering very severe infections early one, and very high mortality of 38 percent.

So, what's going on in these early stages? And I had hypothesized, and I'll just mention this for the context of what I'm going to show you, but we hypothesize that maybe IgM, there's an effect in IgM because we know that the types of infections that patients were getting were these invasive infections with encapsulated

organisms, IgG takes time in these patients to -- IgG levels to carbohydrate antigens are delayed in people, and we thought that maybe IgM was in some compensating for this.

And in these patients, maybe there was a defect here of the IgM that might play a critical role in sort of limiting the severity of infections in the early years, through a few different mechanisms that have been well studied. And I won't get into because I'm going run out of time.

But I wanted to show what we did, because I think it might open, you know, maybe some ideas of maybe what could be discussed at a meeting like this in the future, looking at arrays. And so what we wanted to approach was the idea that we didn't know what to really look for in such patients and that might be some of the questions that we are discussing today: what do you look for?

And there is some strength in numbers, we are in a systems biology realm, you know, and so what we had utilized for this experiment was an array that had 610 carbohydrates, we were

really trying to study the anti-carbohydrate immunity. And we were able to take patients to earmark onto these arrays. Use a bacterial carbohydrate structural database to identify what pathogens or what bacteria are expressed in these specific carbohydrates, and then go back and compare the two groups of patients.

And what we were able to find was that the IgG that was in these patients ultimately as they grew older, was not significantly different, but what we were able to see is that the IgM was quite impaired in these patients to carbohydrates, and particular 18 carbohydrates that were on streptococcal -- strep pneumo staph aureus, the two major pathogens that the patients were getting invasive infections with.

And if you compare it with children that don't have these immune deficiencies, but have gotten these infections, typically the IgM levels go up. So, it was really quite profound when you compared it with children with dissimilar infection history.

And I just want to leave with kind of what this data looks like, in the last 30 seconds,

is that, you know, you are getting larger data, and so you can establish a cutoff that, you know, maybe instead of trying to pick a handful of things, you might want to -- You know, and it doesn't take any more work or effort to necessarily add onto an array like this, to look at 5 or 50.

But, you are able to really get strength in numbers in a lot of data, and maybe some of the same questions will be there, what you put on the array, but perhaps from a clinical standpoint you can, you know, think about it in terms of, you know, getting a broader view of what the product might be during and how broad it's able to, you know, protect.

So, I just wanted to leave that for some discussion, and hope maybe, maybe something in the future can be done to sort of look at these things in the long term. So, thanks a lot.

(Applause)

DR. BOYLE: Dr. Maglione, and thank you one more time. That was phenomenal. If I can invite backup to the stage here, Dr. Thomas Kreil of Shire, who will be talking about antiviral

antibody levels across IG products. Dr. Kreil, take it away.

DR. KREIL: Thank you, John. All right. Good morning, everybody. So, after we've figured out that we still need to understand more, what I would like to do in the coming minutes or so, is review with you some of the things we have actually learned already about antiviral antibodies in IG products. But then toward the backend of this presentation, I would like to share with you, particularly importantly I think what we are currently looking into, because certainly we are not at the point where we understand in a sufficient level of depth, what is in these products.

So, this is just for a starting point, I'm not going to tell this audience what IGs are, but I think there's a few features that would be good for us to keep in mind when we think about IGs. So they are the product of a number of thousands of donors, and the idea is that it would sample a very broad spectrum of antibodies into these products, they are polyclonal, and that's the purpose of these products, and then the

manufacturing process produces from this plasma donation a final vial of IG.

So, one could argue that where you are starting with so many thousands of donors, then really every vial should be the same, because it would just average out everything, but that I believe is not correct. And there's a number of different reasons why I believe that there is differences between these final vials.

The first one is determined by the exposure of the plasma donors themselves, they may be earlier infected with some agents, and later as vaccines become available see the dead antigen, and really it makes a difference in terms of the immune response that you elicit. We had the example yesterday of measles where the titer is generated by infections, were so much higher than what is now generated by vaccination.

The quality of the immune response is different too, because it's in the vaccine antigen, the surface proteins typically after infection, you have all the antibodies against the internal proteins that are expressed during infections that are not part of the vaccine.

So there is a difference with respect to exposure, and there we are seeing changes. The second thing that influenced the antibodies specificity clearly is geography of sourcing, and currently I think in this world there is a large proportion of plasma source from the United States, but we do know that there is plasma from Europe also fractionated into IGs and the antibody spectrum may be quite different because different viruses circulate in these different geographies, with differences in their trend over time as well.

And then the third thing that we need to keep in mind is the manufacturing process itself, because there may be, or not, enzymes at work at the IGs, and there is subclasses of IGs that are more sensitive to these proteolytic events, IgG is fairly easily lost, but there is various infections that we know of, and most of the protection is actually carried by IgG3, or it may be IgG1 for others.

So another aspect that needs to be kept in mind, so there is a fair amount of complexity, and that's why actually more than a decade ago,

we started to take a look at some of these specificities to just better understand. And this is one of the first things we have taken a look at; hepatitis E virus, the incidence in the United States you see on the picture has to declined massively after a vaccine has become available, and has been strongly recommended through the ACIP, so it's widely deployed here in the United States.

A very similar situation in realty like we have seen with measles virus, the vaccine became available, the incidence in decline, so with that reflected then in the antibody concentration in the IG products. And yes, it is. So, this is the HAV antibody concentrations in plasma manufacturing pools, and as you can see, over time, they are in decline. It's important to note also that in Europe the titers are roughly two-fold that in the United States.

And the reason for that is that the vaccination is not quite as universally deployed in Europe as compared to the United States. So, in other words, there are still people who go through the infection, it's a self-limiting

fulminant infection in immune-competent people, so that's why they perfectly qualify as a plasma donor, but they would bring in higher titers.

And that presence of the antibodies in the plasma pool does translate into the immunoglobulin product. So, this is here, a product from Europe, the titers are on average, higher. This is a product from the United States on average the titers are lower, just like we've seen it in the plasma earlier. The important thing to keep in mind here though is that the lowest level of antibodies in IG that we've seen in the study was around six IU per ml, with the assumption, based on literature, that the protective level is 10 ml IU, so we are far beyond protective titer still.

It's, I would argue, a fairly similar situation with measles maybe more close with measles than with HAV, but in some ways it's a very similar occurrence. So, now we have two examples of antibody titers going down by vaccination, now I will reiterate what I said yesterday, at the same time we have more herd immunity through that vaccination, and so there will be less exposure

of people who depend on IG supplementation as well.

But then, it doesn't go down always. There are actually instances where we see that antibody titers go up, and there is a very interesting example to take a look at. Interesting insofar as in '99, there was a new virus introduced to this continent, and it met a completely serum naïve population.

And so over time, we can actually follow what happened, how many of the people went through the infection with that virus, and would that translate into a seroconversion of the population.

And this is one way of looking at it, in '99, we just had 60 cases of West Nile Virus infection here in New York, in '99 all the IG preparations we could test from U.S. plasma didn't have a titer against West Nile virus obviously because the virus was not in circulation.

If we had jumped in here to 2003, the virus had at the time made its way into the Midwest with a fairly high instance here, and that's when

you start to see some noise here, some low titers, but still you can determine them by a neutralization assay.

And then as we went on in 2007, the virus wasn't then making pretty much all of the United States and that was reflected in fairly high titers in all lots of IG produced from U.S. plasma. Another way of looking at this is, this is the cumulative incidence of West Nile virus infections in the United States, and the big, big, big year has been 2002.

This was the big jump where actually we came up to 0.2 percent of the population of this country having gone through West Nile virus infection, most of them have been, you know, asymptomatically, so nobody would have realized. And that's exactly what is reflected in the IG lots (inaudible) to the United States plasma.

I think it's intriguing to see here, 2002, you see this big jump that is not translated into the lots manufactured in 2002, and that's due to the fact that the collection, and then the manufacturing process, it takes a year before the product is on the market, and that's why in 2003

now, you see that same jump that you see in here.

So, in some ways, IG did reflect the seroconversion of the United States population in some ways I would argue IG is even good as a sentinel, testing one lot of IG is going to give you information about the serious data potentially of 10,000 people.

Now, the other question, probably the more important question, there is antibody, but is that antibody meaningful? And there is a mouse model for West Nile virus infection where you can show that West Nile virus in that model, in a fairly dramatic setting, causes lethal infection.

But if you treat these mice with European IG you'll see that in Europe we didn't have the virus in circulation, and so there's no protective antibody that is different to here, just taking one of the IVIG lots from the United States, where there is a protective level of antibody, even against this very significant and severe challenge.

So, it does translate into protection against this infection. Another thing that we

were able to learn too is, we were fractionating an IG lot into the subclasses contained in this lot, and then the pure subclass fractions were investigated for neutralization of West Nile virus, and you can see here the IgG3 neutralizes the virus -- excuse me -- IgG1 neutralizes the virus better than the IG preparation.

So if you correct that for roughly 60 percent IgG1 that is in the IG preparation, you realize that all of the neutralization capacity in this IG preparation against West Nile virus is carried by IgG1. We know that against CMV for example, IgG3 is a lot more important, so I think it's important to understand also which fraction of the IG lots are actually going to do the trick for the virus you are looking at.

And going on to another Flavivirus because West Nile virus is just one of around 50 Flaviviruses that we know; there is a like twin brother circulating in Europe, tick-borne encephalitis virus, and particularly in Central Europe therefore there is two vaccines available that are widely used, so in Austria where I come from we have a vaccination rate of north of 80

percent, and that now is reflected in IG products from European plasma where you see very robust neutralization titers.

The virus has another relative that is called Far Eastern or a Siberian serotype of tick-borne encephalitis virus, so it does circulate in Russia, and if you get access to IG produced in Russia then you see that also there is some level of neutralization there. Now, in Russia people don't vaccinate 80-plus percent of the people, you only get the people who are actually going through the infection.

In U.S. IVIG, there is also clearly not a neutralization of that virus because the virus is not in circulation, there is a twin brother, Powassan virus that circulates to some degree around New York area, but it's difficult to see whether there would a serological crossway activity between these viruses. So that's the influence, as I said earlier, of the geography of plasma sourcing.

You can antibodies against what is circulating in that very geography. So, is there any other viruses that we needed to take a look

at, there is a recent review from a large population of people with immune deficiency out of Japan, and the author has identified two viruses of particular concern, particular concern for the subset of patients that they've reviewed with cellular immune defects, and that is respiratory syncytial virus and rotavirus, it's a very different classes of viruses the one respiratory, the other gastrointestinal, and I think that needs to be kept in mind too, because there may be quite different mechanisms at play.

In that study they also have taken a look, for example, for influenza virus infections, and they could not find the difference between people with our without cellular immune defects with respect to that virus.

Now, taking a look at these two viruses, you actually find that it's not that easy and straightforward always to take a look at these virus, and I'm going to start with rotavirus. Rotavirus first, theirs is a number of different species, A through H, in humans it's mostly either the programmatic, but it can B and C too, but then

within those, there is a lot of different serotypes, and if you are looking for a serological reactivity for neutralization of the virus, then actually you need to figure out which viruses we are going to use, and that is going to determine which antibodies you are going to see.

Just for something that is more well-known, influenza viruses are categorized for the hemagglutinin and the neuralamenities, so two different surface proteins determine two different serotypes, and the same is the case with rotavirus, the P and G protein can be different, so you are looking at a whole array of viruses in reality. And just taking one isolatant to the lab and doing some assay, is potentially not going to tell you the true story about rotavirus antibodies.

The other thing that complicates the picture here is that there is at least two vaccines available in this country, one being a Pentavalent, the other a Monovalent, and so it's quite conceivable that use of this vaccine is going to drive out certain serotypes that are covered by the vaccine, and maybe those serotypes

will then be replaced by other serotypes because that void of infection is easily filled by similar viruses.

So in other words, it's very, very complex to think through that, and then the final complexity is the laboratory assays because rotaviruses need to be proteolytically activated so that they become infectious, but that proteolytic activation requires use of a proteolytic enzyme in your cell- based assay, and the cells don't like that so much.

So, it's a very complex biological system at the end of it, and so clearly, an assay for a rotovirus neutralizing antibodies, would not lend itself for a QC setting to test every lot of IG. That's when we started to take this into a more systematic approach. So, which are the viruses that we would be interested in, and that would actually lend itself to be investigated in a reasonable format in the lab?

So, we are looking here at the seroprevalence levels, whether they are stable or changing, are they similarly, ideally on a global scale, because obviously we would like to do the

same release assay for all the plasma- derived IG you generate. Then is there some (inaudible) of these viruses in the health of people with immune deficiency? And then is there, in the general adult population, an impact with this virus?

And then ideally here, there is a no, because if there is some impact then we need to expect that there will be vaccine developed and then we are in the same story again like we had with HAV or measles, once the vaccine is deployed the titers go away.

And then the final question is: is an assay available that is such that you can actually validate it for a QC released testing? And ideally that would occur in the BSL2 setting, because not everybody has access to BSL3 or even, more than that, as we discussed yesterday, with polio virus.

And making some assumptions along all of these arguments, there is three viruses that made it to the top of our list. This is human adenovirus, respiratory syncytial virus, and paraninfluenza viruses. I'm actually quite pleased to hear in the talk just before that RSV

and parainfluenza really are viruses of interest. And that's when we are taking those into the lab, and we are trying to taking in more solid look into the neutralizing capacity of IG lots there, so it took -- a convoluted thing technically, because first you need to have access to a virus.

Now, that virus, that's the first question you need to ask yourself, is that just a laboratory isolet that may have been presaged so long, that it doesn't have -- that it has little resemblance to a natural isolet. Once you have the virus you need to bring it into the lab, you need to establish seeds, Andrew yesterday called it, good virology practices, that's what you need to apply here. Then you do a benchmarking of what the infectivity really looks like, and then you bring it into a neutralization assay, the details of which I'm not going to bore this audience with.

So taking a look at human Adenovirus 5, there is 50 serotypes; that in itself is a complication because Adenovirus

is one pick that you might pursue, but it doesn't go into -- it's not going to tell you anything about the other 50 serotypes. But for

Adeno 5 at least, there is a seroprevalence roughly of two-thirds of the population both in Europe and the United States, so it might be a candidate there.

In terms of clinical utility there is a vaccine, or as I understand it, for at least military use, but that, for type 4 and 7, so ideally 5 should stay unimpacted. And there is report of infection of people with immune compromised systems of this virus. So, it's something that we can take a look at, and again that technical detail, because of the advanced time, I'm going to skip.

And this is now we see, taken now two dozen lots of U.S. plasma derived IG here, and European plasma-derived IG here, you see that there is a very high average neutralization titer. The variation is rather little. It may look like much, but frankly this is a factor of less than two, and two full dilutions go into this assay, so really a factor of two is miniscule differences.

On a scale of a neutralization titer of 2,500 I think it's a high titer and it's a fairly

consistent titer, and it will be equivalent between Europe and the United States. So, that might be something that we could think about.

I think more interestingly, respiratory syncytial virus, so there is two different serotypes, and they are different with respect to the clinical picture that they result in. The serotype E can be clinically quite impactful, B is mostly asymptomatic. The interesting feature about respiratory syncytial virus is that re-infections occur, so immunity over times may wane.

And so it keeps in circulation even in people who have gone through the infection. In terms of clinical utility, yes, certainly, this is something that we see occurring in people with compromised immune systems, and because of the potential impact there is vaccines in development and there is an available monoclonal antibody that's Synagis. Still, you can actually take a look at these viruses; there is isolets that are available from both the serotype A and the serotype B that can be taken into the lab and propagated fairly easily.

There are still questions: Are these isolets that are available through the strained collections similar enough to the naturally circulating isolets? But let's assume it is, because it's a serotype, so the neutralizing antibody titer should be not different by more than four fault to all the viruses that fall within that serotype.

Now, this is what the results look like, this is the serotype B, and again, this is the one that is clinically not so impactful, what you see is that there is a significant difference in titer between the United States plasma-derived IGs, versus European. Why? I do not have an idea. It's just a different level of circulation into these different geographies that's the base assumption I guess.

But that is certainly something we are going to take more of a look at. I mean, it's a fairly limited sample of IGs we've tested here, but there seems to be a difference that I cannot explain. To keep in mind a comparison, this neutralization titer of 1 in 3000 is quite significant, but the Synagis, the monoclonal

antibodies were 70,000. So, it makes sense because that one specificity translates into a very specific activity of that product.

This has taken the look at probably the more important serotype, because that's the one that might generate disease actually, it's serotypes A, and what you see here is the titers are quite a bit lower as compared to the serotype A -- excuse me -- as compared to serotype B, and now here, the titers are equivalent between Europe and the United States.

How to explain it? Well, future will tell, but it's just a fact here. The titers that we see against serotype A are lower, as is also the neutralization titer for the Synagis, so the virus is just a little more difficult to neutralize it would appear. Again, not to read too much into this data factor of two is not too big in these assay systems.

The final example I would like with this community here is, human parainfluenza virus 3. Again, there are different serotypes but three is the one that is clinically the most impactful, that's why we've chosen to pursue this one. In

terms of the seroprevalence around the world, as far as we understand it, it's constant, it's universal.

In the United States, people by the age of 5 have gone through infection, and therefore all seroconverted. But still various issues people with compromised immune systems might have with that virus, so it is worth taking a look about the neutralizing antibody titers against this virus. And again, I'm not going to dig into the virology, that probably only nerdy virologists like myself find exciting.

That's the result that you get. This is the neutralizing antibody titers and again, a couple of U.S. American lots versus European, the titers are very high, they are fairly consistent, again within a factor of two that's very little variation there. And it is something that might be of interest for people with a clinical perspective in mind. So, parainfluenza virus 3 might be another candidate to consider.

In summary, this is what the picture looks like for the top three of our list, and it was somewhat an arbitrary selection because we

could have done all 10 had we had a little more lab capacity. But that's the picture. So, 1 in 3,000 is something that seems to be a fairly uniform neutralizing antibody titer against viruses that are widely in circulation in the community with the one exception of the RSV A, that being one, however, that we should be taking a look at because this virus, as I said, is more pathogenic version of the respiratory syncytial viruses.

So, in summary, I think what we can say is, we do not have a sufficient level of understanding about virus antibody titers in IVIG. I think more studies need to occur, and I think it is experimentally quite feasible to do these studies. What we have seen is that the epidemiology of these viruses in the dormant populations do change, and that is translated into the final IG products, for viruses like hepatitis A virus, and measles virus where a vaccine has replaced the early infections. We have seen antibodies like titers in decline that is totally different to what we've seen for West Nile virus for the United States have actually

seroconverted to that new virus.

And I've put Zika virus here with a question mark, because according to the latest information there is now a few thousand Americans who have come back from exposure during cruises in the mid-Americas or similar. I think at some stage we will see a certain level of seroconversion against Zika virus in the United States, plasma supply as well. So, we are running and monitoring on that.

And I believe that the understanding of these virus antibodies in IG is important for more than one application, long term, with polio virus and measles virus at some stage eradicated, then all the donor population is not going to have any contact to those viruses any more, if they've been eradicated for 10 years, 20 years then we are not going to even continue the vaccination. So, at some stage I think we'll have to figure out what else to look at for ascertaining the biological activity of every log that we release.

For more detail on hepatitis A virus and measles virus, we've published both of these, and our measles virus, as I've told you yesterday,

vaccination is not an option to maintain the titers long-term, it's just not feasible.

West Nile virus, I think there is more in that story because we've learned a huge deal, it's really important here to see the seroconversion of the United States, I think that's a fairly unique event that a totally seronaive population is witnessing the introduction of a totally new virus.

And you could see the seroconversion very nicely, which brings me to believe that actually IGs could be used as a sentinel, and then this paper here, we've actually tested your European plasma-derived IGs for West Nile virus antibodies, and were able to see circulation of West Nile virus in Europe before the circulation -- before the surveillance systems picked up, that there was really a West Nile virus circulation; because you can, with one IG sample, survey potentially 5,000, 10,000 donors.

And then is also important to take a look what the subclasses do, because not in every manufacturing process every subclass is translated into a final product, and as we've

shown here, this is IgG1 that is dominant with respect to function. For CMV, we've done similar work, and it shows that IgG3 is more important there.

And then the data that I've just shared with you on human adenovirus 5, parainfluenza virus and respiratory syncytial virus, Maria is smiling up there, because she's going to write it up soon. Thank you very much. (Applause)

DR. BOYLE: Thank you so much, Dr. Kreil. And now, as I switch PowerPoints here, I'd like to invite Dr. Mel Berger of CSL Behring, to come on up. Dr. Berger is going to talk about vaccine-induced antibodies in IG products.

DR. BOYLE: Dr. Berger?

DR. BERGER: Thanks, John. So, I'd like to thank the organizers for inviting me, and all of you for staying. And there's a couple of disclaimers, this is my own opinions and not official positions of CSL.

And so I was asked to review antibodies against vaccine antigens, and that's what I've done, or that's what I've tried to do, and so this is a paper from Montreal in which the

investigators looked at 44 lots of one product from one company, and looked at antibody titers by ELISA, and what you see is pneumococcal antigens going across the first two major rows.

And you see that the coefficient variations of the different lots are all less than 25 percent. So, I think there is a protective level of antibodies, and there is not very significant lot-to-lot variation. At the bottom we look at tetanus diphtheria, and Haemophilus polysaccharide, again, these are all ELISAs, again, if we figure out what this would mean to a patient, and the patient would end up with protective levels even with the lowest titer lots.

For tetanus there's a little variation, a little higher coefficient of variation, but otherwise diphtheria and Haemophilus, diphtheria is a little high at 29, Haemophilus is

percent which is certainly in the same range as all of the pneumococcal titers, whether they are in Pneumovax or Prevnar, and I don't know the total time range over which these 44 lots were produced. But certainly based on this, if I was

in practice I would not be calling a manufacturer and saying, can I have lot 17 because it's better.

Now, this is some work from Korea looking at ELISA titers against Haemophilus polyribose phosphate, the surface polysaccharide of type B Haemophilus, the antibody titer by ELISA, and the serum bactericidal index using a standard complement source.

Again, you see that the coefficient of variation is quite small, but these are multiple lots of two different Korean products, each line represents a different lot. You see that for ELISA against the Haemophilus polysaccharide, and for the serum bactericidal index, again with an external source of complement, all other lots are quite tight. And they also looked at pneumococcal serotypes, and I don't know when the plasma for these products was collected versus when Meningococcal immunization was introduced in Korea, you see a slightly greater range of titers, but still most of the products are protected against most of the serotypes.

There's only about one exception which I'll show you on the next slide. Again, all the

Meningococcal sero groups, all the Meningococcal results here are serum bactericidal index using rabbit complement as a common curing reagent. So, this looks at the, just a mathematical manipulation of the results on the lots, are showing, again, the concentrations of antibody to the polysaccharide by ELISA on the left, bactericidal index on the right using rabbit complement.

And you see again, the different lots, and there are two products here of different companies, but they are all quite good, and they are all quite well above the protective range, or the upper limit of protection which will be 0.15, and 4 or the serum bactericidal index, exactly how they calculated those numbers I don't know.

Now, this shows the serum bactericidal index, against the four different meningococcal serogroups, and again they look pretty tight but not quite as tight as in the previous antigens, and you see that several of the lots of the one product shown by the -- there's two products, right, one is shown by open circles, one is shown by squares, a few of the lots may actually -- two

of them would not have provided protection at the standard doses, and one lot is right on the border line.

So, this data is a little more spread and, again, confidence that if there was meningococcal type C, and the environment of and immune deficient patient you might think about raising the dose. This is another look at haemophilus titers, this is actually; Dorothy was associated with this work.

So, here each group of figures is a different, and although any given product has a quite narrow range of antibody content, you see that two of the products, would have given titers to a patient that are below the protective range. One of these heat-treated, and one of these is a product which at the time was treated with pH4 and pepsin.

So as Tom was pointing it out, these things are treated in different ways, and it may change the titers in some products, and so I think that product G is not available anymore, I'm guessing what that product might have been, product B I don't know about, but the other

products all would have given a patient protective antibody titers.

Now, this is the same hepatitis A data that Thomas showed, and my interpretation of it is exactly the same as his, which is to say if we use something which had a certain rate of natural infection, which has been reduced by a vaccine, the antibody titers in the plasma pools will drop and the antibody titers in the products will drop.

So, I think that as far as the standard, saying what can we use across time, to see that our products were all potent? Something that for which a vaccine is developed, I think probably loses its value as a standard.

Now, this is just to remind me that I'm going to transition over to influenza, and look at the question that patients frequently ask me, and I never really looked up the answer until preparing for this meeting.

But the question goes something like: well, if the process from vein to vein takes a year or more, and if we are supposed to get a new flu vaccine every fall, then how am I protected against the flu by last year's vaccine? And I

would say, well, I think there's possible -- first of all, it's possible that you may respond to proteins better than polysaccharides, it's possible you may respond to the vaccine, so take the vaccine, the benefit, the possible benefit of the vaccine outweighs the risk, but most importantly be sure every one of your immediate contacts gets the vaccine. That would be the most reliable thing I could say.

So, this shows both group cohorts against the -- with antibody titers in the donor pools of different age groups, and this is the birth decade, and you see that people who were born in 1910 to 1920 had higher titers against the 2009 pandemic virus. And of course the reason it was the 2009 pandemic virus, is because people born from, let's say, 1950 or especially 1980 to 2009, have very low titers. Right?

Depending on the donor pool the IVIG might be still provide robust protection. This is to look at variations across lots, and what you have here is the symbols represent different viral strains, and these are all type A, H3 and 2 viruses, and what you see is, in the top

hemagglutinin inhibition assay, and in the bottom, these are culture neutralization assay.

And you see across lots from -- this is Japanese venoglobulin, you see that across lots, the titers tend to be quite consistent. You also see that looking at the common antigenic drift from year to year; there is protection of one year's strained against the drift in the next year string. So, the protecting gradually goes up, here and over the course of about five years.

So, this would be my answer to the patients: If we see a normal annual antigenic drift you would be protected by IVIG made from plasma drawn a year or so ago. Now, this is looking at -- some data looking at what would happen with pandemic strains, and this is looking at -- and I put this data up, in order to make a couple of points, one is to show that the protection of IVIG against influenza is from the F(ab')2 part the antibody binding -- the antigen binding part of the antibody, not the glycosylated FC, because there are some papers which claim that there's a kind of nonspecific inhibition of influenza viruses by the FC, which

is probably really competition in the neuraminidase assay.

And so what you see here is ferrets being nasally inoculated with H5N1, and you see that the IVIG is quite effective at getting all the animals to survive, the diluent is not effective at all, and all of the animals have succumbed by day 4. On the right, you see that this is conveyed by the F(ab')2 not by the FC, which is in blue.

Now, this looks at the hemagglutinin inhibition titer of different viruses, the pandemic H1N1, the seasonal H1N1, a type B, and the H5N1, and again, maybe I shouldn't have used the word inhibits here, this is a hemagglutinin inhibition titer, and you see that there is antibody against both the pandemic H1N1, and the standard seasonal H1N1. And one of the IVIG preparations here was prepared in 2004, which is shown in the black dots, and the 2009, before the appearance of the Swine H1N1, which was supposed to be pandemic that year.

So, patients had antibodies against the H1N1 strains -- but not patients -- IVIG and

antibodies against the H1N1 hemagglutinin, but not against the H5 hemagglutinin; and so this next slide shows that IVIG inhibits the neuraminidase and the neuraminidase assay. So, what this means is that when we look back at the protection of the -- against infection we had protection by an antibody by an IVIG preparation which locked the antibody against the hemagglutinin, because the protection, as it turns out, is conveyed by F(ab')2, against the neuraminidase.

And it has nothing to do with the hemagglutinination inhibition, which of course is the most common assay used if you just say that somebody respond to the vaccine. So, this points out a way in which a functional assay can differ from an antibody titer. Okay, which is the point; this is one reason why we should be looking at functional assays, not just binding assays.

This again, looks at antibodies against a pandemic 2009 H1N1, classical swine H1N1 in IgG prepared, this is so -- it's the pandemic 2009 virus looking at IVIG prepared from plasma obtained before 2009, and you see different lots here are prepared -- the three top lines are

prepared in Japan; the two bottom lines are prepared in the U.S. Again, there was preexisting antibody against the 2009 pandemic H1N1, which is shown on the left. And there were antibodies that were potent both in hemagglutination inhibition, and in viral neutralization.

So, about influenza, plasma from adults and IVIG made from it, contains antibodies with broad specificity, against seasonal influenza strains that have normal year to year antigenic drift. Antibodies against the reassorted pandemic Swine A, which appeared in 2009, were already present in plasma pools obtained before that virus appeared.

Some influenza viruses can be neutralized in biological assays, by antibodies to the neuraminidase, even if antibodies against the hemagglutinin are not detected in the standard hemagglutination inhibition assays.

And the overall, my overall conclusions are that lot-to-lot variations in titers against most of the antigens, are within about plus or minus 25 to 30 percent, and since for most of these

things, or for many of these things, we are doing twofold dilutions. This represents less than one dilution or, in other words, it's probably insignificant.

So, regardless of the titer, and in an individual lot, most immune deficient patients will achieve protective titers on doses of 4 or 500 milligrams per kilogram every three or four weeks or so. Antibodies against haemophilus polysaccharide may provide a useful potency standard. We've had the vaccine now for some time, that's a very immunogenic vaccine; immunologists don't often use the conjugated haemophilus PRP vaccine to evaluate immune function, because it's very immunogenic.

And we do have at least serum bactericidal activity, and I think Moon is going to tell us probably about opsonophagocytosis assays. Immune deficient patients probably do get protection against seasonal flu from IVIG, due to exposure and immunization of the donor pool with previous viruses which differ only by the conventional antigenic drift.

And so I was asked to review antibodies

against vaccines, and that's what I've tried to give you with at least some interesting data about. If I was asked a different question, which is: if I was sitting in this seat, what would I recommend as a standard? I think that -- I certainly understand and I hope I've illustrated at least one situation where the binding and the functional antibodies can be quite different.

So, I certainly agree with the point of looking at functional antibodies. I would say what we need, is something for which there is not a vaccine, and no plans for vaccine, but a high prevalence in the environment, and maybe CMV meets that criterion, and although most people don't think much of CMV, we do have, you know, pathogenic consequences, especially in immunosuppressed transplant patients, in pregnant women, and so on.

So, thanks very much. And I guess we are going to have questions later. (Applause)

DR. BOYLE: Thank you so much, Dr. Berger. He's coming on up. Dr. Ricardo Sorenson, thank you so much, of Louisiana State University. He's coming up to talk about

pneumococcal infections, pneumococcal immunity assessment and recommendations for IG product evaluation.

DR. SORENSON: Good morning and thank you very much for giving me the opportunity to share a few thoughts about the topic that I have thought a lot about from the clinical side. I will briefly go over something that we need to really consider when we think about what should be in a gamuglobulin, pneumococcal infection and antigens and immunity. How do we evaluate this evaluation and IG products.

I will offer you a few conclusions of my own. So, infections with pneumococci are quite varied so again that poses a problem because which antibodies or which strength are we going to look at. Over 90 capsular serotypes based on the shape of the surface polysaccharides but there are some serotypes that have variable virulence and antibiotic resistance. They once said that the most frequent ones of the serotypes that are in the 23 virulence polysaccharide vaccine and the ones of those that have developed antibiotic resistance, those are the ones that go

into the conjugated vaccine for good reason because they are the most difficult to treat. The two vaccines that we have available is the 23 valent, PPV-23 from here on, and then conjugate vaccines. They have been produced by different manufacturers and the conjugate has been always this polysaccharide conjugated to a different type of protein. That has led to the fact that the conjugated vaccine 15, at the bottom there, is not on the market. Because the requirement is that the antibodies to the serotypes in the PCV-15 have to be equivalent or better than the ones in the PCV-7. Somehow when you have 15 coupled to another type of protein that is now used, the antibodies to serotype 6B and 9V have not been equivalent.

So, the infections, this is also something to consider because there is some difference in the amount of antibodies that we need to protect against mucosal infections, otitis media and sinusitis, against global pneumonia and then against invasive infections and I will explain that a little bit more. For us clinicians, the big problem actually is

sinusitis. Because that is the hardest to treat type of infection.

So, what antigens are involved here. These pneumococci have a very specific shape and there is, as you can see there, capsule, a cell membrane and a cell wall. I will detail that a little bit further. The antigens that are relevant are these polysaccharides. These little figures are supposed to be polysaccharides and that are on the capsule of the protein. The antibodies that we measure in the vaccines are based on developing antibodies against these polysaccharides. They are also proteins but they are not being considered anymore although there was a lot of interest in developing an anti-capsular protein vaccine which would happen easier than developing vaccines against polysaccharides. But the way it went was to conjugate the polysaccharide to a protein and that sort of facilitates the response to the conjugate of polysaccharide and protein.

The other element of interest that we will mention in this figure is this C-carbohydrate, C polysaccharide. Although it

is not on the surface of the bacteria and therefore it is not a target for antibodies, it produces a lot of antibodies after natural infection and it can confuse what you are measuring depending on how you prepare the antigen for the assay that you will use. This is a very old slide and it just tells you that neutrophils and bacteria ignore themselves unless there is compliment and antibody. So, it is very important to have those two elements present. That is shown here. This is also a very old slide, centuries, last century and it shows that antibody alone it uses phagocytosis but that this is accelerated very much if you add complement.

So, this is a little addition when we get to the end because depending on the sub class that you have, the interaction with complement may be different. That will make it different, will make the potency different. Here you see an IgG interacting with the surface and (inaudible). I show these slides because they are neat. I don't know how they get them done but it shows that what we draw is actually visible with the right

technology.

So, let's get to the key issue. How do we evaluate immunity. And where do we use, why is this evaluation of immunity against pneumococci particularly important. It is obviously a key for industry developing vaccines and they can discuss endless of hours, I have witnessed, about which assay and how good this vaccine is versus the other vaccine. It is difficult discussions but they have contributed significantly to one element. They have concluded already that opsonophagocytic that Dr. Nahm will discuss in a while is the way to go. Because all other tests are not completely reliable but I will document that a little bit better.

Our, the immunologists, the clinical immunologist's interests in these antibodies is because they are measuring antibodies against pneumococcal polysaccharides, is a key part of the evaluation of some of the antibody mediated immunity. We will measure immunoglobulins and the other thing that we measure is antibodies against individual serotypes of pneumocox.

Because we have a multi-valid vaccine so you can give one vaccine and measure up to 23 serotypes and because there are some things specific about these antibodies. We have that going on for many years and the progresses mean that it has become totally confusing by now. I will explain what I mean with that but it is something we are learning about the validity of the different tests that we can use.

And then, obviously, the idea here is to see how can we evaluate IgG preparations for their potency against pneumococcal antibodies, pneumococcal infections. Which are the main targets for measuring antibodies. It is purified vaccine serotype polysaccharides isolated and prepared individually. So, what is easy to obtain commercially because it is the serotypes that go into the 23 virulent polysaccharide vaccine are those same 23 virulent polysaccharides. They are easily accessible, you can buy them. They're expensive but they are available.

In many places, in Europe and in many countries, instead of measuring antibodies

against each single polysaccharide, they talk about the global test where they use the antigen, the entire mixture of the 23 serotypes that are in the vaccine. I'm going to be done with that because it is no good. But it is still promoted. I have recently reviewed manuscripts but they still say this is the way to go because it is much cheaper. Surface proteins which, I think, would be of interest, have really disappeared. I don't know anybody that for vaccine or clinical uses or gamuglobulin uses is measuring antibodies against proteins but that may be something we should actually do.

Now which are the methods that we use to measure IgG antibodies against surface polysaccharides on pneumococci. They are basically one which we call the WHO, ELISA, meaning World Health Organization ELISA. That defines a specific way of performing the ELISA, I'll get back to that. The multiplex that was addressed by Dr. Hajjar, was thank you very much because you saved me a lot things here to say and I fully agree with the work that you did. And then there is this global test that I already said

is no good.

We measure conventionally, the IgG because this is what provides long term protection. And in the case of gamuglobulin, this is what goes into gamuglobulin. Although IgM and IgA are also developed and some patients that are unable to go to develop IgG actually can develop a very vigorous IgM response. But it is of no help for the discussion today. And here, figures of four different polysaccharides and the point I want to make is they are different, obviously. They are biologically different and the fact that an individual is able to recognize this structure and develop antibodies to this structure, must not mean that they also will develop antibodies to the others. Obviously, since we have such a large number of polysaccharides, 23, everybody has been interested in identifying if an antibody against one of these polysaccharides is always representative for what will happen with antibodies to the rest. For instance, if the response to serotype 14 is good, that means that there will be antibodies against the other

polysaccharides. But that is not the case. At this point, nobody can identify a single serotype, a single polysaccharide where at the antibody presence or absence is sort of a prognosis for a response to all the others.

Here is again, the capsule of polysaccharides. There is one thing, the group carbohydrates that I showed you, need to be absorbed. Any lab that doesn't do this will measure antibodies to polysaccharides that are not on the surface. So, they have no protective value. You also need to observe the serotype 22F because there is a lot of close reactivity. This is a slide showing that you may have, if you do not absorb the antibodies against polysaccharides, here you have these high concentrations of antibodies after absorption and this case, against polysaccharide 22F decreases significantly. In the new testing that it is not clear how many of the commercial labs are following this process or not and it may be part of the differences that Dr. Hajjar has showed us. I don't know if you've had a chance to ask them if they did the absorption step or not.

So, the WHO ELISA is the test that we prefer because it was developed for the introduction of the conjugated vaccines. The first 7 valent conjugated vaccine was introduced about 20 years ago, it was very important to have a test that could be reproduced in every place of the world. And the World Health Organization invited 22 labs to participate in a very extensive study. We participated. And all I want to tell you, this is the best described test ever. The booklet to perform the test is this fat and everything down to the pipette you use has to be standardized. It is the results are all calculated on an FDA provided reference sample. The FDA 89-SF. In the meantime, most labs have produced their own reference but it is based on this reference sample. The results are expresses in micrograms per ml.

And here we get to the first tricky part. We immunologists, for reasons that I won't bother you with but translating results from all tests that were based on measuring antibody nitrogen with radiation. We derived the number 1.3 and said that's what we need. A few people in

the OS and the infectious disease side in other countries agreed that 1.3 has anything to do with real protection. So, that is for the infectious disease group, it's.5 or even.35. The difference is that it is much easier to protect against invasive infections even at pretty low concentrations of antibodies you don't get bacteremia, you don't get sepsis, you don't get meningitis. In the very large study of 22,000 kids in California that approved the first conjugated vaccine, that was enough. Getting to those levels was enough to offer very significant protection against invasive disease. As I mentioned to you for immunologists, we want to get rid of otitis and of sinusitis. And there, it is our clinical experience but that has not been documented in any further detail is that you need 1.3.

The multiplexed bead-based assay that Dr. Hajjar described is not described in the same detail as the WHO ELISA and that's a big problem. Because we don't know how they are being done. Here is one very brief table of what Dr. Hajjar said, LUMINEX 1, we're calling in short LUMINEX.

One LUMINEX, two ELISA inability. This was an attempt to find some form of functional assay. If you just look at any, let's say here, 18 C, 1 LUMINEX 0.5 the other one 4 ELIZA.68. So, what is the diagnosis. We do this test to diagnose specific antibody deficiencies. I will tell you something else. We have rules that the insurance companies read and then they tell us you cannot use gamuglobulin. But the problem is, if you study a normal population, we have done it in children in the Netherlands, they just published a paper on adults. There are as many patients that have a deficient antibody production as the adult, as the healthy control population. Healthy people, among the healthy population, you get as many individuals that have a specific antibody deficiency. So, that puts in doubt the whole issues of (inaudible).

So, here is the comparison of the WHO and the LUMINEX. The problem with the WHO ELISA is that it is cumbersome and that is why the commercial labs have all gone to do LUMINEX. But you see that that's a problem and I'm running out of time. The important test that we need to get

to is opsonophagocytosis. Dr. Nahm will explain this in much more detail. In general, when the OPA, that's how these things are called, the vaccinologists have OPA on all sides. When the OPA is compared to the WHO ELISA the results are generally very comparable. But here, opsonophagocytosis, if serum is involved, it involves IgM, IgG and IgA antibodies compliment phagocytic cells and antibodies to polysaccharides and proteins. So, it involves different targets than ELISA WHO. If you do IgG preparations as only IgG antibodies, this (inaudible). The problem is that what Dr. Berger also mentioned for other situations, there is not a very strict relationship between the weight by volume ELISA and the opsonophagocytosis.

This is the classic paper by Dr. Sandra Steiner from the CDC. What it shows that if you have adult controls and elderly, the ELISA titers in the light bars in the elderly, I don't want to tell you about what it is but it is not good news for us, are very good but our opsonophagocytosis

titers are very bad. In (inaudible) individuals they are the same. And clinically, we see patients that have excellent ELISA titers against all pneumococcal serotypes. They have many infections like described by Dr. Maglione suggestive of antibody deficiencies. And if we can trick the insurance company to allow us to give gamuglobulin, those patients improve.

So, in the end, our antibody testing ability is poor. I will jump over this because let me get to the summary and conclusion since I think I am over time. What I think that needs to be done, I'm very impressed by what was done in the collective worldwide effort to establish the right kind of antibody measure meant after vaccination with the conjugated vaccine. Something similar for the opsonophagocytosis needs to be -- first of all, it has to be opsonophagocytosis because I hope I provide you even more evidence that right now our antibody testing is no longer reliable. I have fought with my colleagues in all the meetings of immunologists saying that the rules that we are writing are incorrect and we are making it

difficult for our patients to get gamuglobulin. Because the insurance companies say okay but they have antibodies so you cannot give gamuglobulin. I think that is a mistake. Opsonophagocytosis needs to be standardized with as much detail as the WHO ELISA with universally available control serum samples, pneumocultures and phagocytic cells. We have to select some relevant serotypes and there should be a reliable phagocytic cell line. Thank you.

DR. BOYLE: Thank you so much, Dr. Sorensen. Now finally for this first part, I'd like to call up Dr. Moon Nahm of the University of Alabama who will be talking opsonophagocytosis, as has been mentioned before, and other methods to detect functional antibodies against incapsulated bacteria. Dr. Moon.

DR. NAHM: Good morning. Thank you very much for inviting me. It's a real pleasure to be here and talk to you all. I am Dr. Moon Nahm. I'm from the University of Alabama at Birmingham. I first must acknowledge my lab group who have been essential for me to maintain

the reference laboratory for NIH as well as WHO. And also, for the materials that I will present today.

Before I begin, I'd like to disclose that our university is licensed to be various reagents to commercial entities but my work has been supported by funding from various sources including from funding from NIH for a long time.

Today, I'll be talking about pneumococcal OPA for IVIG. I will talk about three different aspects. One is the need for OPA and the other one is a development of a multiplex OPA and then the last one, the application of OPA to IVIG. I don't have to spend too much time on this. You all know that the pneumococcus is a well-known gram positive bacterium that reside in the nasopharynx in most individuals without causing any disease but find a susceptible individual that invade to deeper tissue causing pneumonia, sepsis or meningitis.

These diseases also with high morbidity and mortality and so a large number were old adults and young children die of pneumococcal infections. The striking feature of

pneumococcus is that it has a carbohydrate capsule that shield the pneumococcus from the host immune system and the pneumococci can proliferate and cause disease. If we have antibodies to carbohydrate capsule then antibodies can fix compliment and then opsonize the pneumococcus for host of

(inaudible) which can then recognize, ingest and then destroy the bacteria.

Following this principle, we have developed two different types of vaccines that are designed to induce antibodies in our body against a capsule. The polysaccharide vaccine which has been available for a long time, contains the capsule polysaccharide from 23 most common pathogenic types. This vaccine is only useful for adults but not in young children and therefore, people spent a lot of effort to develop so-called conjugate vaccines by conjugating capsule polysaccharide to carrier proteins. This conjugation process is difficult to achieve so the first product had only seven polysaccharides, now the currently used

conjugate vaccine has

different serotypes. This vaccine turned out to be extremely effective in young children and is widely used throughout the country.

Now, as we developed this vaccine, what we noticed is that sometimes pneumococcal antibodies that are detectable by WHO ELISA do not work. This is illustrated with the antibodies 19F and 19A. As you may know, 7 valent conjugate vaccine contained in 19F polysaccharide and then did not contain 19A. Because it is assumed that these polysaccharides are so similar to each other except at this group right here, that the 19F would elicit a cross reactive antibody to 19A and then provide cross protection.

Indeed, when you measure the antibody levels using WHO ELISA, we couldn't demonstrate very high levels of a 19A antibody. But when we studied it the same sera, the sera from children immunized with PCV-7, we found that the opsonic titer against 19A was almost zero. So, based on this, we predicted that PCA-7 would provide no protection against 19A and that clinical

experience with this vaccine confirmed that prediction.

So, as a result of this, vaccine developers clearly recognized the important of OPA so there has been a development of opsonophagocytosis assay. As you know, the classical OPA is done in a test tube that contains a target bacterium along with the phagocytes such as neutrophils and complement which is usually obtained from rabbit and then test serum which is obtained from a vaccinated individual. And then incubate this make sure for a while and then the bacteria will be opsonized by antibody and complement and destroyed by the phagocytes and then determined number of surviving bacteria by plating the reaction mixture in an agar plate.

But this method is not an easy one. It is very tedious to do the assay because you have to count a large number of colonies manually and also you have to do this assay for 7 to 13 different times because the conjugate vaccine contained many serotypes. Also, the vaccine evaluation is done in infants who can give only small amount of serum samples. Doing all these

tests with these tiny amounts of serum samples was an impossible test to do.

So, the classical OPA was deemed it to be impractical for vaccine studies and was not used. Some time ago, I was thinking about the cross protection against the 19A and the 19F and came up with the idea for multiplexing opsonophagocytosis assay. We call this multiplex OPA as MOPA. The way you do it is you have two different target bacteria test tube. One is a (inaudible) to penicillin and the other one is a (inaudible) to kanamycin and then do the opsonization assay altogether but plate the reaction mixture in penicillin plate and kanamycin plate and then you can throw out the different serotypes.

This idea can be generalized to many different serotypes. I have been working more than 20 years to develop a multiplex opsonization assay that is practical for vaccine studies which we have attained now. I have to say, over the years, I have been communicating with the people in the IVIG field and I had to tell them that the assay was not ready but now I think the assay has

advanced enough and may be useful. So, we had to, over the 20 years, develop the multiplex assay

(inaudible) multiplex assay for many different serotypes. We have assays for more than say 30 different serotypes and the assay throughput is extremely fast. I would say our assay throughput is as fast as ELISA, so it is extremely fast. We have been improving the robustness of the assay and we have been gradually automating and the assay is now becoming standardized.

As a result, the FDA licensed the PCV-13 for adults based only on the opsonophagocytosis assay results. And then that required (inaudible) at all. So, the problem with the OPA is that there is a significant lab to lab variation. The vaccine field tried very hard to remove this variation. So, they created a reference sera and a calibration sera so there is a new reference serum called, 007sp. This is replacing 89sf you have heard about because 89sf

is now gone and you it is not available so you will have to use 007sp. And then the international collaboration determined the impact of a standardizing OPA with this reference serum on interlaboratory assay variation. It took several years, about five years, involved six different labs from three different continents. We published the results recently. The first thing we worried about is when all six laboratories analyzed one serum sample, in this case, 007sp, we were worried that we would have a wildly different result because of the different labs that are using different assay results. But the collaborators were very surprised to find that OPA results by six different labs without any prior attempt to standardized produced the reasonable comparable results.

And then, we decided to apply a standardization. The idea with standardization is really straight forward normalization. That is, in each assay, we included this serum 007sp and we also added a test sample and the results of the test sample is normalized against the

result of the standardized serum using this basic equation shown here. And then impact of standardization was very stark. Here is the distribution of the results by six different labs. The X axis shows the consensus values for these about 20 individual serum samples. The Y axis shows individual values reported by individual labs. After normalization, after standardization, as you can see, there spread becomes very, very small, indicating that standardization helps a great deal.

So, with this, MOPA has come a long way. We know that we have standard serum available and we made a huge amount of 007sp so that it will last many decades. Now Korean FDA is involved in creating a calibration serum set maybe about a dozen or two dozen serum sets so that they have assigned values and they can be used to establish assays in one's laboratory. We still believe that there is room for improving assay precision and my laboratory is now trying very hard to improve the assay precision. Just to give you a rule of thumb in precision of the assay, I would say the CV would be in the order of 30 to 40

percent. I'd like to bring it down to about the 10 to 20 percent range. With 30 to 40 percent CV, that is the equivalent to the classical way of thinking, say one titer above and below. We like to make our assay better than the classical one titer above and below.

We are trying to correlate the OPA values with the degree of protection but I know to do this, I will have to work with you if there are any other people, clinical immunologists who work with us, to create the threshold of values for protecting against different diseases or different age groups. By the way, I heard that the OPA values are low in older individuals compared to younger individuals. We investigated this issue and what we found was that old people do not make IgM antibodies. So, when you measure only IgG antibody levels, then young and old people look fine. But when you measure the functional capacity, IgM antibodies contribute quite a bit and as a result, old adults look to have less opsonic capacity. But if we remove all the IgM from young and old then their opsonic titer is equivalent. In the future we can monitor primary

immunodeficient patients on IVIG.

So, in summarizing, I told you that there is need to develop OPA assay during the vaccine development. We have been working to develop multiplex OPA for a long time. Now we have a fairly robust high throughput assay. The protocol has been posted on our website in extreme detail. It's about 50 pages long and it is so popular that we published it in four different languages. So, you can always check our website and get the protocol. By the way, we also wrote the WHO ELISA protocol so these two protocols are on the same website. I told you about the remaining issues for OPA.

Now, just to briefly touch on the application OPA on IVIG but I don't think I really have to talk too much about it because this has been -- these two studies have been mentioned already. There was one U.S. study done in 2004 by Dorothy Scott along with Carl Frasch. They studied the five lots from seven manufacturers and this is the data summary involving OPA. What I noticed was that there was a significant manufacture to manufacture variation. Now, with

the recent Korea study, this study was done by one of my ex-students, Dr. Kim. She studied pneumococcal, meningococcal and the hemophillos antibodies using functional assays. She found that and she studied the two IVIG preparations available in Korea. The IVIG preparations had relatively high titer of opsonic activity for many different serotypes.

In summary, I think there is a lot of OPA's ready to be used for IVIG studies and I'll be happy to help any group for these applications. Thank you very much.

DR. BOYLE: Thank you so much, Dr. Nahm. As you can see, we're off by a few minutes but we are going to stick to schedule as this room is no longer ours at noon. I think we're talking about a 10 minute bio break with the speakers getting the restroom first. But if the speakers can be up here and mics on at 11:05, we're going to rock and roll from there.

(Whereupon, at 10:55 a.m., a recess was taken.) 11:05 a.m.

DR. BOYLE: Dr. Scott will be here in a moment. As you can see, we are tight on time.

For those of you who did write down questions, and they are ever so relevant questions, if you can try to work them in to the three questions that we'll be asking here today, that would be enormously useful. So, we'll give things just one more moment here but again, as you have questions, let's try to wrap them into the questions that we're here to discuss today. If I can remind everybody that the first question that we're going to be addressing is, which antibody specificities are relevant for PI patients. Among these, please comment on test method availability and robustness. Keep that in mind for one more moment. Mark.

DR. BALLOW: Mark Ballow, from USF in St. Pete, Florida. I have two questions that are related to that question. I want to ask PJ this question. You presented the frequent infections that CVID patients and SKID patients have historically by pulling out the literature. The question I have for you is that patients on replacement gamuglobulin, adequate replacement gamuglobulin, do we have any data on the types of infections both bacterial and viral that those

patients are prone to? I'm not talking about GI because that's a different issue. Norovirus and salmonella and stuff, that's a different scope. I'm talking about sinopulmonary because that may tie into Dr. Kreil's work that he presented about what might be suitable as a potential pathogen to look at too standardized in products. That's the first question.

MR. MAGLIONE: So, I think that's, of course, a really, really good question. As far as the data that I'm aware for what happens post IgG replacement. We know that there is good data on pneumonia improves. It seems like there are a couple of studies that have really shown that. I know from the experience that I've had with Mt. Sinai and as fellow with Charlotte and now seeing patients there as well on my own, sinus infections seem to still be a regular issue. I think that the things that we expect -- when we discuss with our patients, I can say what we say and hopefully it's the same things you're saying and if not, please I would definitely want to learn from it. We typically advise that severe infections, things like sepsis, pneumonia, those are the

things we expect to improve with the IgG replacement. Sinus infections are a bit more difficult and I think the expectations are a bit different there. I think those types of infections persist.

Now, the types of infections, in terms of whether these are viral exacerbations, bacterial, I don't think has really been great studies for that. There are a few studies in Europe that have looked at evidence that we are probably under recognizing the importance of respiratory viral infections. I think that is probably a good point for discussion here further whether we really should be considering some of those as possible things. That maybe that IgG replacement antibody levels are inadequate and maybe that's why we see some efficacy in terms of a biologic trough and increasing the dose. Maybe you're getting higher levels of those titers that are not included as highly or not being really evaluated as much. I think the short answer is there is a lot that we need to do there and hopefully we'll do those studies.

DR. HAJJAR: If I may to add, I echo PJ.

So, we recently we're funded by the U.S. immune deficiency network to look at this specific question and hopefully will be presenting our findings during the AAAAI meeting in the upcoming spring. We found exactly what PJ was talking about, streptococcus pneumonia, hemophillos influenza and Moraxella keteralis continue to be the pathogens in patients with CVID who are actually on immunoglobulin replacement therapy. Obviously, this is data from the US IDNET so this is not immediate chart review but it gets us as close as possible to the U.S. population. We have some more data that hopefully will be coming out when the publication is ready but I think it speaks to the same findings and we found both pneumonia, otitis media and sinusitis, those pathogens were kind of common.

DR. GOLDING: Just to follow up on that, this is Basel Golding, FDA. I'm wondering based on the last session and the fact that sinusitis is a common clinical problem, in our trials designs that we fall licensure we haven't included sinusitis because of the difficulty of diagnosis as acute and chronic and so on. That

we haven't used that as a clinical measure of efficacy of the immunoglobulin. But now that we have tests like the OPA or MOPA, I'm wondering if levels of those antibodies in the patient on treatment, whether that would be a good surrogate that we should consider measuring in our clinical trials. Is there data out there that correlates, for example, sinusitis and measuring of MOPA or OPA against strep pneumonia.

DR. NAHM: That's a very good question. We have been busy in the vaccine field so we developed the assay and applied the assay to the vaccine field. The immune deficiency field is really virgin territory. I am very happy to collaborate with any of you to advance in this area.

To supplement what I told you, I focused on the pneumococcal OPA but this OPA can be adapted to any gram positive bacteria and also the same technology can be adapted to bacterial assays such as gram negative organisms like a hemophillos, meningococci, shigella, whatever. And the assays are extremely efficient. This assay is a very broad analytical platform that

should be applied to primary immune deficiency area.

DR. BALLOW: My second question has to do with a statement that was made that some of the specific antibodies to the virus has fallen to different IgG subclasses. Like CMV was three and I can't remember, adeno was one I think you mentioned. So, I think this may be important because IgG three has the shortest half-life and we've certainly seen in some patients, in fact, some of my colleagues actually use IgG 3 as a surrogate marker for adequate immunoglobulin replacement in patients with immune deficiency.

So, it might be important to choose a surrogate marker that actually falls within the IgG subclass, not only for lot specificity and control but also for treatment. To ensure, for example, whatever the respiratory virus whether it's para influenza, RSV, I don't know which subclasses they tend to favor but it may result in a more clinical efficacy in some of our patients who are experiencing continued respiratory tract infections.

DR. SORENSON: Just a comment, I don't

think there will ever be a reason to check immunoglobulins for those antibodies. But I do agree with you that we have to find only one type of specific antibody deficiency. Which should be called SPAD, really because it is specific pneumococcal antibody deficiencies. Antibody deficiencies that cause severe infections against all kinds of other viruses and bacteria, certainly are very important but nobody has accepted them as a diagnostic entity but we know they exist. And there, I don't know if we can really expect the industry to provide us with the titers against all these viral pathogens that were mentioned. There are patients that do not respond to Hepatitis B, for instance, although it is not necessarily causing disease. There are patients that do not respond to very specifically against some pathogens.

I think it is a diagnostic issue and I would stay away from asking the industry to provide everything that they

(inaudible).

DR. BALLOW: Ricardo, that's not what I was driving at. I was driving at a potential

pathogen to replace measles antibody as a surrogate marker for lot to lot potency. Why not choose a potential surrogate marker that really may have some clinical relevance. Obviously, measles probably doesn't in our population but it may be that adenovirus or Para flu or RSV could not only serve as a marker for lot potency but also have some importance clinical relevance as well. That's what I was driving at.

DR. SCOTT: I guess go ahead.

DR. KREIL: So, the one comment I would like to make is, I hope I have not mislead the audience to believe that I've suggested that any of these three candidates is ready for prime time. I think there needs to be a search, long term, to do something else but Polio because Polio really is not in circulation. Measles circulation is very, very limited. So, long term, I do believe that there will be a need to come up with a replacement and quite obviously it would be interesting to have something that would not only ascertain biological activity of every lot. But even be more meaningful beyond that, I do not believe we're at the point where we have enough

information to make that choice yet.

DR. SORENSON: Actually, I would suggest that you pay attention to the clinical side of things too. Because friends, I could tell you that antibodies to measles are highly efficient. We have maternal antibodies to cross that cross to placenta prevent the measles infection of the vaccine that produces a response. It is not the same for Polio. Measles is given at 12 months, Polio at 2 months because antibodies that the mother also has against Polio do not prevent the mucosal infection that Polio causes which is a different infection from Measles. It may be helpful to talk about those issues and see which type of antibodies are important for different situation and not every virus is the same, certainly. They are all very different in the way they cause infections and the way that we should use to prevent them.

DR. GOLDING: So, Dr. Kreil is saying that there is nothing really for prime time. What is the situation with CMV, would that be a good candidate? Is there a commercial assay for CMV and I'm hearing that IgG 3 is important, would

that be a possible candidate?

DR. KREIL: So, we and others have done lots of work on CMV as well. The reason why I've shown not to bring it up here is a discussion of which cell line you're using. You get different readouts if you're using endothelia or fibroblast cells. It is a question of which CMV virus you're using because there are certain lab adapted strains that behave totally different than other viruses have been reflective of the natural isolets. So, the experimental complexity around CMV, I think, has not been mastered yet. It is difficult to conceive for me how in a QC setting it will.

There are certain elements of it that we understand like IgG 3 seems to play a more important role for defense against CMV. On the other side, there is a lot of question marks around the assay systems.

DR. BERGER: I think you could pick an assay system and agree to standardized preparations or agree to that as a standard without totally settling the question, is this the most relevant for X, Y or Z clinical

infection. As a standard for a drug potency, I think you could decide, okay in and this, fibroblast line, you should have this titer. This would be a minimal acceptable titer in this fibroblast line or in this other cell line.

DR. SCOTT: I think it's a very good discussion about what exactly we're looking for. So, if we go back to the wish list, relevance is up there and that is because that is part of what potency assays are supposed to encompass. One argument that people have made is that almost any specificity is relevant if you can demonstrate functionality because that tells you about the intactness of the antibody.

But the historical selections were appropriate for the time, actually. We would prefer to have something that is at least moderately relevant to the primary immune deficient population. Maybe not the most relevant, certainly certain ones come to mind that we've mentioned. Strep pneumo may be a respiratory virus, para influenza, RSV, adenovirus, hemophillos influenza and CMV, are things that we've talked about more than we've

talked about other things. I should add influenza. It's interesting but maybe has its other logistical problems.

I would say this at the end but I think I should say it now. Maybe we can all think about taking a relatively short list and looking at the feasibility and looking at the assays and selecting some to test more generally among several different groups just to look at our products and see where they are and see how difficult or feasible the assays are. I agree that at some point, we're going to look at replacing these assays. That point is not going to be tomorrow but if we start to get the work done, we'll find out some interesting things and we might be able to make something that will help clinically or help the clinicians at least, improve their treatment with immunoglobulins. So, we want it all, we might not get it all but I think we should look for something where we can get it all if possible.

DR. MOND: I think one has to be careful in choosing a list that's not too short. Because you might compromise, you might choose something

that's CMV, or EBV, whatever you choose, to the detriment of something that might have a lower titer. So, you might select an IVIG that has a high titer to X and that might be to the detriment of having it may be a low titer to Y which is also clinically relevant. So, the list couldn't be too short. In other words, if you choose respiratory viruses or those that cause pyogenic infections, it might be to the exclusion of the enteroviruses. So, I think we have to be very careful with the list one chooses.

DR. SCOTT: Yes, thank you for reminding me about the enteroviruses because certainly those are pathogens of concern. I think we also have to consider the extent to which there is any evidence that immunoglobulins are helpful or useful in enteroviruses. So, in a sense, if they're not very helpful or useful, then that makes their potency test, that kind of potency test a bit less relevant like maybe Polio.

DR. BLAESE: One of the criteria I think I've heard mentioned several times is we should think of avoiding picking candidates where there is a vaccine or likely to be a vaccine

available for that particular agent. Because we've been through this with Measles and Polio and there is no sense in going down that road again if we can avoid it.

DR. SCOTT: The irony is, things for which we're developing vaccines are things that we think are really important. So, in some sense, we have Diphtheria which is a potency specification that gives very good titers all the time. And some vaccines might be capable of doing that, I think. Tetanus was mentioned, the HIB vaccine was mentioned, so there are some that probably aren't going to fall to a level that is considered ineffective in the immunoglobulins given at typical doses.

You're absolutely right, I think the bigger goal is to choose something that is not expected to fluctuate substantially over 10, 20 or even 30 years.

DR. GOLDRING: The thing about choosing something that is a vaccine has a practical advantage in the sense that I think our office of vaccines at the FDA pretty much know what they're doing. And in order to license a

vaccine, if the antibody is used as a surrogate for efficacy or part of the study, they need to have validated assays. So, if we look at the vaccines and the validated assays that are available for vial vaccines, we may find something that is relevant and feasible. I understand Mike's concern that we would like to choose something that when you vaccinate with it that you get long lasting antibody in your blood for a long time so we don't have a problem with the donors, the same problem that we're having with Measles. It's not a perfect world, we may have to choose something (inaudible) and relevant despite them.

DR. NAHM: And also, with the bacteria, there are some cross reactive species of bacterium that seems to stimulate. For instance, for hemophillus influenza, most adults are having antibodies to it because they are immunized to it with e-coli expressing similar capsule. So, there is a relatively high level of anti hemophillus antibody in adults even with or without the vaccine.

DR. SCOTT: I just have a question, Dr.

Nahm, is the cross reactive antibody as effective as the vaccine induced antibody or do we know that? Invitro.

DR. NAHM: So, that's a hemophillus influenza case. The hemophillus influenza antibody response is usually comprised of three IgG clones in a person. IgG 2 and the one is IgG 1. One IgG 1 and the one IgG 2 clone is specific to hemophillus influenza polysaccharide. But the third one is generally cross reactive with the e-coli K100 polysaccharide. And the K100 polysaccharide and hemophillus type e polysaccharide differ in one linkage group. So, they are very similar.

They examine the functionality of those two antibodies and it is true that the hemophillus specific antibodies are slightly more potent than the cross reactive antibody. But the cross reactive antibody seems to be sufficiently functional.

DR. SCOTT: Thank you very much.

DR. MOND: I have a question and it is for PJ and not related to any of those questions.

DR. SCOTT: Okay well, what I'd like to

do is stick to the questions as well as we can up here so that we can leave with good conscience a few minutes before noon. I want to ask the audience and the speakers if you have additional comments related to question one which is, which specificities seem to be most relevant for PI patients. I think we've heard a lot of useful information but any last comments.

DR. MISZTELA: Maybe this is a comment on Dr. Kreil's presentation. What we have seen that actually when we look at different plasma source from different geographic locations, you end up with varying levels of specificity. So, perhaps this is exactly what is needed for PI patients. I'm not being too specific about different subclasses of antibodies but best protection is obviously short when you have plasma donors from different geographic regions which go through different infections. So, perhaps this is something to think about a bit further. I speak on behalf of PP8. Obviously, we have a vested interest in making sure that plasma is available globally in Europe and in the U.S. As Dr. Kreil has shown, there are

differences in protection afforded between the regions.

This also relates to the session of yesterday where we were thinking of selecting high titer populations. So, my thought was, are we actually disadvantaging the patients if we only select for a specific population. Because it will likely that these people have some sort of specificities in their antibodies that maybe don't provide a cross cover. It's perhaps selecting for specific populations may not be a good idea. This is just a general comment.

DR. SORENSON: I could answer in part. I think we are not ready for that. We may be getting there because actually what we are seeing with genetic testing now is that you can't begin to specify which infections agents affect certain types of genetic defects. Down to incredible specificities where everybody that has salmonella gets one type of (inaudible) deficiency. But then there is the other thing that you mentioned that in Latin America, my friends are studying that there the same hyper IgM patients get different infections. So, I think

that eventually there may be the time where you say well, for this kind of deficiency be defined by immunological or by genetic means or by both. We need this kind of specificity but I don't think we are there yet.

DR. SCOTT: Well, that reminds me of snake antivenoms where you need to have the snakes that are specific for the actual region where the antivenom is going to be needed. I don't know, it's a funny comparison. But the beauty of the immunoglobulins is they have so many specificities and they represent so much. Nevertheless, there are these regional differences and I think we even saw them in Dr. Kreil's presentation looking at the different regions of the U.S. where West Nile Virus serology was higher than in other regions. But it is something we can look at and it is something that is possible to study. I think it would be difficult, as you say, to right off the bat, be able to make those kinds of inclusions.

DR. BERGER: There are these things that change rapidly, emerging infections that spread rapidly. I don't see how we can always be

ahead of them. I think there was at least one treatment protocol using IVIG obtained in Israel to treat West Nile when it started to appear in the U.S. I think we had questions like when Zika was very high in Brazil but not yet very important in the U.S. What do you tell an immunodeficient patient in Brazil who is dependent on IVIG made from American plasma.

So, that's one kind of issue of things that change rapidly. Just like the issue of vaccines is not so much something that we had a vaccine, obviously the vaccine against the Diphtheria toxoid has been around for a long time and we probably have pretty stable immunity. It is a question of a vaccine which is changing, a vaccine which changes the epidemiology of the disease in a contemporaneous period. Similarly, with regional things, some regional things, I think, are more stable than others which are rapidly moving.

DR. SCOTT: These are good points but in the view of time, I think that we are actually on which antibody specificities are likely to be highly consistent across products. I wonder if

the speakers and the audience have anything to add to the ones that we've already mentioned.

DR. BALLOW: I want to come back to another potential pathogen that we've haven't talked about very much and that's Moraxella. Can you refresh my memory, I may have missed this in our presentation. Measurement of Moraxella, is there a biologic assay, opsonophagocytosis. Dot, did you look at that in IV products?

DR. SCOTT: We did not.

DR. BALLOW: Does anyone have data? What it's like in the normal population and what it might be in immunoglobulin preparations. Because that may be a relevant again, for two reasons. One, because of clinical relevance and also we're not going to develop a vaccine to Moraxella. We may not run into the same problems as Measles.

DR. NAHM: People are working on Moraxella vaccines. In a decade, there may be a new story.

DR. MOND: I think I may have some information on question two. We have gone through selecting thousands of donors and looking

at their antibodies to RSV. We find that for the most part, they're pretty homogenous in their antibody titer. There are 7 percent or maybe 8 percent that are regarded as very high titer to RSV. The other 92 to 93 percent fall within a relatively restricted range. So, if you're asking for an antibody specificity that is highly consistent, I would say RSV is one of those.

DR. SCOTT: So noted. Actually, previously noted. I've been adding things to the list ever since I mentioned it.

DR. KREIL: Just one point that I'd like to throw in here. The consistency would come primarily, I believe, through a large seroprevalence. If you depend on a smaller segment of your donor population then you will inevitably run into these issues when through just the location of your sourcing establishments, you may tap into donor populations of different sero status. So, I would think that something like

or 70 or even north of that percent seroprevalence is something that would be go to make sure that there is a consistency among

products.

DR. SCOTT: All right and question three actually combines question one and two. Again, I think we've been talking about it all along which may warrant further studies in IG products. I'll see who else has made a short list and see if it looks like mine.

DR. KREIL: Well, I've shown my list so I'm just going to reiterate. RSV, PAV, Adeno influenza types. That's something we can take a look at this by the fact with the antigenic drift, you're going to have issues there. CMV, HSV is certainly something we can take a look at. Those would be my hit list for a number of reasons. Seroprevalence generally very high, assay systems that can be mastered in a QC setting. Respiratory pathogens seem to be the one of higher concern as compared to gastrointestinal, for example. That's why I think these would be the ones I'd be interested in.

DR. GOLDING: My assessment is that the strep pneumonia and the hemophillus influenza should be at the top of the list. I assume there is some consensus on that.

DR. SCOTT: Well, to be fair, I think we just should not really go to top of the list yet but just list approximately five would be nice or more if there seems to be justifications. And that's what later, we need to go through to look more closely at feasibility, availability of reference standards, type of assay and so forth. It's not cast in stone but I think always strep pneumonia and H influenza and now possibly Moraxella need to be on that list, no question, because they still are problematic. H flu and strep pneumonia were extremely problematic before there were immunoglobulins for people to have.

We need to keep in mind, we're not only talking about what afflicts patients now but also what is being prevented in patients now by the products that we have. And we know that people still do get these illnesses but not to the extent that they used to, strep pneumonia and H flu. But yes, I agree, they're on the list and it sounds like assays are becoming increasingly available and increasingly feasible to do in a number of labs. I guess our time is up but we can take a few

more minutes because it is not yet noon.

DR. SORENSON: It may be useful to watch antibiotic and antifungal resistance because those will be the bacteria of the pathogens for which you need to have antibodies. If you look at the picture of new antibiotics being developed, my understanding is that it is pretty bleak. There are not too many drugs in the pipeline. There are more and more pathogens that are becoming multiple resistant. It may be that suddenly it will be important to develop an antibody against pathogens that so far, we haven't even thought about.

DR. SCOTT: I think we've had a lovely list of viruses and in addition to strep pneumonia, H flu and Moraxella, are there other bacteria pathogens that we perhaps ought to consider.

DR. SORENSON: Microplasma actually is very important in antibody deficiencies. It is probably under diagnosed because we have no good means to identify it. We treat for microplasma whenever the antibiotics seems to fail. We assume that it could be microplasma. More often

than not, it works.

DR. SCOTT: And the third category is vaccine related viruses or bacteria. Again, we've mentioned Tetanus, HIB. Any others? Where people were already given vaccines and ought to have immunity that is transmitted to the IGs. You don't have to come up with something. If you can't think of anything it's okay. I just don't want to miss out with this audience.

DR. GOLDING: What about Hepatitis A. It was mentioned in some of them. There is a vaccine and it is infectious and we do have outbreaks in the United States. B is another possibility although that is more related to certain risk factors.

DR. KREIL: So, for Hepatitis B, there is no assay system. The assay system for Hepatitis A virus, that is something that is going to be quite difficult to establish in an acute setting. It is virology a complex animal. It's the first virus that we used to call a lipid envelop virus that we now realized that will be lipid envelop under certain circumstances and therefore, less successful to antibodies. So,

if you want to test antibody with that virus in the lab it's going to be not feasible.

DR. SCOTT: Parvo virus B-19.

DR. KREIL: And there is a (inaudible) for parvo virus B-19 but it is run on cells that are erythropoietin dependent precursor cells in acute setting, impossible.

DR. SCOTT: Okay if there aren't any additional comments, well if there are, quickly raise your hand. Okay, I think I will try to summarize what we've just talked about at today's session. If Mr. Boyle, Mary Gustafson or someone from PPTA and if Stacy is here for NIAID, I welcome you to make any final remarks and so forth. I'll go first and then perhaps there are a few other folks that would like to say something.

I think this session was extremely helpful and I also think that compared to the 2000 session that we had, we've been able to focus more on what we think may be important and the feasibility of those assays. We should be able to move forward with the help of some of the folks who are here. I don't think that this is something that FDA needs or should do in isolation

in terms of at least seeking potential candidates that are promising as new and more relevant and more possible potency assays for immunoglobulin.

So, there are several concepts that came up and several other criteria that were spoken of. One is the need for stability of whatever specificities there are and expected stability of the levels of that antibody in the donor population over a long period of time. Perhaps the number was suggested that 50 to 70 percent seroprevalence might be good to aim for.

We didn't really discuss it but we say two very good examples of how functional assays have an advantage over binding assays both with the influenza and with the 19F 19A pneumococcal antibody differences that are seen in opsonophagocytosis but not by a ELISA. These are very good to know and we continue to keep them in mind but we are rather devoted at any rate to having functional assays.

We also heard a very important talk about the insufficiency of the Luminex commercial assays to give consistent results across laboratories which affects clinical decision

making but also is a word of caution in terms of this kind of assay as a potential one to evaluate potency. Now, maybe it can be improved but on the other hand, Dr. Nahm who is a world expert in opsonophagocytic assays and who has devoted his career to understanding immunity to very important pathogens. He is here and he has offered to collaborate with anybody in this room so you might get a lot of takers on this. I urge people to take advantage of his expertise and his offer to assist or to participate, collaborate in research. I think it is rather certain that we will want to do so as we move forward.

We heard about assays that are useful and feasible including opsonophagocytosis assays which are adaptable to other bacterial species as well as pneumococcus. And antiviral assays that likewise are functional and appear to be feasible although not ready for prime time at this point. That's okay because we don't know what we want to look at in more detail specifically.

We talked about interregional variation, that goes back to the need for stable titers geographically depending on where the

plasma comes from within any given country in addition to the need for stable titers with respect to whether or not vaccines are being developed. When we get into the same position that we're in with Measles, ten years after, we figure out a new potency assay.

I think I will conclude there. I think we have a lovely list of things to assess for feasibility not in the lab but first as a group. I appreciate the trip that all of the speakers have had to make. Some of you from very far away, some of you directly from extremely far away. Thank you for coming to help us and help the products and help the patients ultimately in this workshop. I would like to also thank our staff who has been through a lot and done a perfect job on making this workshop possible and smooth for all of us.

DR. GOLDING: I think we should also give a round of applause to Dot for all her work in setting this up.

DR. SCOTT: Thank you, it was far from just me. I'm the figurehead. So, I think perhaps John, you'd like to say a few words?

DR. BOYLE: Sure, I'll keep this quick for people who have to get to Dulles and National and elsewhere. I just want to reiterate to everyone here what was said early on. Although immunoglobulin has so many uses for the PI community, it is both lifesaving and lifelong. To be perfectly honest, everything that has been discussed here today would be somewhat surprising to those who are immunoglobulin. They take for granted that the antibody makeup is and ever shall be as strong as it needs to be. We know that is not the case. A little bit of insider baseball here. I just applaud everyone from the last -- a show of hands, who was at the last, the 2007 workshop that we had. So, we have a little bit of institutional knowledge that is spanning the decades here. We have a lot of new faces and we're just grateful that we have so many new people in here helping to address this. I'm going to be on immunoglobulin as far as I know, for decades to come and we will have these every ten years as we need to because the issue is ongoing and something could become the new Polio or the other ones that are being considered here.

So, we are grateful for all those that are here and for the other voices that we may need to bring in because this is what keeps us safe. So, thank you to all who have traveled and given time and energy. Those of PI, especially those who are on immunoglobulin, they don't know the work that has been done here but an idea if we're going to work with all of our partners to make sure that they understand that these conversations are ongoing. There are challenges ahead but they've got some great partners in government agencies and the medical community, of course, and our industry partners who are working together on this. So, thank you all.

DR. SCOTT: Thank you, John. PPTA, I should mention and IDF have both supported this workshop. NIAID has brought a great staff to support this beautiful venue. Does PPTA have any remarks?

DR. CERVENAKOVA: Yes on the part of PPTA, I'm really grateful and we are grateful to FDA for organizing this workshop. We are happy to be a part of that. Thank you very much everybody who participated from the companies as well as

physicians who came and addressed very important issues. To IDF representatives who are here and we are going to work with you on the future of the industry and it will be useful for the patients. Thank you. One thing not to forget that we are planning to do a publication out of this workshop so we are asking everybody to get back in touch with us, with Dorothy and myself, and let us know who would like to contribute. There is a supplement that we are planning to publish in transfusion. Thank you.

DR. SCOTT: One housekeeping remark and that is remember to turn your badge in at the exit. They'll probably make you do that but just in case. Thank you everybody, have a great trip home.

(Whereupon, at 11:57 a.m. the PROCEEDINGS were adjourned.)

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CERTIFICATE OF NOTARY PUBLIC

COMMONWEALTH OF VIRGINIA

I, Carleton J. Anderson, III, notary public in and for the Commonwealth of Virginia, do hereby certify that the forgoing PROCEEDING was duly recorded and thereafter reduced to print under my direction; that the witnesses were sworn to tell the truth under penalty of perjury; that said transcript is a true record of the testimony given by witnesses; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this proceeding was called; and, furthermore, that I am not a relative or employee of any attorney or counsel employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.

(Signature and Seal on File)

Notary Public, in and for the Commonwealth of Virginia

My Commission Expires: November 30, 2020

Notary Public Number 351998