Use and interpretation of diagnostic vaccination in primary immunodeficiency: A working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology

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A major diagnostic intervention in the consideration of many patients suspected to have primary immunodeficiency diseases (PIDDs) is the application and interpretation of vaccination. Specifically, the antibody response to antigenic challenge with vaccines can provide substantive insight into the status of human immune function. There are numerous vaccines that are commonly used in healthy individuals, as well as others that are available for specialized applications. Both can potentially be used to facilitate consideration of PIDD. However, the application of vaccines and interpretation of antibody responses in this context are complex. These rely on consideration of numerous existing specific studies, interpolation of data from healthy populations, current diagnostic guidelines, and expert subspecialist practice. This document represents an attempt of a working group of the American Academy of Allergy, Asthma & Immunology to provide further guidance and synthesis in this use of vaccination for diagnostic purposes in consideration of PIDD, as well as to identify key areas for further research. (J Allergy Clin Immunol 2012;130:S1-24.)

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The majority of patients given a diagnosis of primary immunodeficiency disease (PIDD) have some impairment of humoral immunity. These most typically include quantitative deficiencies of antibodies, qualitative deficiencies of antibodies, or both. Patients with antibody deficiencies often present with recurrent respiratory tract infections, but there can be a wide array of infectious susceptibilities, as well as other presenting or subsequent comorbidities. Therefore the assessment of humoral immunity is a critical component in the evaluation of patients suspected of having a PIDD. Importantly, indications for and

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Abbreviations used

AAAAI: American Academy of Allergy, Asthma & Immunology
ACIP: Advisory Committee on Immunization Practices
ADA: Adenosine deaminase
AT: Ataxia telangiectasia
CVID: Common variable immunodeficiency
FDA: US Food and Drug Administration
HDCV: Human diploid cell vaccine
HIB: *Haemophilus influenzae* type b
KLH: Keyhole limpet hemocyanin
MCV: Meningococcal conjugate vaccine
MPSV: Meningococcal polysaccharide vaccine
PCECV: Purified chick embryo cell vaccine
PCV: Pneumococcal conjugate vaccine
PIDD: Primary immunodeficiency disease
PPV: Pneumococcal polysaccharide vaccine
PRP: Polyribosylribitol phosphate
RFFIT: Rapid fluorescent focus inhibition test
SAD: Specific antibody deficiency
SBA: Serum bactericidal assay
THI: Transient hypogammaglobulinemia of infancy
WAS: Wiskott-Aldrich syndrome
XLA: X-linked agammaglobulinemia

interpretation of humoral immune testing must rely on clinical correlation because an overriding theme of PIDDs is the susceptibility to infectious disease, the atypical manifestations of infectious disease, or both.

Presently, there are a variety of laboratory-based tools available for the evaluation of suspected PIDDs with deficits in humoral immunity. These include direct genetic diagnosis of single-gene disorders,[1,2] flow cytometric analysis of lymphocyte subpopulations,[3] and quantitative and qualitative evaluation of serum immunoglobulins.[4] Although age, sex, environmental exposures, medications, and geography can influence some of these measures, these tests are, in the vast majority of cases, objective and useful for providing definitive diagnoses. However, the evaluation of immunoglobulin quality is complex and can be difficult to assess. Considerations involve antibody repertoire, antigen-specific immune responses, development of immunologic memory, and specific avidities for antigens. This is of critical relevance because subjects incapable of generating protective antibody responses are more susceptible to infection and, under many circumstances, can benefit from immunoglobulin replacement therapy.

Therapeutic immunoglobulin preparations are expensive and of limited supply, thus further necessitating careful evaluation of patients for antibody deficiency states that might require immunoglobulin replacement therapy. Qualitative assessment of antibody function is an evolving topic. The procedure presently involves the use of *in vitro* assays with the objective of determining whether the specificity of the *in vivo* antibody response is appropriate. Additionally, results can provide a reasonable correlate for protection against infection. Because a variety of tests and measures are available, the thoughtful selection of an approach is important.

Qualitative antibody responses are routinely assessed by measurement of antibody specificity for fairly standardized antigens to which a significant proportion of subjects are exposed. Prophylactic vaccines provide a relatively ubiquitous source of standardized antigenic exposure. Vaccines licensed for prophylactic use in the United States at the time of the writing of this document are listed in Table I. In most subjects vaccines are administered with stringent regulation of dosage, adjuvant content, route, and schedule. Thus evaluation of the vaccine response through measurement of antibody titers provides some measure of antigen standardization between patient populations. However, there are variations in the approach to and interpretation of these measurements that present complexities through which the clinician must navigate. These include the age of the patient, which can influence both the response to vaccine challenges and the manifestation of the PIDD. Some PIDDs and diagnostic approaches are specific to children, whereas others are more common in adult patients. Throughout this document, concerns relevant to pediatric and adult patients are specifically noted as they relate to the individual vaccines used to elicit humoral immunity.

When poor antibody response is perceived, it is standard practice to provide an antigenic challenge (through "booster" immunization) to determine whether a subject retains the ability to generate a qualitative antibody response. Although the process of diagnostic vaccination is routine, there are many variables for clinical consideration. These include which vaccines or antigens to use, how to administer and use them, which tests to use to measure responses, and how to interpret the data in the context of complex clinical scenarios. As a result, the interpretation of diagnostic vaccination can result in more questions than answers.

In an effort to provide guidance for practicing allergists/immunologists (and others clinically evaluating patients with potential PIDDs) in assessing antibody quality with regard to vaccination in potentially immunodeficient patients, a working group of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology (AAAAI) was formed and charged in December 2007. It included members of the Primary Immunodeficiency Committee, as well as members of the Vaccines and Biological Threats Committee. The group was assembled with the task of developing individual summary statements relating to topics pertinent to diagnostic vaccination. The work in generating the statements was assigned to specific subcommittees and occurred between October 2008 and April 2009. These were then subjected to at least 2 rounds of blind review, after which they were revised and edited. Each statement was categorized according to the quality of the supporting evidence and assigned a strength of recommendation (Table II). This process was completed in August 2010, and then the document was submitted for independent peer review through the Practice and Policy Division of the AAAAI in March 2011, revised, and then completed in December 2011.

Although it is clear that many questions remain, the intent of this effort is to promote clarity and facilitate evidenced-based practice in this diverse clinical arena. The dynamic market landscape of vaccines, which include changes in licensure, availability of new vaccines, and innovations in diagnostic testing, will necessitate ongoing changes to this document and its recommendations.

The summary statements are presented in the following text divided according to 4 broad topic areas. The first section (I) is the use of common vaccines to measure humoral immune function. The second section (II) relates specifically to the use of pneumococcal polysaccharide vaccine for measurement of humoral

TABLE I. Vaccines currently licensed for use in the United States

Vaccine	Trade name	Live vaccine	Manufacturer	Notes
Adenovirus type 4 and type 7 vaccine, live	No trade name	**Yes**	Barr Labs	Oral
Anthrax vaccine adsorbed	Biothrax	No	Emergent BioDefense Operations Lansing	Adsorbed
BCG live	TICE BCG	Yes	Organon Teknika Corp	
Diphtheria and tetanus toxoids	None	No	Sanofi Pasteur	Adsorbed
Diphtheria and tetanus toxoids adsorbed	No trade name	No	Sanofi Pasteur	Adsorbed
Diphtheria and tetanus toxoids and acellular pertussis	Tripedia	No	Sanofi Pasteur	Adsorbed
Diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed	Infanrix	No	GlaxoSmithKline Biologicals	Recombinant
Diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed	DAPTACEL	No	Sanofi Pasteur	Recombinant
Diphtheria and tetanus toxoids and acellular pertussis + hepatitis B + poliovirus	Pediarix	No	GlaxoSmithKline Biologicals	Adsorbed recombinant (hepatitis B) Inactivated (poliovirus)
Diphtheria and tetanus toxoids and acellular pertussis poliovirus vaccine	KINRIX	No	GlaxoSmithKline Biologicals	Adsorbed and inactivated
Diphtheria and tetanus toxoids and acellular pertussis + poliovirus and *Haemophilus* b conjugate	Pentacel	No	Sanofi Pasteur	Adsorbed, inactivated, *Haemophilus*–tetanus toxoid conjugate
Haemophilus b conjugate vaccine	PedvaxHIB	No	Merck & Co	Meningococcal protein conjugate
Haemophilus b conjugate vaccine	ActHIB	No	Sanofi Pasteur, SA	Tetanus toxoid conjugate
Haemophilus b conjugate vaccine	Hiberix	No	GlaxoSmithKline Biologicals, SA	Tetanus toxoid conjugate
Haemophilus b conjugate and hepatitis B	Comvax	No	Merck & Co	Meningococcal protein conjugate, hepatitis B (recombinant)
Hepatitis A	Havrix	No	GlaxoSmithKline Biologicals	Inactivated
Hepatitis A	VAQTA	No	Merck & Co	Inactivated
Hepatitis A and hepatitis B	Twinrix	No	GlaxoSmithKline Biologicals	Inactivated (hepatitis A), recombinant (hepatitis B)
Hepatitis B	Recombivax HB	No	Merck & Co	Recombinant
Hepatitis B	Engerix-B	No	GlaxoSmithKline Biologicals	Recombinant
Human papillomavirus (types 6, 11, 16, 18)	Gardasil	No	Merck and Co	Recombinant quadravalent
Human papillomavirus (types 16, 18)	Cervarix	No	GlaxoSmithKline Biologicals	Recombinant bivalent
Influenza A (H1N1) 2009	None	No	CSL Limited	Monovalent
	None	No	MedImmune	Monovalent
	None	No	ID Biomedical Corporation of Quebec	Monovalent
	None	No	Novartis Vaccines and Diagnostics Limited	Monovalent
	None	No	Sanofi Pasteur	Monovalent
Influenza virus H5N1	No trade name	No	Sanofi Pasteur	
Influenza virus, types A and B	Afluria	No	CSL Limited	Trivalent
	FluLaval	No	ID Biomedical Corp of Quebec	Trivalent
	Fluarix	No	GlaxoSmithKline Biologicals	Trivalent
	Fluvirin	No	Novartis Vaccines and Diagnostics Ltd	Trivalent
	Agriflu	No	Novartis Vaccines and Diagnostics S.r.l.	Trivalent
	Agriflu	No	Novartis Vaccines and Diagnostics S.r.l.	Trivalent
	Fluzone and Fluzone High-Dose	No	Sanofi Pasteur	Trivalent
Influenza vaccine, types A and B	**FluMist**	**Yes***	MedImmune	Intranasal trivalent

(Continued)

TABLE I. *(Continued)*

Vaccine	Trade name	Live vaccine	Manufacturer	Notes
Japanese encephalitis virus	Ixiaro	No	Intercell Biomedical	Inactivated, adsorbed
	JE-Vax	No	Research Foundation for Microbial Diseases of Osaka University	Inactivated
Measles virus	**Attenuvax**	**Yes**	Merck & Co	
Measles, mumps, and rubella virus	**M-M-R II**	**Yes**	Merck & Co	
Measles, mumps, rubella, and varicella virus	**ProQuad**	**Yes**	Merck & Co	
Meningococcal (groups A, C, Y, and W-135) oligosaccharide	Menveo	No	Novartis Vaccines and Diagnostics	Diphtheria CRM197 conjugate vaccine
Meningococcal polysaccharide (serogroups A, C, Y and W-135)	Menactra	No	Sanofi Pasteur	Diphtheria toxoid conjugate vaccine
Meningococcal polysaccharide vaccine, groups A, C, Y and W-135 combined	Menomune-A/C/Y/W-135	No	Sanofi Pasteur	
Mumps virus vaccine, live	**Mumpsvax**	**Yes**	Merck & Co	
Pneumococcal vaccine, polyvalent	Pneumovax 23	No	Merck & Co	
Pneumococcal 7-valent conjugate	Prevnar	No	Wyeth Pharmaceuticals	Diphtheria CRM_{197} protein conjugate
Pneumococcal 13-valent conjugate	Prevnar 13	No	Wyeth Pharmaceuticals	Diphtheria CRM_{197} protein conjugate
Poliovirus	IPOL	No	Sanofi Pasteur, SA	Inactivated (monkey kidney cell)
Rabies	Imovax	No	Sanofi Pasteur, SA	
	RabAvert	No	Novartis Vaccines and Diagnostics	
Rotavirus	**ROTARIX**	**Yes**	GlaxoSmithKline Biologicals	Oral
	RotaTeq	**Yes**	Merck & Co	Oral, pentavalent
Rubella virus	**Meruvax II**	**Yes**	Merck & Co	
Smallpox (vaccinia)	**ACAM2000**	**Yes**	Sanofi Pasteur Biologics	
Tetanus and diphtheria toxoids	No trade name	No	MassBiologics	Adsorbed for adult use
	DECAVAC	No	Sanofi Pasteur	Adsorbed for adult use
	TENIVAC	No	Sanofi Pasteur (not available)	Adsorbed for adult use
Tetanus toxoid	No trade name	No	Sanofi Pasteur	Adsorbed
Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis	Adacel	No	Sanofi Pasteur	Adsorbed
	Boostrix	No	GlaxoSmithKline Biologicals	Adsorbed
Typhoid Ty21a	**Vivotif**	**Yes**	Berna Biotech	Oral
Typhoid Vi polysaccharide	Typhim Vi	No	Sanofi Pasteur, SA	
Varicella virus	**Varivax**	**Yes**	Merck & Co	Oka strain
Yellow fever	**YF-Vax**	**Yes**	Sanofi Pasteur	
Zoster	**Zostavax**	**Yes**	Merck & Co	Oka strain

*Boldfaced vaccines represent those that are live. Specific guidance in the use of live vaccines in immunocompromised patients is recommended as directed in the licensing information for the individual vaccines and as per this document's Summary Statement 8.

immunity. The working group determined that the pneumococcal polysaccharide vaccine warranted a full section because of the historical emphasis placed on its use, as well as its application in certain health care coverage guidelines. This section on Pneumococcal vaccination includes the topics of preexisting anti-pneumococcal titers, as well as titers used to measure resistance to infection. The third section (III) addresses the use and interpretation of responses to meningococcal vaccination. The fourth section (IV) is focused on the use of neoantigens and alternative vaccines in measuring humoral immune function. The fifth and final section (V) covers measurement and variability in the response to currently available vaccines, including the variability defined in the limited studies of immunodeficient populations.

With these specific areas of focus, the document consists of a series of 70 summary statements that are first listed and then reiterated along with a more detailed explanation, including key supporting references. This format is similar to that used in other key documents in the field of primary immunodeficiences[4] and is intended to serve as a lexicon for practitioners seeking further guidance on the topic of diagnostic vaccination as it applies to PIDDs. The present effort is not intended as a guideline for establishing individual PIDD diagnoses; for that, the reader is referred to the Joint Council on Allergy, Asthma & Immunology Practice Parameter on PIDD.[4] In this light the present document should be viewed as additional guidance on the specific topic of use and interpretation of vaccination responses in consideration of PIDD and not taken to replace anything stated in the present or future PIDD practice parameters. Because certain topics are relevant to more than 1 summary statement, the reader is encouraged to review the listing of summary statements before deciding which of the detailed statements are relevant to a specific diagnostic consideration.

TABLE II. Categorization of evidence and basis of recommendation and strength of recommendation

Ia	From meta-analysis of randomized controlled studies
Ib	From at least 1 randomized controlled study
IIa	From at least 1 controlled trial without randomization
IIb	From at least 1 other type of quasiexperimental study
III	From nonexperimental descriptive studies, such as comparative, correlation, or case-control studies
IV	From expert committee reports or opinions or clinical experience of respected authorities or both
A	Based on category I evidence
B	Based on category II evidence or extrapolated from category I evidence
C	Based on category III evidence or extrapolated from category I or II evidence
D	Based on category IV evidence or extrapolated from category I, II, or III evidence
NR	Not rated

LISTING OF SUMMARY STATEMENTS

I. Use of common vaccines for measurement of humoral immune function

Summary Statement 1: The most commonly used vaccines for B-cell functional analysis are US Food and Drug Administration (FDA) approved and used worldwide in children to prevent communicable diseases. (Ia A)

Summary Statement 2: The diagnosis and treatment of common variable immunodeficiency (CVID) has traditionally included assessment of vaccine responses. (IIa B)

Summary Statement 3: There are 4 primary immunodeficiencies that largely depend on qualitative analysis of vaccination responses. (IV D)

Summary Statement 4: Several genetically definable primary immunodeficiencies have been associated with poor polysaccharide antibody responses, and vaccination with pneumococcal polysaccharide vaccine (PPV) can be of diagnostic utility. (IIa B)

Summary Statement 5: Antibody responses to T cell–independent (polysaccharide) antigens should not be a component of routine investigation for antibody deficiency in children less than 18 months of age still in the midst of receiving their primary vaccination series. (IIa A)

Summary Statement 6: Certain immunodeficiencies are drastic, and pursuing evaluation of humoral immune function through vaccine antigen challenge would delay necessary therapy. (IV D)

Summary Statement 7: The use of polysaccharide vaccines as a diagnostic tool must integrate numerous criteria. (IIa B)

Summary Statement 8: The use of live viral vaccines should be avoided in patients with certain immunodeficiencies. (IIa B)

II. Use of the pneumococcal polysaccharide vaccine in evaluation of humoral immune function and in diagnosis of functional antibody deficiency

Summary Statement 9: Pneumococcal vaccines are recommended for all children, adults older than 65 years, and certain high-risk groups. (Ib A)

Summary Statement 10: Pneumococcal vaccines are usually well tolerated. (Ib B)

Summary Statement 11: Different titers of pneumococcal antibodies might serve different anti-infective purposes. (IIb B)

Summary Statement 12: Pneumococcal antibody titers vary over time in healthy subjects. (IIb B)

Summary Statement 13: Pneumococcal antibody titers might be of value to determine the response to documented past pneumococcal infection if the infecting serotype is known. (IV D)

Summary Statement 14: Pneumococcal IgG antibody responses are generally assessed by means of ELISA or related immunologic assay. (NR)

Summary Statement 15: Functional assays for detecting specific anti-pneumococcal antibodies also exist and might provide a better measure of anti-pneumococcal antibody quality. (IV D)

Summary Statement 16: PPV is widely used diagnostically in both adults and children having completed their primary pneumococcal conjugate vaccine (PCV) series who are suspected of immunodeficiency to ascertain response to polysaccharide antigens. (Ib A)

Summary Statement 17: PCV7 and PCV13 are used occasionally in the diagnosis of immunodeficiency. (IIb C)

Summary Statement 18: Measurement of individual pneumococcal serotype titers before and after immunization and enumeration of the number of serotypes responding is an accepted technique to evaluate humoral immune function. (IIb B)

Summary Statement 19: Measurement of pneumococcal antibody titers to either vaccine should be done 4 to 8 weeks after vaccination. (Ib A)

Summary Statement 20: A protective (normal or adequate) response to each pneumococcal serotype is defined as a titer equal to or greater than 1.3 μg/mL antibody. (IIb C)

Summary Statement 21: A normal response for a single serotype present in a pneumococcal vaccine is defined as the conversion from a nonprotective to a protective titer. (III D)

Summary Statement 22: The number of pneumococcal serotypes that are protective after a vaccine can be used to define a normal (adequate or epidemiologic) response. (IV D)

Summary Statement 23: Certain pneumococcal serotypes are considered to be more reliably antigenic than others. (Ib A)

Summary Statement 24: The higher the preimmunization titer for a specific pneumococcal serotype, the less likely that the titer will have a significant increase after vaccination. (III C)

Summary Statement 25: Most patients with a prevaccine titer of greater than 1.3 μg/mL can mount a 2-fold increase in titer on immunization. A minority of patients with high initial titers will be capable of mounting a 4-fold increase in antibody titers after vaccination. (III C)

Summary Statement 26: The probability of a 4-fold antibody response approaches zero if the preimmunization titer is between 4.4 and 10.3 μg/mL, depending on the pneumococcal serotype. (III C)

Summary Statement 27: Secondary immunodeficiencies might affect antigen-specific responses and diminish the response to the pneumococcal vaccine. (NR)

Summary Statement 28: Immediate repeat booster doses of PPV are ineffective (and not recommended and might promote hyporesponsiveness). (Ib B)

Summary Statement 29: Patients who have previously received PCV7 or PCV13 can be given PPV23. (III C)

Summary Statement 30: A diagnosis of specific antibody deficiency (SAD) can be made if the response to PPV23 is deficient but the responses to protein antigens (eg, tetanus toxoid or diphtheria toxoid), conjugate vaccines (*Haemophilus influenzae* type b, PCV7, or PCV13), or both are intact and total immunoglobulin levels are normal. (III C)

Summary Statement 31: PCV7 or PCV13 protein conjugate vaccines can be administered to patients who have a poor response to PPV23. (III C)

Summary Statement 32: The degree of polysaccharide nonresponsiveness in selective antibody deficiency can be classified into 4 phenotypes. (IV D)

Summary Statement 33: Further clinical research is warranted to refine best practice applied to patients with specific phenotypes of selective antibody deficiency. (NR)

III. Use of meningococcal vaccine to measure humoral immune function

Summary Statement 34: In the United States there are currently 3 meningococcal vaccines licensed for use in children aged 2 years and older and adults. (Ia A)

Summary Statement 35: The 3 meningococcal vaccines contain the same serogroups. (NR)

Summary Statement 36: MCV4 is a protein conjugate vaccine, and MPSV4 is a polysaccharide vaccine. Therefore they differ in the mechanism of immune response. (Ib A)

Summary Statement 37: There are different methodologies for assessing the immunogenicity of meningococcal vaccines. (Ib A)

Summary Statement 38: All of the currently licensed meningococcal vaccines in the United States have been found to be immunogenic. (Ib A)

Summary Statement 39: Meningococcal polysaccharide vaccine is less reliable in young children. (Ib A)

Summary Statement 40: Meningococcal polysaccharide vaccination can result in hyporesponsiveness to subsequent meningococcal vaccination. (Ib A)

Summary Statement 41: There are commercially available laboratory tests for meningococcal antibody titers. (III C)

Summary Statement 42: An increase in titers of at least 2 meningococcal serogroups is expected after vaccination of an immunocompetent subject. (IV D)

Summary Statement 43: Immunogenicity might depend on several factors (which could have relevance if additional manufacturers begin to produce these vaccines). (IIb C)

Summary Statement 44: Given that there are commercial laboratories that measure meningococcal antibody titers and both vaccines have been proved to be immunogenic, responses could be used in the clinical evaluation for immunodeficiency. (IV D)

Summary Statement 45: There are specific considerations regarding the immunogenicity of certain meningococcal serogroups should they be available in vaccines. (III C)

IV. Use of alternative vaccines and true neoantigens in evaluating defective humoral immunity

Summary Statement 46: Immunization with neoantigens can be used in the evaluation of specific antibody response in the setting of immunoglobulin replacement therapy. (III C)

Summary Statement 47: Sufficient experience does not exist regarding the use of routine vaccines in the context of a patient with primary immunodeficiency receiving immunoglobulin replacement therapy to assess antibody response. (IV D)

Use of bacteriophage φX174 to measure humoral immune function

Summary Statement 48: The only neoantigen that has been extensively studied to assess human antibody responses is the T cell–dependent antigen bacteriophage φX174. (III C)

Summary Statement 49: Immunization with the neoantigen bacteriophage φX174 and subsequent evaluation of specific antibody responses might be included in the diagnosis of primary immunodeficiency to assess antigen-specific class-switching and the kinetics of the antibody response, including in the evaluation of patients who are already receiving immunoglobulin supplementation. (III C)

Summary Statement 50: Immunization with the neoantigen bacteriophage φX174 is relatively labor intensive and is performed as research. (IV D)

Summary Statement 51: Keyhole limpet hemocyanin (KLH) is a potential alternative to φX174 as a neoantigen. (IV D)

Use of human rabies virus vaccine as an alternative neoantigen to evaluate humoral immune function

Summary Statement 52: Rabies virus vaccines are available and used in the United States as postexposure prophylaxis. (Ib A)

Summary Statement 53: Rabies virus vaccination is generally well tolerated. (Ib A)

Summary Statement 54: Cell culture–derived rabies virus vaccines as pre-exposure vaccines elicit adequate humoral immune responses. (Ib A)

Summary Statement 55: Rabies virus vaccines can be used as a neoantigen to assess humoral immune responses in healthy subjects. (IIb B)

Summary Statement 56: Although rabies virus vaccines can elicit lymphocyte proliferative responses after immunization, the rabies virus nucleocapsid can produce a superantigen response by human T cells that might compromise its utility to assess cell-mediated immune responses as a neoantigen. (IIb B)

Summary Statement 57: Rabies virus vaccine can be used as a neoantigen to evaluate humoral immune responses in patients with secondary immune deficiency; however, the degree of the response might be linked to the dose (micrograms of protein) of the vaccine. (IIb C)

Summary Statement 58: Rabies virus vaccine can be used as a neoantigen to evaluate humoral immune responses in patients with primary immune deficiencies. (IIb C)

Summary Statement 59: A single injection of rabies virus vaccine might be useful in eliciting a measurable antibody response, but further study of this intervention in primary immunodeficiency diagnostic evaluation is needed. (IV D)

Summary Statement 60: Rabies virus vaccination can potentially be used to assess humoral immune function in a patient receiving immunoglobulin replacement therapy. (III C)

Summary Statement 61: Testing for rabies virus vaccine–specific antibodies is available, but the general application of specific methods in patients suspected of having primary immunodeficiency needs to be established. (IV D)

Summary Statement 62: In contrast to rabies virus vaccine, it is unlikely that meningococcal vaccine will be a suitable neoantigen for patients receiving immunoglobulin replacement therapy. (IV D)

Summary Statement 63: The use of *Salmonella typhi* Vi vaccine has future potential as a diagnostic and alternative polysaccharide antigen in patients with primary immunodeficiencies, but sufficient data are not presently available to support its use. (IV D)

V. Variability in immunogenicity among currently available vaccines

General considerations

Summary Statement 64: The FDA requires that vaccine manufacturers must test each lot and demonstrate conformance to established standards for that vaccine. (NR)

Summary Statement 65: When assessing vaccine lot consistency, it is important to understand the interrelationship between efficacy, immunogenicity, and potency. (IV D)

Summary Statement 66: Vaccine lot consistency is generally based on measures of potency. (Ib B)

Summary Statement 67: Vaccine potency is dependent on numerous factors. (III C)

Summary Statement 68: Although potency measurements are considered to be standardized, they do not guarantee lot consistency as it relates to immunogenicity or efficacy. Despite meeting potency standards, there are data that suggest lot variation occurs and that vaccine lots have failed. (III C)

Variability in immunogenicity among currently available vaccines specific to assessing immunodeficient populations

Summary Statement 69: Tetanus toxoid vaccines demonstrate no significant immunogenic variability and are good diagnostic tools for evaluation of immune competence to T-dependent antigens. (Ib A)

Summary Statement 70: Protein-conjugated *Haemophilus influenzae* type b (HIB) and pneumococcal vaccines show variability in immunogenicity because of the protein carrier and nature of the antigen. (Ib A)

I. USE OF COMMON VACCINES FOR MEASUREMENT OF HUMORAL IMMUNE FUNCTION

A substantial number of vaccines are licensed for prophylactic use in the United States (Table I), and many are part of required or recommended vaccination series. Multiple PIDD diagnoses depend in part on the evaluation of the responses to these routine antigenic exposures. For direct guidance regarding the diagnosis of specific PIDDs, the reader is referred to the current (and any future) Joint Council of Allergy, Asthma & Immunology Practice Parameter on PIDDs.[4] The following summary statements (Summary Statements 1-8) are on general considerations of the most commonly used vaccines as they apply to PIDDs for diagnostic purposes.

Summary Statement 1: The most commonly used vaccines for B-cell functional analysis are US Food and Drug Administration (FDA) approved and used worldwide in children to prevent communicable diseases. (Ia A)

TABLE III. Immunologic characteristics of major diagnostically applied vaccines

Vaccine	T-cell independent or dependent	Peak antibody levels	Protective levels
HIB conjugate	Dependent[6]	6 mo (3-4 wk after third dose)[7]	1.0 μg/mL[8]
Meningococcal conjugate	Dependent[9]	2-4 wk[10]	2 μg/mL[11]
Meningococcal polysaccharide	Independent[9]	2-4 wk[5]	2 μg/mL[11]
Pneumovax conjugate	Dependent[12]	4 wk	See Summary Statement 20
Pneumococcal polysaccharide	Independent	4 wk	See Summary Statement 20
Rabies	Dependent	21 d after third dose for pre-exposure prophylaxis[13]	0.5 IU[14]
Tetanus	Dependent	2-3 wk after initial series[15]	0.15 IU/mL[16]

Vaccines can be safely used to assess humoral function. In general, the use of vaccines as a diagnostic tool requires information about the following: safety, immunogenicity, assays for antibody measurement, and normal response. The most commonly used vaccines for B-cell functional analysis are FDA approved and used worldwide in children to prevent communicable diseases (see Table I for an overview). Therefore the safety of these vaccines has been extensively evaluated and continues to be monitored by health and governmental agencies.

Diphtheria and tetanus toxoid vaccines are the most commonly used vaccines to assess antibody production to protein antigens. These antigens are usually regarded as T-dependent antigens requiring T- and B-cell cooperation. Pure nonconjugated PPVs are the most commonly used vaccines to assess antibody production to polysaccharide antigens and are often referred to as T independent (although this applies most directly to the IgM response). These vaccines are less commonly used in children but are believed to trigger immune responses differently from those accessed by protein-based or conjugated vaccines. Importantly, the vaccines commonly used for diagnostic purposes have particular immunogenic characteristics, as described throughout this document (examples are shown in Table III).[5-16]

Summary Statement 2: The diagnosis and treatment of common variable immunodeficiency (CVID) has traditionally included assessment of vaccine responses. (IIa B)

Vaccine responses in this heterogeneous group of patients have been extensively studied in small numbers of subjects. One study reported a response rate of 23% to polypeptide vaccine antigens and an 18% response rate to nonconjugated PPVs.[17] Another study characterized the response to meningococcal polysaccharide vaccination and found a response rate of 64% within the CVID cohort.[18] A third study compared the response to the HIB conjugate vaccine in healthy adult subjects and adult patients with CVID and demonstrated variability in response to the HIB conjugate but hyporesponsiveness in almost all patients.[19] Variable vaccine responses can be observed in at least some persons with the diagnosis of CVID, and some degree of responsiveness is not necessarily contradictory to this diagnosis. Interestingly,

2 groups have found a correlation between lack of vaccine responsiveness (specifically to PPV) and diminished percentages of IgM memory B cells.[17,20] Taken together, these data emphasize the utility of vaccine responses for their diagnostic and potential therapeutic modality in patients with CVID. However, vaccine responsiveness is not used alone as the diagnostic criterion because decrease in specific antibody levels is of primary immunologic importance and susceptibility to infection is of primary clinical importance. That said, specific antibody levels are most often decreased or absent in patients with CVID. Diagnosis-specific definitions are provided elsewhere.[4]

Summary Statement 3: There are 4 primary immunodeficiencies that largely depend on qualitative analysis of vaccination responses. (IV D)

Because of the presence of quantitatively normal B-cell population counts and variable quantitative serum immunoglobulin levels (decreased to normal values), 4 primary immunodeficiency syndromes solely depend on qualitative analysis of vaccination responses: transient hypogammaglobulinemia of infancy (THI); IgG_1, IgG_2, or IgG_3 subclass deficiency; selective IgA deficiency; and selective antibody deficiency. Responses to protein or protein-conjugated antigens are typically conserved in these conditions. Responses to polysaccharides can be impaired in patients with IgG subclass 2 and selective antibody deficiency. The true incidence of these diagnoses is not known; however, existing registry data and expert opinion suggest that these are among the most common primary immunodeficiency syndromes.[21,22] The diagnosis of IgG subclass deficiency is associated with immunoglobulin levels for any of the first 3 IgG subclasses that lie greater than 2 SDs below the age-specific mean reference ranges, which need to be considered as a percentage of the total IgG level.[23] IgG_4 levels are commonly low and frequently unmeasurable and thus are not germane to the topic of IgG subclass deficiency.[24] Although presently controversial as an independent diagnosis,[4] deficiencies in IgG types 1, 2, or 3 or a combination of these might be associated with recurrent sinopulmonary infections.[25-29] Because a deficiency in an individual IgG subclass can occur in as many as 2% of the healthy population, careful immunologic consideration of these subjects and strong clinical correlation are indicated.[4]

Young children presenting with recurrent respiratory tract infections and immunoglobulin levels of less than the age-matched reference ranges in the presence of otherwise normal T- and B-cell numbers often undergo further evaluation, which includes vaccine challenge. Immunologists rely on the vaccine response to help make the distinction between significant PIDDs and transiently low immunoglobulin levels, as seen in patients with THI, or delayed maturation of antibody responsiveness. If the vaccine response is interpreted as normal, a diagnosis of some form of delayed maturation of antibody responsiveness might be likely, as can occur in patients with THI. A subset of pediatric patients who have marginally poor vaccine responses and otherwise lack sufficient evidence for a specific diagnosis of PIDD might have normalization of laboratory values over time, which is likely indicative of some form of delayed maturation of antibody responsiveness and can also be consistent with THI.[30-32] In patients with delayed maturation of antibody responsiveness, repeated evaluation over time of their vaccine responses is necessary to assess for normalization of their responses. Likewise, the other common humoral immunodeficiencies (ie, selective IgA deficiency with IgG subclass deficiency and selective antibody deficiency) rely on accurate interpretation of vaccine responses for appropriate diagnosis and management.

Summary Statement 4: Several genetically definable primary immunodeficiencies have been associated with poor polysaccharide antibody responses, and vaccination with pneumococcal polysaccharide vaccine (PPV) can be of diagnostic utility. (IIa B)

The response to PPVs, along with the clinical history, is important in guiding the medical management of these patients.[33,34] In both patients with Wiskott-Aldrich syndrome (WAS) and those with ataxia telangiectasia (AT), several small studies have reported poor responses to polysaccharide vaccines in the majority of patients.[35,36] A small study in patients with AT demonstrated that initial vaccination with PCV and subsequent vaccination with PPV could lead to higher antibody levels to PCV-specific serotypes and non-PCV serotypes.[37] The titers were determined by using ELISA with a PPV mix as the antigen. This intervention can be considered for therapeutic utility in increasing *Pneumococcus* species–specific antibody levels and thus irrespective of diagnostic efforts. Poor response to PPV has also been described in patients with 22q11.2 deletion (DiGeorge) syndrome,[38,39] although the incidence of impaired polysaccharide response was considerably lower in patients with 22q11.2 deletion compared with that seen in patients with WAS and those with AT. However, there might be selection bias for patients with 22q11.2 deletion with polysaccharide antibody impairment followed in an immunology clinic when considering the relative frequency of this microdeletion in the general population.[40] Because the majority of patients with 22q11 have a milder deficiency of immunity when compared with patients with WAS or AT, vaccination might represent an important therapeutic intervention in this population, including those outside of the pediatric age range. These 2 genetic diagnoses are offered as examples because there are numerous others to which this rubric could apply.

Summary Statement 5: Antibody responses to T cell–independent (polysaccharide) antigens should not be a component of routine investigation for antibody deficiency in children less than 18 months of age still in the midst of receiving their primary vaccination series. (IIa A)

Children less than 2 years of age have been historically reported to have a reduced ability to respond to polysaccharide antigens while possessing strong responses to protein antigens.[41] After this age, polysaccharide-specific antibody responses gradually mature. This ontogeny of anti-polysaccharide antibody responses in part explains the susceptibility of children to invasive disease caused by encapsulated bacterial pathogens, such as HIB and *Pneumococcus* species. These have served as a rationale for the development of protein-conjugated vaccines, which are standard components of the pediatric vaccination schedule. The poor immunologic antibody response to polysaccharide does not relate to the specificity of the antigen but is due to age-dependent immunologic maturation. However, the appearance of isohemagglutinins in the serum can act as a surrogate marker for the development of polysaccharide-specific antibody responses.[42] Interestingly, allogeneic or autologous bone marrow transplant recipients show the same pattern of early recovery of protein antibody responses and delayed ontogeny of polysaccharide antibody responses.[43] However, other historic and more recent data suggest that children as young as 6 months can effectively respond to polysaccharide vaccination.[44-46] (This is more specifically addressed in Summary Statement 16.) Given that some

polysaccharide vaccines (see Summary Statements 28 and 40) are hypothesized to interfere with the performance of conjugate vaccines, the most prudent recommendation is to not use polysaccharide vaccines routinely for diagnostic purposes in young children still in the midst of or too soon after their PCV vaccination schedule (currently 18 months of age).

Summary Statement 6: Certain immunodeficiencies are drastic, and pursuing evaluation of humoral immune function through vaccine antigen challenge would delay necessary therapy. (IV D)

Patients with severe T-cell immunodeficiency or absence of B-cell development secondary to gene mutations present with severe hypogammaglobulinemia, absent production of specific antibodies, or both. In these patients serum IgG levels might reflect placentally transferred maternal antibodies during the first 3 to 6 months after full-term birth. If a diagnosis of a severe PIDD is established through phenotypic or genetic means, replacement therapy should not be delayed further, irrespective of transferred maternal IgG. In a patient with a severe PIDD and very low IgG levels, measurement of humoral immune function through vaccine challenge is therefore not essential but can provide supporting evidence for the primary diagnosis if vaccination has already been performed. In this case antibody testing can be performed at the same time as other immunologic testing without further delaying any intervention. However, once the diagnosis is recognized, delay in providing therapy to determine vaccine response is not justified. Certain rare exceptions within these diagnoses do exist as a feature of mild variants of these known diseases but again are the rare exception in these cases and should not be anticipated.[47-49]

As an additional general guideline, after conditions leading to secondary hypogammaglobulinemia (eg, protein-losing enteropathy or nephrotic syndrome) have been ruled out in patients with infections and suspected immunodeficiency, it is expert opinion that IgG levels of less than 200 mg/dL in an infant warrant initiation of immunoglobulin replacement, if clinically appropriate, and that preceding evaluation through vaccine antigen challenge is not necessary. However, if an immunodeficiency diagnosis is probable, replacement therapy should be provided irrespective of the IgG level, and a specific value of 200 mg/dL would not apply.[23] This would include suspected cases of X-linked agammaglobulinemia (XLA) in which there are less than 2% B cells present or early diagnoses of severe T-cell defects. In older subjects the level of 200 mg/dL does not apply and might be considered too low of a threshold. However, in patients with protein-losing conditions, it is recommended that antibody specificity still be evaluated.

Summary Statement 7: The use of polysaccharide vaccines as a diagnostic tool must integrate numerous criteria. (IIa B)

Numerous considerations need to be taken into account when using pure polysaccharide vaccines, including previous vaccination history, the patient's age, outcome of repeat vaccinations, order of vaccinations, preexisting titers, and definition of "normal" or "protective."[19,50-52] These issues are complex and are the topics of subsequent summary statements in this document (in particular Summary Statements 14 and 20).

Summary Statement 8: The use of live viral vaccines should be avoided in patients with certain immunodeficiencies. (IIa B)

Certain primary immunodeficiencies are associated with susceptibility to viral infection and impaired cell-mediated immunity, such as severe combined immunodeficiency.[53-55] Others, such as XLA, have a specific and abnormal susceptibility to certain types of viruses.[56] In patients with these disorders, vaccination with live viral vaccines (Table I) should be avoided because these are capable of resulting in clinically relevant infection. It is always safer to withhold live viral vaccination while diagnostic considerations are in progress and when a combined or T-cell immunodeficiency has been diagnosed (except where specific recommendations are available). More specific guidance on this topic is provided elsewhere.[4,57]

II. Use of the pneumococcal polysaccharide vaccine in evaluation of humoral immune function and in diagnosis of functional antibody deficiency

Summary Statement 9: Pneumococcal vaccines are recommended for all children, adults older than 65 years, and certain high-risk groups. (Ib A)

Pneumococcal vaccination should be provided to certain subjects in accordance with the Advisory Committee on Immunization Practices (ACIP). Two types of pneumococcal vaccines are available. These are (1) PCVs (Prevnar 7 and Prevnar 13; Wyeth Pharmaceuticals, Madison, NJ) and (2) PPV23s (Pneumovax; Merck & Co, Whitehouse Station, NJ).

Prevnar 13 (PCV13) was licensed in February 2010 and will replace PCV7 (Prevnar 7) by using the same schedule for initial and booster immunizations. PCV13 contains 6 additional conjugated capsular polysaccharide antigens not present in PCV7. Therefore PCV13 should be viewed as the primary PCV relevant to considerations applied to patients under evaluation for PIDDs relative to the remainder of this document. However, with that qualification, much of the PCV data available for consideration relative to this document are derived from the use of PCV7, and thus PCV7 is discussed extensively throughout.

PCV7 is composed of purified capsular polysaccharides of 7 pneumococcal serotypes conjugated to CRM197, a diphtheria toxoid protein. PCV7 includes serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. PCV13 contains the 7 serotypes in PCV7 plus 6 additional serotypes (serotypes 1, 3, 5, 6A, 7F, and 19A). PPV23 (Pneumovax) is composed of purified capsular polysaccharides of 23 serotypes, including **1**, 2, **3**, **4**, **5**, **6B**, **7F**, 8, 9N, **9V**, 10A, 11A, 12F, **14**, 15B, 17F, **18C**, **19A**, **19F**, 20, 22F, **23F**, and 33F. (Serotypes in PCV13 are shown in boldface, and serotype 6A is not present in PPV23.[58])

Previously PCV7 and now PCV13 are recommended for vaccination of all infants at 2, 4, 6, and 12 months of age. PCV7 and now PCV13 are also used in children 24 to 59 months of age who have not been previously immunized or had incomplete vaccination before age 24 months and therefore are considered to be at high risk of acquired invasive pneumococcal disease.

PPV23 is currently the most useful agent for evaluating clinically relevant T-independent antibody responses in infection-prone patients (see Summary Statement 16).[59] The pneumococcal vaccine contains 25 μg each of 23 purified capsular polysaccharide antigens (*Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20 22F, 23F, and 33F).[60] PPV23 is recommended for patients older than 65 years or high-risk pediatric patients in an effort to reduce their susceptibility to infection (irrespective of any effort to pursue the diagnosis of an immunodeficiency). PPV23 use can also be considered in high-risk patients previously

given PCV7 or PCV13 to broaden their coverage. High-risk patients, such as those with sickle cell disease, asplenia, asthma, diabetes mellitus, cochlear implants, cerebrospinal fluid leaks, HIV infection, nephrotic syndrome, other immunodeficient states (primary or secondary), or chronic heart, lung, or liver disease; American Indian/Alaskan native children; and solid organ transplant recipients, should receive pneumococcal immunization in accordance with published recommendations.[58]

Summary Statement 10: Pneumococcal vaccines are usually well tolerated. (Ib B)

Pneumococcal vaccines are usually well tolerated.[58,61-63] Adverse events have been described and include localized redness, swelling, and occasional fever for 1 to 2 days after vaccine administration. Anaphylactic reactions are very rare. Some patients with preexisting antibodies associated with previous receipt of pneumococcal vaccines might have exaggerated local reactions; these can be treated with nonsteroidal anti-inflammatory medications and local comfort measures, including warm or cold compresses.

Summary Statement 11: Different titers of pneumococcal antibodies might serve different anti-infective purposes. (IIb B)

Pneumococcal antibody levels, as measured in sera, that are required to prevent sinusitis, otitis, bronchitis, and pneumonia might need to be higher than those required to prevent hematogenously invasive pneumococcal disease because adequately protective serum antibody levels might not get into extravascular locations at high enough levels.[61,63-65]

Summary Statement 12: Pneumococcal antibody titers vary over time in healthy subjects. (IIb B)

After pneumococcal polysaccharide vaccination, serum antibody titers in most patients decrease after several months to years, frequently decreasing to prevaccination levels by 5 years after vaccination in subjects less than 65 years of age.[64,65] In those 65 years or older, the decrease to prevaccination levels can occur within 2 years. In general, PCVs are thought to be more effective in defense against the serotypes of *Pneumococcus* species contained in these vaccines because of a greater responsiveness afforded by the conjugated diphtheria toxoid immune stimulation effect.[58,61,62,66-68]

Healthy subjects immunized more than 5 years previously might have waning antibody levels; therefore nonprotective titers in these subjects are generally not evidence of antibody immunodeficiency.[69] Healthy nonimmunized subjects often have protective antibody levels to some serotypes but not to others as a result of clinical or subclinical infection; absence of some antibody serotypes in the nonimmunized subjects does not indicate immunodeficiency. In contrast, failure to demonstrate sufficiently increased titers after immunization could be indicative of immunodeficiency.

Summary Statement 13: Pneumococcal antibody titers might be of value to determine the response to documented past pneumococcal infection if the infecting serotype is known. (IV D)

Patients with pneumococcal infection are expected to have measurable titers against the serotype of the infecting bacteria. This can represent a specific antibody response and would be expected to occur in an immunologically healthy subject. Similarly, many patients with PIDDs and antibody defects would be expected to respond imperfectly to infection with a particular pneumococcal serotype. This suggests the value of subtyping pneumococcal organisms when identified in the context of a severe infection to assess for appropriate responsiveness. However, subtyping might not be readily available in all centers. These data should be pursued when attainable, but this practice is not considered a standard of care.

Summary Statement 14: Pneumococcal IgG antibody responses are generally assessed by means of ELISA or related immunologic assay. (NR)

There are a number of methods used for the detection of *Pneumococcus* species–specific IgG.[61,63] Pneumococcal serologic assays are performed for 2 main reasons: (1) to assess whether seroconversion occurs for the purposes of protection and (2) to asses for humoral immune competence. Studies have demonstrated differences in the quality of anti-pneumococcal antibody by using various assays, and achieving certain titers presumably translates to protection from disease, although the values might be different in the different assays (see specific summary statements addressing this topic below). For vaccine trials, serotype-specific IgG assays were developed. A consensus methodology exists for this purpose with internationally available standards. However, pneumococcal IgG can suffer from poor specificity if the test antigen contains both serotype-specific polysaccharide and C-polysaccharide. Antibodies to the latter are not protective.

Currently, the most commonly used techniques for measuring anti-pneumococcal antibodies include ELISA or fluorescence multi-analyte profiling with Luminex technology (Luminex, Austin, Tex). Before specific antibody detection, the techniques include adsorption with polysaccharide C and serotype 22F to eliminate nonspecific cross-reactive antibodies.[70]

Testing is available from several commercial laboratories. Antibody titers to at least a subset of serotypes present in PCV7, PCV13, and PPV23 should be performed 4 to 8 weeks after pneumococcal vaccination. Prevaccine titers allow for determination of the extent of increase in response caused by the vaccination.

Assessment of antibody responses to pneumococcal vaccines serves 2 purposes: (1) to determine whether the subject is capable of mounting protective antibody responses and (2) to determine the magnitude of the response. Defining protective antibody levels and even "normal" ranges for pneumococcal IgG is problematic because the protective level can be different depending on the serotype being assessed; this also varies by age. Historical studies evaluating immunogenicity do reflect some lack of consensus regarding not only cutoff levels for protection but the number of serotypes defining responders and nonresponders. Some have proposed that for children 24 months through 5 years of age, a normal response to PPV is defined as "protective" antibodies to 50% or more of the serotypes tested, with at least a 2-fold increase in the titers.[71-75] For subjects aged 6 to 65 years, a normal response has been defined as protective antibodies to 70% of the serotypes tested, with at least a 2-fold increase in the titers. Additional and more current perspectives on these historical interpretations of response are provided in other summary statements that follow (see Summary Statements 22 and 32).

Summary Statement 15: Functional assays for detecting specific anti-pneumococcal antibodies also exist and might provide a better measure of anti-pneumococcal antibody quality. (IV D)

Although not commercially available, the opsonization phagocytic assay measures the functionality of the anti-pneumococcal

antibodies. Some subjects have had high levels of pneumococcal antibodies determined by using ELISA methodology and low values (poor opsonophagocytosis activity) on the opsonization phagocytic assay.[76,77] Thus the presence of anti-pneumococcal antibody and its function can be discordant. Whether this disparity has clinical correlation is not yet known.

Absorption with the serotype 22F polysaccharide improves the correlation between ELISA titers and opsonophagocytosis.[70]

Summary Statement 16: PPV is widely used diagnostically in both adults and children having completed their primary pneumococcal conjugate vaccine (PCV) series who are suspected of immunodeficiency to ascertain response to polysaccharide antigens. (Ib A)

PPV23 is routinely used in the evaluation of patients with suspected antibody deficiency, both primary and secondary.[4,78] Numerous facets are involved in interpreting the responses. as discussed in the following sections and in a number of references.[4,51,59,71-75,78-80] Although subjects less than 2 years of age have been considered hyporesponsive by some sources,[58] population immunization studies performed by the World Health Organization in the 1980s using the 23-valent nonconjugated pneumococcal vaccine,[46] as well as more recent investigations,[44,45] have demonstrated that children tested as young as 6 months of age could mount pneumococcal antibody responses, which in the case of the original reference demonstrated reduced incidence of pneumococcal disease. Thus PPVs should be used for diagnostic purposes, as clinically indicated, but should be generally avoided during the timeframe of and a period after the primary PCV series because of largely theoretic concerns for interference with the efficacy of the PCV (derivative from studies in adults [see Summary Statements 28 and 29] and experience with meningococcal vaccine [see Summary Statement 40]).

Summary Statement 17: PCV7 and PCV13 are used occasionally in the diagnosis of immunodeficiency. (IIb C)

Previously PCV7 and now PCV13 can be used in infants and children less than 60 months of age who lack protective antibody titers to the pneumococcal serotypes contained in these vaccines. Three immunizations are recommended for children less than 24 months of age, and a single immunization is recommended for children 25 to 60 months of age or adults.[58] These vaccines can be used in addition to the usual vaccine antigens used for the determination of T-dependent antibody response, such as tetanus toxoid, diphtheria toxoid, and conjugated *H influenzae* vaccines.[4]

PCV7 or PCV13 can also be used in subjects older than 2 years (including adults) with a poor response to PCV23 to determine their response to protein-conjugate antigen.[73,74] A single dose is recommended.[58] For immunodeficient HIV-infected adults, 2 doses 1 month apart were used.[81] It is important for the prescribing provider to be familiar with the FDA-approved indications for these vaccines because some uses might represent an "off-label" indication.

Summary Statement 18: Measurement of individual pneumococcal serotype titers before and after immunization and enumeration of the number of serotypes responding is an accepted technique to evaluate humoral immune function. (IIb B)

The number of individual serotypes currently used for diagnostic purposes varies from 4 to 23. However, 12 to 14 are most commonly used by allergists/immunologists. Although diagnostic approaches to humoral immunodeficiency are likely to change over time, at present, the quantitative measurement of pneumococcal IgG titers is a well-accepted standard approach.[4]

Summary Statement 19: Measurement of pneumococcal antibody titers to either vaccine should be done 4 to 8 weeks after vaccination. (Ib A)

Vaccination response is best measured more than 4 and less than 8 weeks after the immunization was provided.[82] If prior antibody titers are available, the assays ideally should be performed in the same laboratory.

Summary Statement 20: A protective (normal or adequate) response to each pneumococcal serotype is defined as a titer equal to or greater than 1.3 μg/mL antibody. (IIb C)

The protective level for each pneumococcal serotype is set at 1.3 μg/mL, as measured by using a reliable quantitative technique.[4,59,71-76,78] This consensus value has been used in several studies,[59,72-74,78] but a value of 1.6 μg/mL has been used in other studies, and some commercial laboratories use a value as low as 1.0 μg/mL or as high as 2.0 μg/mL. Lower values have also been suggested.[83,84] Clearly, controversy remains on this topic. Some commercial laboratories now use individual values determined as the means obtained for each serotype from a large number of measurements and define protective values as being in the statistically relevant range. Furthermore, several studies have shown that maximum titers achieved with each serotype can differ from each other. When reported, the conversion factor for nanograms of antibody nitrogen per milliliter (ng N/mL) to antibody micrograms per milliliter is as follows: 160 ng N/mL = 1.0 μg/mL. The reported thresholds also vary depending on whether the serotype-specific assay used C-polysaccharide and 22F adsorbents.[70]

Summary Statement 21: A normal response for a single serotype present in a pneumococcal vaccine is defined as the conversion from a nonprotective to a protective titer. (III D)

Although the definition of what constitutes a protective titer is an active area of research, it is important to appreciate the value of when a subject is able to increase the level of specific antibody from one not considered protective to one that is protective. The quantitative increase in a particular titer is the subject of much investigation and is addressed in other statements within this document (see Summary Statements 24-26).

Summary Statement 22: The number of pneumococcal serotypes that are protective after a vaccine can be used to define a normal (adequate or epidemiologic) response. (IV D)

Defining protective antibody levels and even "normal" ranges for pneumococcal IgG is problematic because the protective level might differ depending on the serotype assessed, and this also varies by age. Studies evaluating immunogenicity reflect the lack of consensus regarding not only cutoff levels for protection but the number of serotypes defining responders and nonresponders. Although based on limited evidence, some have proposed that a normal response to PPVs for children from 24 months through 5 years of age is conversion of 50% or more of the serotypes tested with at least a 2-fold increase in the titers. For subjects aged 6 to 65 years, a normal response is defined as conversion of 70% of the serotypes tested with at least a 2-fold increase in the titers.[59,72-74,78]

For a current interpretation of these historical recommendations, see the summary statements below, with particular reference to Summary Statement 32 in this section. It is important to acknowledge that this particular guideline regarding the utility of response to pneumococcal serotypes has always been offered as

expert opinion based on experiential observations of at-risk patients or those who have received a diagnosis. Substantive additional research is needed to truly define whether a particular threshold equates with normal or abnormal immunity. Importantly, the application of any guideline or interpretation of data needs to be in the context of clinical correlation, which, in the case of humoral immunodeficiency, is that of susceptibility to or atypical manifestations of infectious disease. A more detailed perspective of this working group is offered in Summary Statement 32.

Summary Statement 23: Certain pneumococcal serotypes are considered to be more reliably antigenic than others. (Ib A)

Pneumococcal capsular serotypes can vary in their immunogenicity.[51,79,80,85] For example, the serotype 3 polysaccharide is immunogenic even in young children who are unable to respond to other serotypes, whereas serotypes 6B and 23F are often poor immunogens. Thus the response to 1 or a select few pneumococcal serotypes cannot be taken as representative of protection or antibody immunocompetence. Attempts at defining responders versus nonresponders have been fraught with heterogeneity, and generalizable rules in the context of diagnostic vaccination are not possible.

Summary Statement 24: The higher the preimmunization titer for a specific pneumococcal serotype, the less likely that the titer will have a significant increase after vaccination. (III C).

An adequate response to pneumococcal vaccination has historically been defined as a postvaccine titer of greater than 1.3 μg/mL or up to a 4-fold increase in antibody titers over baseline levels. It has been established previously that the presence of a high preimmunization antibody titer does not necessarily neutralize the response to the serotype in the vaccine. Patients are still capable of mounting a biologic response on vaccine administration.[51] However, high preimmunization antibody titers to specific pneumococcal serotypes are less likely to significantly increase after immunization when compared with low preimmunization antibody titers.[4,75]

Summary Statement 25: Most patients with a prevaccine titer of greater than 1.3 μg/mL can mount a 2-fold increase in titers on immunization. A minority of patients with high initial titers will be capable of mounting a 4-fold increase in antibody titers after vaccination. (III C)

It is not uncommon for adults and children to have prevaccination titers of greater than 1.3 μg/mL for several pneumococcal serotypes. Interpretation of the response to vaccination when the preimmunization titer is greater than 1.3 μg/mL is not entirely clear. Few studies assess the postvaccine response when the prevaccination titer is greater than 1.3 μg/mL. In a recent study directly addressing this issue, postvaccine antibody titers increased approximately 2-fold for most of the 14 serotypes analyzed.[52] This was true for both adults and children. Only 10% to 40% of patients attained a 4-fold response when the initial titer was greater than 1.3 μg/mL. Thus in patients who are considered to have a protective prevaccine antibody titer (ie, initial serotype titer >1.3 μg/mL), the postvaccine response can still be used in assessing the immune response. However, for these serotypes, a 2-fold response would be considered appropriate. Importantly, as stated above, the need to interpret these data in light of clinical correlation is essential. Caution is also suggested in the management of patients who only marginally meet responses considered to be adequate.

Summary Statement 26: The probability of a 4-fold antibody response approaches zero if the preimmunization titer is between 4.4 and 10.3 μg/mL, depending on the pneumococcal serotype. (III C)

The probability of a 4-fold increase in antibody titer response decreases as the preimmunization titer increases. Additionally, there is a serotype-specific absolute preimmunization value above which a 4-fold or greater response would not be expected. This value varies between serotypes and ranges from 4.4 to 10.3 μg/mL. This holds true regardless of age, sex, IgG level, or IgG subclass values.[52] This can be simplified by assuming that patients with protective antibody titers retain the potential to mount a 4-fold increase in antibody response as long as the preimmunization titer is less than 4 μg/mL.

Summary Statement 27: Secondary immunodeficiencies might affect antigen-specific responses and diminish the response to the pneumococcal vaccine. (NR)

Antibody response can be altered in patients with underlying medical conditions, including patients with chronic debilitating diseases and patients receiving immunosuppressive medications.[4,74,78] Retesting might be warranted in these patients when their clinical condition improves.

Summary Statement 28: Immediate repeat booster doses of PPV are ineffective (and not recommended and might promote hyporesponsiveness). (Ib B)

It is unnecessary to immediately administer repeat courses of PPV23 because a significant boost in antibody titer is unlikely to occur.

In the context of repeated pneumococcal vaccination, development of hyporesponsiveness has been documented, specifically in adults who have received an initial vaccination with PPVs followed by a booster with the PPV[86] or a booster with PCVs.[87] Similarly, studies with the unconjugated meningococcal polysaccharide vaccine in infants and children demonstrated evidence of hyporesponsiveness induced by repeated use of this vaccine or subsequent vaccination with a meningococcal conjugate vaccine.[88] Studies with pneumococcal polysaccharide vaccine in similar age groups have demonstrated increases in antibody levels against certain serotypes but lower levels with others. Therefore hyporesponsiveness after repeat dosing of the 23-valent vaccine has been shown, but there is little agreement between studies.[89] This might be relevant in the vaccine-naive patients with recurrent infections undergoing immune evaluation but has not been rigorously investigated. In any case, the repetition of PPV23 is not advised. However, the seriousness of providing a diagnosis is not to be taken lightly, and caution is advised in being sure that the vaccine was properly administered and was of a valid, potent, and unexpired vaccine lot. Similarly, the adequacy of postvaccination testing should be ensured with regard to the reliability of the laboratory and timing of measurement relative to vaccination.

Summary Statement 29: Patients who have previously received PCV7 or PCV13 can be given PPV23. (III C)

Previous administration of PCVs does not preclude the subsequent administration of PPV23. Immunization with PPV23 can increase the titers of the PCV7 or PCV13 strains, as well as immunize against the strains not present in PCV vaccines.[76,79] However, it was observed in subjects older than 70 years of age that an initial dose of 23-valent PPV led to decreased response to the 7-valent PCV. The same type of observation was made with meningococcal polysaccharide vaccine,[90] and these results

are discussed elsewhere in this document. A poor response to PPV23 in this situation in a patient who has had a good response to PCV, however, is suggestive of an SAD.[59,71,75,79,80]

PCV7 administration can serve as a priming event (eg, enhance a subsequent antibody response) to PPV23.[73,74] The priming is serotype specific, so that the titer to a non-PCV7 strain is unaffected.

Summary Statement 30: A diagnosis of specific antibody deficiency (SAD) can be made if the response to PPV23 is deficient but the responses to protein antigens (eg, tetanus toxoid or diphtheria toxoid), conjugate vaccines (*Haemophilus influenzae* type b, PCV7, or PCV13), or both are intact and total immunoglobulin levels are normal. (III C)

SAD, also known as selective IgG deficiency, is a common immunodeficiency manifested by recurrent bacterial respiratory tract infections, such as sinusitis, otitis, bronchitis, or pneumonia, with laboratory findings identifying deficient PPV23 and/or other antigen-specific antibody responses.[4,59,71,74,91,92] SAD can be isolated or present as a component of other primary or secondary immunodeficiencies (eg, IgG subclass deficiency, WAS, partial DiGeorge syndrome, HIV, and splenic deficiencies).[72-74] Some children with the diagnosis of SAD not complicated by another primary or secondary immunodeficiency will demonstrate normal immune responses in later childhood and thus will "outgrow" this illness. Importantly, the diagnosis of SAD by itself is not an indication to progress to immunoglobulin replacement therapy. Immunoglobulin replacement can be effective in patients with SAD in the appropriate clinical context, specifically one in which the susceptibility to infections is impressive, other comorbid diagnoses have been managed, and antibiotic prophylaxis has been suboptimal.

Summary Statement 31: PCV7 or PCV13 protein conjugate vaccines can be administered to patients who have a poor response to PPV23. (III C)

A response to PCV suggests that the subject is able to respond preferentially to protein antigens but does not alter the diagnosis of selective antibody deficiency.[91,92] PPV vaccination can boost the preexisting antibody response to the serotypes present in the PCV vaccine.[73,74]

Summary Statement 32: The degree of polysaccharide nonresponsiveness in selective antibody deficiency can be classified into 4 phenotypes. (IV D)

A recommendation is offered for 4 phenotypes of polysaccharide nonresponsiveness after vaccination with PPV23 (Table IV). These are as follows:

- *Memory phenotype.* These individuals have an adequate initial response to PPV23 (>50% protective for children 2-5 years of age and >70% protective for those 6-65 years of age) but lose this response within 6 months. They might respond to a second administration of PPV23 after 1 year.
- *Mild phenotype.* These patients have either multiple vaccine-containing serotypes to which they did not generate protective titers (≥1.3 μg/mL) or an inability to increase titers 2-fold (≥50% for children under 6 years and ≥70% for patients 6-65 years of age), assuming the prevaccination titers are less than the threshold levels specified in Summary Statement 26 in the presence of a history of infection.
- *Moderate phenotype.* These patients have fewer than the expected number of protective titers to specific serotypes for their age (50% for children <6 and 70% for patients 6-65 years of age) but demonstrate protective titers (≥1.3 μg/mL) to 3 or more serotypes.
- *Severe phenotype.* These patients have protective titers to no more than 2 serotypes, and the titer, if present, tends to be low (<1.3-2.0 μg/mL).

A PCV booster can be considered for any of these patients at any age. The vaccine response might be both therapeutic (by providing antibody to pneumococcal serotypes) and diagnostic (a failure to respond to PCV7 or PCV13 suggests a global antibody deficiency). Overdiagnosis of humoral immunodeficiency must be avoided.

Patients with any of the above might warrant prophylactic antibiotics, immunoglobulin replacement therapy, or both given the appropriate clinical context. Immunoglobulin replacement therapy should always be considered in patients with severe and moderate phenotypes and might be appropriate for those with memory and even mild phenotypes, depending on the clinical characteristics and/or response to antibiotic prophylaxis and optimal management of comorbid conditions.

Importantly, this series of phenotypes represents the consensus of the working group, and further research will likely result in refinement and improvement of this guidance. Additional caution is recommended in patients who only marginally meet the standards of protective responses after vaccination. These patients should be monitored clinically and not necessarily dismissed as having adequate immunity. Finally, clinical correlation is essential, as referred to above, in that a hallmark of humoral immunodeficiency is susceptibility to infectious disease, atypical manifestations of infectious disease, or both. It is under circumstances of appropriate clinical correlation that this approach should be applied.

Summary Statement 33: Further clinical research is warranted to refine best practice applied to patients with specific phenotypes of selective antibody deficiency. (NR)

Although the use of immunoglobulin replacement therapy is substantiated in experimental studies (evidence level IIb) and specific recommendations for the use of prophylactic antibiotics exist, it will be important to study the context of the different subcategories.

III. Use of meningococcal vaccine to measure humoral immune function

Summary Statement 34: In the United States there are currently 3 meningococcal vaccines licensed for use in children aged 2 years and older and adults. (Ia A)

Menomune (MPSV4; Sanofi-Pasteur, Lyon, France) is a polysaccharide vaccine that has been available in the United States since 1981. It is approved for patients 2 years and older. It is the only FDA-approved meningococcal vaccine for patients older than 55 years.

Menactra (MCV4, Sanofi Pasteur) and Menveo (MCV4, Novartis) are protein conjugate–based vaccines. Menactra was licensed for use in 2005 for patients between 11 and 55 years old but has since been approved for use in children as young as 2 years. Menveo (MCV4, Novartis) was licensed in 2010 for use in patients between ages 2 and 55 years. The MCV4 vaccine is recommended as a routine vaccination for children during the 11- to 12-year-old office visit, with "catch-up" administration for children starting in high school. It is also recommended in

TABLE IV. Summary of PPV23-deficient response phenotypes

Phenotype*	PPV23 response, age >6 y	PPV23 response, age <6 y	Notes
Severe	≤2 protective titers (≥1.3 μg/mL)	≤2 protective titers (≥1.3 μg/mL)	Protective titers present are low
Moderate	<70% of serotypes are protective (≥1.3 μg/mL)	<50% of serotypes are protective (≥1.3 μg/mL)	Protective titers present to ≥3 serotypes
Mild	Failure to generate protective titers to multiple serotypes or failure of a 2-fold increase in 70% of serotypes	Failure to generate protective titers to multiple serotypes or failure of a 2-fold increase in 50% of serotypes	2-Fold increases assume a prevaccination titer of less than cutoff values in Summary Statement 26
Memory	Loss of response within 6 mo	Loss of response within 6 mo	Adequate initial response to ≥50% of serotypes in children <6 y of age and ≥70% in those >6 y of age

*All phenotypes assume a history of infection.

children younger than 11 years with high-risk conditions. The vaccine does have specific prophylactic/therapeutic uses in patients with PIDDs (notably complement deficiency) and secondary immunodeficiencies. For further information on this topic, the reader is referred to the most recent guidelines on management of PIDDs.[4]

Summary Statement 35: The 3 meningococcal vaccines contain the same serogroups. (NR)

Menomune (MPSV4), Menactra (MCV4), and Menveo (MCV4) are quadrivalent and contain *Neisseria meningitides* serogroups A, C, Y, and W-135.

Summary Statement 36: MCV4 is a protein conjugate vaccine, and MPSV4 is a polysaccharide vaccine. Therefore they differ in the mechanism of immune response. (Ib A)

Although all 3 meningococcal vaccines currently approved for use in the United States to prevent invasive meningococcal disease contain the same 4 *N meningitides* serogroups, they might stimulate an immune response through differing mechanisms. Menomune (MPSV4) is a polysaccharide-based vaccine, whereas Menactra and Minveo (MCV4) are protein conjugate vaccines. Polysaccharide-based vaccines can initially lead to a T-cell or thymus-independent immune response. T cell–independent antigens stimulate mature B lymphocytes but not T lymphocytes. This immune response is of limited duration because of poor memory B-cell induction with polysaccharide antigens.[93] This can lead to a weak or absent booster response, even with multiple doses of the immunization.[94,95] In fact, patients who have received the polysaccharide meningococcal vaccine have exhibited a state of hyporesponsiveness or decreased response on revaccination with both the polysaccharide and protein-conjugated meningococcal vaccine.[90,96,97] This is likely to be most pronounced in group C polysaccharide.[98] Several studies in adults have demonstrated a reduced response to a second dose of meningococcal polysaccharide vaccine compared with a previously unimmunized group.[86,90,99] This phenomenon has also been noted in children.[100,101]

Children younger than 2 years might be unable to mount a strong T cell–independent immune response and therefore might not be effectively vaccinated with polysaccharide immunizations (see also Summary Statement 16). Several conjugated meningococcal vaccines were developed and used widely in Europe and Canada to overcome the limitations of the polysaccharide meningococcal vaccine.[98] There are currently 2 approved conjugated meningococcal vaccines in the United States at this time.[102] Menactra and Minveo (MCV4) contain *N meningitides* serogroups A, C, Y, and W-135, which are covalently linked to diphtheria toxoid and CRM197 protein, respectively. Conjugate vaccines are formed by conjugating the polysaccharides to a protein carrier, which shifts the immune response from T-cell independent to T-cell dependent. This results in a more effective vaccine, largely by stimulating the production of memory B cells, with a broader range and higher affinity of antibody responses and improved immunologic memory.[93,98] It does not appear that immunization with the conjugate meningococcal vaccine leads to a hyporesponsive state, as has been noted with the polysaccharide vaccine.[86,100,103,104]

Summary Statement 37: There are different methodologies for assessing the immunogenicity of meningococcal vaccines. (Ib A)

Immunologic evaluation of meningococcal vaccine response is typically assessed through measurement of serogroup-specific total IgG antibodies by using ELISA and assessment of serum bactericidal activity with the serum bactericidal assay (SBA), which determines antibody function. Other methodologies have been reported but have not been widely available.

Frequently, studies testing meningococcal vaccine efficacy and immunogenicity use SBAs. Many consider human SBAs the gold standard as a measurement of protection against meningococcal disease and vaccine efficacy. Currently, these bactericidal assays to determine meningococcal vaccine response are more time intensive and are not commercially available.[105] In contrast, serogroup-specific meningococcal polysaccharide IgG antibody assays are available commercially. Multiple studies have concluded that SBA titers correlate directly with serotype-IgG ELISA concentrations after administration of a meningococcal vaccination.[105-107]

Summary Statement 38: All of the currently licensed meningococcal vaccines in the United States have been found to be immunogenic. (Ib A)

Several conjugated meningococcal vaccines used in Europe and Canada have been found to be immunogenic in adults and children as young as 2 months of age.[95,101,108,109] Menactra and Minveo are the only conjugated meningococcal vaccines approved for use in the United States. Multiple studies have evaluated the immunogenicity of the vaccines by assessing functional activity with an SBA. These studies have confirmed a clinically relevant immune response in subjects 2 to 55 years old.[102,110-114] This response has been documented for all 4 serogroups (A, C, Y, and W-135) found in the conjugate vaccine currently licensed in the United States.[102,104,114] In children less than 2 years old, this has been studied most often in vaccines containing serogroup C. Results have demonstrated the conjugated immunizations are safe and immunogenic in this age group.[95,109,112] Children who received meningococcal conjugate

vaccines have been found to have higher titers of anticapsular and bactericidal antibodies compared with those seen in subjects challenged with meningococcal polysaccharide vaccines.[90,100] This difference is less clear in adults because they often respond well to the polysaccharide-based vaccine.[108,115] Studies have also shown that long-term antibody persistence is higher in persons who received the conjugated vaccine.[95,101,109,116,117]

Summary Statement 39: Meningococcal polysaccharide vaccine is less reliable in young children. (Ib A)

The immunogenicity of Menomune, a quadrivalent polysaccharide meningococcal vaccine, has been well established. Studies have been performed in persons in all age groups, including infants and young children. As with other polysaccharide-based vaccines (see also Summary Statement 16), there is an age-related decrease in responsiveness, and children less than 18 to 24 months of age are reported to generate a less effective response. An immune response similar to what has been found in adults is not achieved until 4 to 5 years of age.[98,115,118,119] As with the conjugated vaccine, a response was seen with all 4 serogroup components in the immunization.[5,98,102,118,120]

Summary Statement 40: Meningococcal polysaccharide vaccination can result in hyporesponsiveness to subsequent meningococcal vaccination. (Ib A)

The polysaccharide–based meningococcal vaccine can lead to a state of hyporesponsiveness with future exposures to either the conjugated or nonconjugated meningococcal vaccine. The effect can persist for at least 12 months and could potentially confound further immunologic workup with vaccine responses.[96,97,109,121,122]

Immunologic refractoriness has been described several years after polysaccharide vaccination. Adult patients "boosted" with one fiftieth of the usual dose of meningococcal polysaccharide vaccine showed evidence of immunologic refractoriness if they had received the licensed meningococcal polysaccharide vaccine 4 years earlier. In contrast, antibody responses were noted in all subjects who had received the investigational meningococcal A and C oligosaccharide-protein conjugate vaccine.[86]

The effect of this refractory period on the possible risk for meningococcal infection, as well as the use of other immunizations in evaluation of the immune system, is a theoretic issue and in need of further investigation.

Summary Statement 41: There are commercially available laboratory tests for meningococcal antibody titers. (III C)

Meningococcal antibody titers are available from several commercial laboratories. The method used is the multianalyte immunodetection that measures serum IgG antibodies recognizing polysaccharide antigens from the 4 *N meningitides* serogroups included in Menactra, Menveo, and Menomune.

Summary Statement 42: An increase in titers of at least 2 meningococcal serogroups is expected after vaccination of an immunocompetent subject. (IV D)

A 2- to 4-fold or greater increase of at least 2 serogroups is believed to be the expected response when comparing postvaccination with prevaccination results. This has not been rigorously studied in relation to the workup of the immune system and in the diagnosis of immunodeficiency. Levels are expected to peak around 4 weeks after vaccination.[5,98,120] Specific recommendations regarding the number of serotypes and the minimal titer achieved to be considered a normal immune response needs further study to allow for the widespread use of this vaccine in the diagnosis of primary antibody immunodeficiency.

Summary Statement 43: Immunogenicity might depend on several factors (which could have relevance if additional manufacturers begin to produce these vaccines). (IIb C)

Various factors affect the immunogenicity of vaccines, including dose, serogroup, and conjugation status. Conjugation considerations include oligosaccharide chain length, number of conjugation sites, conjugation chemistry, specific adjuvant used, manufacturing process, and formulation. Most of the conjugate vaccines use the same few carrier proteins, which raises the issue of antigenic competition. The repeated use of the same carrier molecule with different polysaccharide vaccines might interfere with the response to an alternative conjugated polysaccharide.[93,123]

Summary Statement 44: Given that there are commercial laboratories that measure meningococcal antibody titers and both vaccines have been proved to be immunogenic, responses could be used in the clinical evaluation for immunodeficiency. (IV D)

The conjugate vaccine might not play a significant role in the workup for immune defects, especially in children. There are many other vaccines included in the recommended immunization series leading to a T cell–dependent, B cell–mediated immune response. The use of those vaccines in the screening of a child for immunodeficiency could decrease the number of needle sticks that would be required. There is no pure polysaccharide vaccine in the routinely recommended immunization series. Therefore MPSV4, the polysaccharide-based pneumococcal vaccine Pneumovax, or both are available tools that assess T cell–independent B-cell immune responses.

Summary Statement 45: There are specific considerations regarding the immunogenicity of certain meningococcal serogroups should they be available in vaccines. (III C)

Another concept to consider is what, if any, role naturally occurring meningococcal antibodies play in the response to meningococcal vaccination.[124,125] Although serogroup B accounts for a significant portion of cases of meningococcal disease, especially in young children, it is not a part of any vaccine currently in use. This is due to its poor immunogenicity and cross-reactivity with glycoproteins expressed on brain cells. There is concern that this could lead to adverse reactions to a vaccine that contains the serogroup. Various formulations for group B vaccines have been studied, including a native outer-membrane vesicle vaccine, but to date, they have lacked efficacy, as measured based on the bactericidal antibody response.[126] If serogroup B becomes part of a licensed vaccine, studies would need to be performed looking into its immune responsiveness.[104,119]

IV. USE OF ALTERNATIVE VACCINES AND TRUE NEOANTIGENS IN EVALUATING DEFECTIVE HUMORAL IMMUNITY

Summary Statement 46: Immunization with neoantigens can be used in the evaluation of specific antibody response in the setting of immunoglobulin replacement therapy. (III C)

The AAAAI's "Practice parameter for the diagnosis and management of primary immune deficiency"[4] recommends the use of standard childhood immunization for assessing antibody responses. Immunization with the neoantigen bacteriophage φX174 is an option in some centers for the evaluation of patients

TABLE V. Neoantigens considered for diagnostic use in patients with primary immunodeficiency

Antigen	Licensed vaccine	Route of administration	Doses needed	Titer measurements	Notes
φX174	No	IV	4	8 over 3 mo	Administered as an IND
KLH	No	SC	Up to 9	2 wk after	Insufficient information available for routine use in patients with PIDDs
Rabies virus vaccine	Yes	IM	1, 2, or 3	Varied	Further study needed in patients with PIDDs
Meningococcal vaccines	Yes	IM	NA	NA	Not recommended per Summary Statement 62
Salmonella typhi Vi	Yes	IM	Unclear	Unclear	Further study needed in patients with PIDDs

IND, Investigational new drug; *IM*, intramuscular; *IV*, intravenous; *NA*, not applicable; *SC*, subcutaneous.

who are receiving immunoglobulin supplementation; however, it can only be used on a research basis because it is not an FDA-licensed vaccine. In the field of primary immunodeficiencies "neoantigen" refers to an immunogen to which the host has not been previously exposed, and there are currently several options available. Under certain circumstances, antibodies to these antigens are underrepresented in the therapeutic polyclonal immunoglobulin pool and thus can be used to immunize a patient receiving immunoglobulin replacement therapy. Various "neoantigens" under consideration in this document are presented in Table V.

Summary Statement 47: Sufficient experience does not exist regarding the use of routine vaccines in the context of a patient with primary immunodeficiency receiving immunoglobulin replacement therapy to assess antibody response. (IV D)

Patients receiving immunoglobulin replacement therapy for antibody deficiency would be expected to have reasonable antibody titers for routine vaccines because of passive transfer. This will likely interfere with the assessment of a patient's endogenous humoral responses. There are no published studies testing the humoral response to childhood vaccines in patients suspected to be immunocompetent who are receiving immunoglobulin replacement therapy. The antibody titers of 5 pneumococcal serotypes in 7 commercial polyclonal immunoglobulin products have been evaluated and have demonstrated significant interproduct variability.[127] In this one study, calculated serum titers that would be obtained in a 20-kg child receiving 400 mg of intravenous immunoglobulin per kilogram of body weight were 0.10, 0.18, 0.17, 0.77, and 0.58 μg/mL for serotypes 4, 6B, 9V, 14, and 19F, respectively.

Use of bacteriophage φX174 to measure humoral immune function

Summary Statement 48: The only neoantigen that has been extensively studied to assess human antibody responses is the T cell–dependent antigen bacteriophage φX174. (III C)

Currently, only the T cell–dependent antigen bacteriophage φX174 has been well documented as a neoantigen to assess human antibody responses.[128] In the original application of bacteriophage φX174, it was administered to patients with XLA and other primary immunodeficiency states.[129] Patients with XLA did not respond to vaccine. The remaining patients made either IgM-specific antibodies only or both IgM- and IgG-specific antibodies. Patients who did not make IgG antibodies to the bacteriophage were more likely to have recurrent respiratory tract infections. This initial experience suggested that the use of this immunization protocol was helpful to predict the risk of infection.

Summary Statement 49: Immunization with the neoantigen bacteriophage φX174 and subsequent evaluation of specific antibody responses might be included in the diagnosis of primary immunodeficiency to assess antigen-specific class-switching and the kinetics of the antibody response, including in the evaluation of patients who are already receiving immunoglobulin supplementation. (III C)

The immunization protocol with bacteriophage φX174 has been used to report humoral immune responses in patients with immunodeficiency diseases, such as adenosine deaminase (ADA) deficiency, and in immunoreconstituted patients after hematopoietic cell transplantation.[128,130] Bacteriophage φX174 was used to compare different treatments used in 10 patients with ADA deficiency. Those who were not treated produced minimal specific anti-φX174 antibodies. Two patients undergoing transplantation with bone marrow from matched related donors and with subsequent normal T-lymphocyte function produced specific antibacteriophage antibodies; however, the switch from IgM to IgG was abnormally low. Four patients receiving transplants of T cell–depleted haploidentical bone marrow stem cells and with subsequent normal T-cell function had low specific antibody responses for at least 3 years after transplantation. Treatment with PEG-ADA, which was used in the other 4 patients, led to some restoration of immune function and resulted in normal specific antibody responses to bacteriophage in 3 of them and a suboptimal response in the remaining patient. However, application of this neoantigen is not a standard of care (see Summary Statement 50).

Summary Statement 50: Immunization with the neoantigen bacteriophage φX174 is relatively labor intensive and is performed as research. (IV D)

The bacteriophage φX174 is not an FDA-licensed vaccine. The available immunization protocol recommends 4 vaccinations with φX174 administered intravenously and 8 specific time points over 3 months for the assessment of the antibody responses. This approach provides kinetic data and information about class-switching and IgM persistence and might provide information regarding antibody affinity maturation.[129] Although this protocol is a well-documented approach, it is rather time and resource demanding as designed and would benefit from further development to be a practical general clinical test. Furthermore, an investigational new drug license and an institutional review board–approved research protocol are required to undertake this evaluation by using φX174. When considering the practical and regulatory aspects, as well as the cost and time involved, there are currently no approaches for the evaluation of an immunologic response to neoantigens that are readily available for routine patient evaluation in a clinical setting.

It is recommended that, when clinically feasible, all diagnostic studies of antibody specificity should be performed before initiating immunoglobulin replacement therapy.

Summary Statement 51: Keyhole limpet hemocyanin (KLH) is a potential alternative to φX174 as a neoantigen. (IV D)

KLH has been used as a neoantigen in the evaluation of human antibody response on a research basis.[131] There are presently not sufficient data to assess the widespread applicability of KLH as a human neoantigen for use in evaluation of patients with primary immunodeficiency. However, it has been studied in patients with secondary immunodeficiency.[132] It is reasonable to consider KLH a potential alternative to φX174 as a neoantigen, although further study is needed in the context of primary immunodeficiency for it to be considered an actual alternative.

Use of human rabies virus vaccine as an alternative neoantigen to evaluate humoral immune function

Summary Statement 52: Rabies virus vaccines are available and used in the United States as postexposure prophylaxis. (Ib A)

Rabies is a zoonotic disease resulting from infection with an RNA virus (*Lyssavirus* species) and causes an acute progressive encephalomyelitis. Rabies is relatively uncommon in the United States; however, rabies poses a risk to international travelers in areas in which it remains endemic. Studies on rabies vaccine safety and efficacy indicate that postexposure prophylaxis combined with wound treatment, local infiltration with rabies immune globulin, and vaccination are extremely effective when all components are appropriately administered.

Two cell-culture rabies virus vaccines are available for use in the United States: human diploid cell vaccine (HDCV; Imovax, Sanofi Pasteur) and purified chick embryo cell vaccine (PCECV; RabAvert, Novartis Vaccines and Diagnostics). These vaccines are formulated only for intramuscular administration in a single-dose vial. Both vaccines induce an active immune response with the production of viral neutralizing antibodies. The antibody response requires approximately 7 to 10 days to develop after completing the immunization series, and detectable rabies virus neutralizing antibodies generally persists for several years (reviewed by Manning et al[133]). In the immunization regimen used for pre-exposure prophylaxis, the vaccine is administered on days 0, 7, and 21 or 28.

Summary Statement 53: Rabies virus vaccination is generally well tolerated. (Ib A)

Local reactions occur with HDCV in approximately 60% to 89% of recipients. This is in contrast to PCECV, with which local reactions, such as pain at the injection site, redness, swelling, and induration, were reported for 11% to 57% of recipients. Local pain at the injection site is the most common local reaction. These local reactions are mild and usually resolve within a few days. Systemic reactions are less common and mild, such as fever, headaches, dizziness, and gastrointestinal symptoms. They have been reported in 7% to 56% of HDCV recipients and 0% to 31% of PCECV recipients. Hypersensitivity reactions have been reported in 6% of patients receiving booster vaccines after the primary rabies prophylaxis vaccination regimen. Rarely, neurologic adverse events after rabies vaccination have been reported, but in none of these cases has causality been established (reviewed by Manning et al[133]).

Summary Statement 54: Cell culture–derived rabies virus vaccines as pre-exposure vaccines elicit adequate humoral immune responses. (Ib A)

A number of studies have provided evidence for the effectiveness of pre-exposure rabies vaccination to elicit an adaptive immune response in human subjects. An adequate humoral immune response, as defined by the ACIP, is an antibody titer of 0.5 IU/mL or complete virus neutralization at a 1:5 serum dilution by using the rapid fluorescent focus inhibition test (RFFIT). Multiple studies comparing different pre-exposure prophylaxis regimens led to the recommendation of vaccination with 3 intramuscular doses of cell-culture rabies virus, which results in neutralizing antibody titers of greater than 0.5 IU/mL by 14, 21, and 28 days after primary vaccination. In some studies immunization with HDCV resulted in higher titers than seen in the group of subjects receiving PCECV at day 28. However, subsequently (eg, days 50 and 92), there was no difference in the geometric mean titers observed between the 2 vaccine types when administered through the intramuscular route.

Although a 3-dose rabies pre-exposure prophylaxis series is the standard regimen recommended by the World Health Organization and ACIP,[134] a 2-dose pre-exposure series has been used in other countries.[134] One study compared 2 doses (days 0 and 28) versus 3 doses (days 0, 7, and 28) administered through the intramuscular route and showed that persistence of titers was greater in those subjects receiving 3 vaccine doses.

Summary Statement 55: Rabies virus vaccines can be used as a neoantigen to assess humoral immune responses in healthy subjects. (IIb B)

Rabies virus vaccines have been used as neoantigens to assess humoral immunity.[131,135] One study of 18 healthy subjects evaluated the antibody response and peripheral blood lymphocyte proliferative responses to rabies virus vaccine after a primary and single-booster immunization (HDCV) administered at a 3-month interval.[131] All subjects mounted an antibody response in the IgG (IgG_1 and IgG_3 subclasses), IgM, and IgA isotypes after a primary and booster immunization. IgG antibody titers showed a mean 31-fold increase 4 weeks after the first vaccine, and a secondary antibody response was observed after the single-booster vaccine with a switch from IgM- to IgG- and IgA-specific antibodies and an increase in antibody avidity. Only 1 subject did not reach the protective IgG antibody level after the primary immunization (0.5 IU/mL). The highest IgG anti–rabies virus antibody level was detected 2 weeks after the booster immunization compared with 4 weeks after the primary immunization. Lymphocyte proliferative responses were also measured after the primary and booster rabies virus vaccinations. Four weeks after the primary immunization, 7 of 18 subjects showed a stimulation index of 3 or greater, and all subjects achieved a stimulation index of 3 or greater at 4 weeks after the secondary immunization.

Summary Statement 56: Although rabies virus vaccines can elicit lymphocyte proliferative responses after immunization, the rabies virus nucleocapsid can produce a superantigen response by human T cells that might compromise its utility to assess cell-mediated immune responses as a neoantigen. (IIb B)

Rabies virus as a neoantigen has been evaluated for an ability to elicit lymphocyte proliferative responses after immunization.[136] Specifically, 3 doses of rabies virus (Imovax IM) administered intramuscularly over the course of a month, with 1 dose each on days 0, 7, and 28, were used. Peripheral blood mononuclear cells were evaluated for proliferative responses to rabies virus 4 weeks after the final rabies vaccine immunization. Although

all vaccines demonstrated an increase in rabies virus–specific antibody (>0.5 IU/mL), only 83% demonstrated a lymphocyte proliferative response after 8 days of culture of their cells with soluble rabies virus antigen. Four subjects did not demonstrate a 2-fold increase in lymphocyte proliferative response to rabies virus antigen because of high preimmunization lymphocyte proliferative responses. These baseline increased lymphocyte proliferative responses to the vaccine antigen might be due to a viral superantigen effect in some subjects. Rabies virus nucleocapsid can elicit a Vβ8-specific superantigen response of human T cells.[137,138] Thus the rabies virus nucleocapsid protein might function as a superantigen for T-cell lymphocyte proliferative responses and might compromise the utility of rabies virus vaccines in assessing cell-mediated T-cell lymphocyte proliferative responses to this neoantigen.

Summary Statement 57: Rabies virus vaccine can be used as a neoantigen to evaluate humoral immune responses in patients with secondary immune deficiency; however, the degree of the response might be linked to the dose (micrograms of protein) of the vaccine. (IIb C)

Patients with secondary immunodeficiency have been evaluated with rabies virus vaccine (dog kidney cell tissue culture derived, 170 μg of protein/mL).[132] In this study 81 control subjects were immunized subcutaneously with 6 different doses of rabies virus vaccine, and specific antibody titers were determined by means of ELISA and immunofluorescence before and 14 days after administration. A rabies virus vaccine dose of 170 μg of protein was sufficient to produce a detectable IgG antibody response in all subjects. Patients considered to have a form of secondary immunodeficiency caused by uremia were also evaluated, and only 3 of 19 responded to the 170 μg of rabies virus protein immunization with rabies virus–specific IgG. When a higher dose of rabies virus vaccine (680 μg) was used, 16 of 20 responded.

Rabies virus vaccination (HDCV) was also studied in HIV-infected children using a 3-dose regimen (0, 7, and 28 days), after which neutralizing antibody levels were measured.[139] Geometric mean titers of rabies antibody in the HIV1-infected children were significantly lower than those in the control groups. Furthermore, those HIV-infected children with 15% $CD4^+$ cells or less had significantly lower antibody titers than children with 15% $CD4^+$ cells or greater.

Thus although rabies virus vaccine could be used as a neoantigen in a secondary immunodeficiency setting, higher vaccine doses can potentially mask a defective response.

Summary Statement 58: Rabies virus vaccine can be used as a neoantigen to evaluate humoral immune responses in patients with primary immune deficiencies. (IIb C)

Rabies virus vaccination has been evaluated in 5 patients with primary immunodeficiency (age 4-13 years).[131] A majority of patients in this study mounted normal primary and secondary IgG anti-rabies antibody responses to rabies virus vaccine. Despite reduced numbers of circulating B cells and a severely decreased immune response after vaccination with tetanus toxoid and conjugated *H influenza* type b polysaccharide, these patients were able to produce normal IgM and IgG isotype antibodies to rabies virus vaccine. Thus it is unclear how sensitive rabies virus will be as a screen for humoral immunodeficiency, although there are likely issues regarding dosing and regimen (see Summary Statements 59 and 60 for consideration of regimen and applicability).

A separate group of patients with a genetically confirmed primary humoral immunodeficiency (CD19 deficiency) were also evaluated with rabies virus vaccination[140] by using a primary and single-booster immunization. All but 1 patient could produce anti-rabies IgG antibodies after the primary immunization, but the secondary IgG antibody response at week 13 was less than the 95% confidence limit of responses from healthy subjects in all patients.

Given the rare but reported adverse events associated with rabies virus administration (see Summary Statement 53), this vaccine is not recommended as a neoantigen challenge for all patients suspected of having a humoral immunodeficiency. Until additional studies of safety are performed, rabies virus vaccination for diagnostic purposes is only recommended as a consideration in challenging diagnostic circumstances in which additional data are needed.

Summary Statement 59: A single injection of rabies virus vaccine might be useful in eliciting a measurable antibody response, but further study of this intervention in primary immunodeficiency diagnostic evaluation is needed. (IV D)

The number of immunizations needed to evaluate the humoral response to rabies virus vaccine is a major issue in considering the application of this vaccine to patients with primary immunodeficiency for diagnostic purposes. A primary immunization was enough to elicit an IgG antibody response in some studies. One primary immunization with 1 booster immunization 3 months later is enough to lead to satisfactory protective levels of IgG antibodies in healthy subjects.[131] However, this might present an unacceptable timetable in the evaluation of a patient with a primary immunodeficiency. Thus more data are needed to determine whether a single rabies virus vaccine dose can discriminate between healthy subjects and those with primary immunodeficiency.

Summary Statement 60: Rabies virus vaccination can potentially be used to assess humoral immune function in a patient receiving immunoglobulin replacement therapy. (III C)

The administration of rabies vaccine to patients with immune deficiency receiving immunoglobulin replacement for the purpose of evaluating their specific antibody responses after immunization with a neoantigen might be useful because therapeutic polyclonal immunoglobulin is not expected to contain significant anti-rabies antibody titers. In 2 studies patients with a primary immunodeficiency[131,140] who were receiving immunoglobulin replacement therapy were given rabies virus vaccine to evaluate their specific antibody responses. This limited experience (obtained outside of the United States) demonstrated some utility to this intervention. However, further study is needed to define any broad applicability in patients with primary immunodeficiency receiving immunoglobulin replacement therapy. This is presently not recommended as a routine test for patients with primary immunodeficiencies receiving immunoglobulin replacement therapy.

Summary Statement 61: Testing for rabies virus vaccine–specific antibodies is available, but the general application of specific methods in patients suspected of having primary immunodeficiency needs to be established. (IV D)

There are several potential issues regarding the testing for anti–rabies virus antibodies. The RFFIT is performed by certain state department of health laboratories. However, this is commercially available in Clinical Laboratory Improvement

Amendments–certified laboratories (an example is the Kansas State Veterinary Diagnostic Laboratory: http://www.vet.k-state.edu/rabies).

By using the RFFIT, the result of a 1:5 titer might not distinguish between those patients with PIDDs and healthy subjects but is considered an adequate response.[133] However, there are several ELISAs reported in the literature[141] that might be easier to perform and provide results that can distinguish between healthy subjects and immune-deficient patients.

Summary Statement 62: In contrast to rabies virus vaccine, it is unlikely that meningococcal vaccine will be a suitable neoantigen for patients receiving immunoglobulin replacement therapy. (IV D)

The increasing application of meningococcal vaccination in the general US population will most probably lead to an increase in meningococcus-specific antibody in plasma pools generated for use in the United States. Thus it is unlikely that meningococcal vaccination will be able to be used as a neoantigen for patients receiving immunoglobulin replacement in the United States.

Summary Statement 63: The use of *Salmonella typhi* Vi vaccine has future potential as a diagnostic and alternative polysaccharide antigen in patients with primary immunodeficiencies, but sufficient data are not presently available to support its use. (IV D)

Salmonella typhi Vi vaccine (available in the United States as Typhim Vi) is an extracted polysaccharide vaccine for intramuscular use as an alternative polysaccharide vaccine for evaluating anti-polysaccharide antibody responses. In licensing studies for this product, 96.3% of 2- to 5-year-old children were reported to have a 4-fold or greater increase in specific antibody levels, with prevaccination and postvaccination mean titers of 0.16 and 3.23 μg/mL, respectively. A study of responses in healthy donors measuring prevaccination and postvaccination sera by using ELISA documented a mean greater than 10-fold increase in children.[142] These authors have proposed that a greater than 3-fold response be considered normal. Because the prevaccination titers to *Salmonella typhi* in the population is generally low, this vaccine presents promise for use in patients with primary immunodeficiency as a diagnostic challenge. Substantial further study is needed to determine how this might be used in practice.

V. VARIABILITY IN IMMUNOGENICITY AMONG CURRENTLY AVAILABLE VACCINES

Although rare, variability among vaccine lots does occur and can result in decreased immunogenicity and even vaccine failure. Conceivably, this could lead to inappropriate conclusions when vaccines are used in the assessment of immune competence. Ongoing efforts to standardize specifications for raw materials, production facilities, manufacturing processes, and control testing of vaccines are robust and imperative. General aspects pertaining to potential variability, as well as issues specific to immunodeficiency populations, are discussed.

General considerations

Summary Statement 64: The FDA requires that vaccine manufacturers must test each lot and demonstrate conformance to established standards for that vaccine. (NR)

The Code of Federal Regulations, Title 21 for Food and Drugs, Chapter I, Subchapter part 610, delineates the "General biological products standards."[143] Therein the regulatory guidelines for production and testing for vaccines are provided and create a standard to which all vaccines available in the United States are to be held. Elements specific to individual vaccines are also provided through this guidance.

Summary Statement 65: When assessing vaccine lot consistency, it is important to understand the interrelationship between efficacy, immunogenicity, and potency. (IV D)

"Vaccine efficacy" is the ability to induce a state of resistance to disease. "Immunogenicity" is the property of a vaccine to induce a distinct immune response. This is typically characterized by clinical laboratory measurements (eg, specific antibody production, cytokine profile, and antigen-specific T cells). "Potency" is used by the FDA to describe the specific ability or capacity of the product (vaccine) to affect a given result.[143] Vaccine potency is therefore relative, as determined by making a comparison with reference material (usually the serial vaccine used to demonstrate efficacy of the vaccine). These comparisons often rely on surrogate markers (eg, measures of immunogenicity) rather than on direct comparison of clinical efficacy.

Summary Statement 66: Vaccine lot consistency is generally based on measures of potency. (Ib B)

Vaccines are initially validated by demonstrating clinical efficacy in patient populations. During this process, measures of immunogenicity (eg, production of serotype-specific IgG) in vaccinated hosts are determined and might later be used as the basis for assessing the potency of subsequent vaccine lots. Other potency measurements might include antigen quantitation or quantitation of replicating immunogens within the final product.[144]

For example, the immunogenicity and efficacy of early pneumococcal polysaccharide vaccines (PPVs) was based on measurement of serotype-specific IgG antibodies and reduction in cases of laboratory-verified pneumococcal pneumonia in specific cohorts.[145-147] Subsequent studies of both the polysaccharide and protein-conjugated pneumococcal vaccines have largely relied only on generation of serotype-specific IgG antibodies to establish lot potency.[148-150] Efficacy is presumed based on comparison of these measures of potency.

Similarly, the efficacy of tetanus toxoid (inactivated tetanus toxin) was originally determined by the survival of immunized guinea pigs or mice after challenge with tetanus toxin.[151] As with the pneumococcal vaccines, immunogenicity and lot potency of tetanus and tetanus-containing combined vaccines are now largely determined by means of quantitation of tetanus toxin in vaccine and *in vivo* measurement of anti-tetanus antibodies.

As noted elsewhere in this document (Summary Statement 37), the development of meningococcal vaccines used both serogroup-specific total IgG antibodies determined by means of ELISA and SBA to assess immunogenicity. Although both can be used to assess lot potency, quantitation of specific IgG antibodies are now most often used because the SBA tends to by more time and labor intensive.

Live attenuated viral vaccines (including combination vaccines) are manufactured to include specific minimum amounts of immunogens (viral particles). This, along with quantitation of virus-specific IgG levels, is often used to assess lot potency and immunogenicity. Occasionally, these data are published to demonstrate the initial lot consistency of a new vaccine, as in ProQuad (measles, mumps, rubella, and varicella; Merck & Co) combination vaccine.[152] It is interesting to note that historical data from efficacy or field effectiveness studies previously conducted for

the component vaccines were (and often are) used to define levels of serum antibodies that correlated with protection against measles, mumps, rubella, and varicella.

Summary Statement 67: Vaccine potency is dependent on numerous factors. (III C)

Early in the development of the PPV it was evident that the amount of antigen influenced immunogenicity.[153] With the development of protein-conjugated vaccines, including that for *Pneumococcus* species and HIB, it was noted that the nature and amount of carrier protein, concomitant vaccines, and timing of a vaccine in relation to previous doses also influence immunogenicity.[148-150,154,155] Over time, it has become clear that many factors might have influence. Antigen quality, product contamination, adjuvant strength, process failures, improper storage, environmental factors, and operator blending errors are all variables that could result in reduction of potency.[144]

Summary Statement 68: Although potency measurements are considered to be standardized, they do not guarantee lot consistency as it relates to immunogenicity or efficacy. Despite meeting potency standards, there are data that suggest lot variation occurs and that vaccine lots have failed. (III C)

Lot-specific variations in immunogenicity of a heptavalent conjugated pneumococcal vaccine have been identified.[156] There are several trials recently completed or ongoing that attempt to address immunogenicity among lots of conjugated pneumococcal vaccines as identified through www.clinicaltrials.gov, such as NCT00680914.

Apparent vaccine failures have also been acknowledged in the literature. Although host factors, such as prematurity, immune deficiency, malignancy, and certain genotypes, are often identified in these cases, confirmation of failure caused by a defective (ie, impotent) vaccine lot is far less frequent but has been documented.[157-159] With regard to diagnostic implications, ensuring the validity of a given lot of vaccine is recommended. It is also important to confirm that the actual vaccine has been adequately and appropriately administered. However, repeat vaccination is not routinely recommended unless a validity concern is identified.

Variability in immunogenicity among currently available vaccines specific to assessing immunodeficient populations

Summary Statement 69: Tetanus toxoid vaccines demonstrate no significant immunogenic variability and are good diagnostic tools for evaluation of immune competence to T-dependent antigens. (Ib A)

The ability to respond to T-dependent antigens (protein antigens or toxoids, such as tetanus or diphtheria) is essentially mature at birth. This is why primary immunization can be administered to infants between the second and sixth months of life. However, purified antigens are not strong immunogens and require the help of adjuvants: tetanus toxoid is not immunogenic in the absence of aluminum salts, and pertussis toxin has adjuvant properties by itself and, when mixed with tetanus and diphtheria toxoids, acts as an adjuvant for the 2 other toxoids. Current tetanus toxoid vaccines are immunogenic in all immunocompetent subjects, irrespective of age, with a protective humoral 5-year time span in 95% of the population,[160] whereas immunocompromised subjects, such as those who have undergone transplantation (solid organ or bone marrow), receive chemotherapy, or are infected with HIV, will demonstrate variable response to tetanus toxoid antigen, depending on the net state of immunosuppression.[161] In general, tetanus toxoid vaccines demonstrate no significant immunogenic variability because of the nature of the antigen and thus are good diagnostic tools for evaluation of immune competence to protein antigens. Thus an absent response should be considered abnormal until proved otherwise.

Summary Statement 70: Protein-conjugated HIB and pneumococcal vaccines show variability in immunogenicity because of the protein carrier and nature of the antigen. (Ib A)

HIB vaccines are designed to produce antibodies to the capsular component polyribosylribitol phosphate (PRP). Because of the poor immunogenic response produced by PRP alone, it has been conjugated to carrier proteins with the purpose of enhancing T-dependent responses and immunologic memory. HIB vaccines show some variability of immunogenicity based on the protein carrier: the mutant diphtheria protein CRM197 (HbOC or PRP-CRM), meningococcal protein conjugate (the outer membrane protein complex of *N meningitides*; PRP-OMP), or tetanus toxoid (PRP-T).[162] However, interchanging conjugate vaccines in primary series does not affect immunogenicity, and the concentration of the antibody after mixed vaccine regimens can be higher than after administration of one type of vaccine for all doses.[163] All the currently licensed HIB vaccines are immunogenic in populations with low levels of late-age HIB disease.[162] When tested in American Indian populations with a high rate of HIB disease, the least immunogenic vaccine using diphtheria toxin as a carrier did not provide effective protection (reviewed by Heath[162]). In general, HIB conjugate vaccines are "good" immunogens, and a poor response is highly suspicious for immunodeficiency.

The 2 commercially available heptavalent PCVs use the mutant diphtheria protein CRM197 (PCV7) or the *N meningitides* outer membrane protein as carrier proteins (Pnc-OMPC). Both conjugate vaccines demonstrate similar immunogenicity when a third priming dose is administered. Clinical trials have demonstrated that 82% to 100% of participants were capable of achieving serum antibody levels of greater than the selected cutoff established by the World Health Organization for all vaccine serotypes.[164,165] On the other hand, PncOMP vaccine is less immunogenic than PCV7, with 82% to 88% of participants achieving protection, as determined by serum titers greater than the chosen cutoff value after 3 priming doses.[166] Furthermore, the polysaccharides used within the conjugate vaccine formulations to protect against serotypes 6B, 23F, and 9V appear to be less immunogenic. However, for serotypes 6B and 23F, antibody concentrations after the administration of a further booster dose were substantially higher despite low antibody levels after the priming series (reviewed by Oosterhuis-Kafeja et al[167]). Thus the interpretation of responses to *Haemophillus* species and pneumococcal vaccines needs to be mindful of these additional variables.

CONCLUSION

The use of vaccine responses as a diagnostic tool is firmly established for the evaluation of patients undergoing immunologic evaluation. They are frequently used in the context of providing a diagnosis or for justifying a particular therapeutic intervention. However, it is important to recognize that a knowledge gap exists regarding the issue of the different titer responses associated with the specific sequence of vaccination

formulations (eg, PCV before PPV or *vice versa*). The effect repetitive vaccinations might have on immune responsiveness, as well as specific cutoff values, and quality measurements that indicate less-absolute forms of PIDD also require further investigation. Furthermore, the normal response to vaccine antigens, in particular polysaccharide antigens, is variable and warrants further investigation to firmly establish the normal range for comparison with and effect of repeat immunizations in patients with PIDDs. In addition, there are several available neoantigens or alternative vaccine antigens for which a routine role in clinical humoral immune assessment might ultimately be found. The clinical immunologist is faced with using the currently available vaccines as tools to interrogate the humoral immune system of the patient suspected to have a humoral immune defect. It is essential to emphasize that in the absence of more direct evidence, the clinical status of the patient must dictate the therapeutic intervention (eg, the institution of immunoglobulin infusions) and not the response or lack thereof to a particular vaccination. However, the summary statements of this working group are provided for guidance and to facilitate rational diagnostic use of vaccine response evaluations.

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