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Assignment of *Neisseria meningitidis* Serogroup A and C Class-Specific Anticapsular Antibody Concentrations to the New Standard Reference Serum CDC1992

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A new standard meningococcal reference serum designated CDC1992 was prepared to replace meningococcal reference sera ECG and PB-2, which are not available in sufficient quantities for continued use as primary reference sera. CDC1992 was prepared from 14 healthy adult volunteers who underwent plasmapheresis 4 to 12 weeks postvaccination with a single dose of a *Neisseria meningitidis* quadrivalent polysaccharide vaccine. Total and/or class-specific meningococcal serogroup A and C anticapsular antibody concentrations (in micrograms per milliliter) were assigned to CDC1992 by using homologous and heterologous enzyme-linked immunosorbent assay (ELISA) formats. The reference serum ECG was used as a reference standard to assign total anticapsular antibody concentrations to CDC1992 by a homologous ELISA format. A heterologous ELISA format, with the *Haemophilus influenzae* type b standard reference serum FDA 1983, was used to assign total and class-specific antibody concentrations to CDC1992. Alkaline phosphatase-labeled mouse anti-human monoclonal antibody conjugates were used as secondary antibodies in both ELISA formats. The total, immunoglobulin G (IgG), IgA, and IgM antibody concentrations, assigned to CDC1992 for serogroup A were 135.8, 91.8, 20.1, and 23.9 μg/ml, respectively, and those for serogroup C were 32.0, 24.1, 5.9, and 2.0 μg/ml, respectively. Meningococcal serogroup A and C antibody concentrations were in good agreement when homologous and heterologous ELISA format results were compared. Total and class-specific serogroup A and C antibody concentrations were determined in six adult quality control serum samples from the Centers for Disease Control and Prevention by using the homologous ELISA and our assigned antibody concentrations for CDC1992. Antibody concentrations in reference sera ECG and PB-2 were measured in order to provide a historical link to previous studies. The general acceptance of CDC1992 as the standard reference serum and the assigned antibody concentrations will allow investigators to compare antibody levels in serum to those in a single reference preparation.

Neisseria meningitidis causes meningococcal disease with substantial morbidity and mortality worldwide. *N. meningitidis* is a leading cause of bacterial meningitis in many countries and is one of the more common causes in the United States (3, 35, 37). Effective polysaccharide vaccines are available for the control of meningococcal serogroup A and C disease in persons 2 years of age and older (11, 19, 20); however, the level of meningococcal anticapsular antibody required for protection is not known. This is due, in part, to the lack of standardized meningococcal antibody assays and reagents and standard reference sera. Estimates of the protective level of meningococcal anticapsular antibody ranging from 0.5 to 2.0 μg/ml have been reported (12, 13, 15, 24).

Recently, advances have been made in meningococcal assay standardization (2, 4, 10). Enzyme-linked immunosorbent assays (ELISAs) have been standardized for quantification of total meningococcal serogroup A and C anticapsular antibodies (4, 10). Reagents, a standard reference serum (designated CDC1992), and a standardized method of data analysis have become available with the introduction of these standardized meningococcal assays (4, 10). However, until now antibody concentrations have not been assigned to the standard reference serum CDC1992.

Two reference sera, designated ECG and PB-2, have been widely used to estimate total meningococcal anticapsular antibody levels in serum samples with unknown antibody levels (unknown serum samples). Each reference serum was used to construct a multipoint reference curve to calculate antibody concentrations in unknown serum samples. ECG was prepared in 1969 from a single donor who had been immunized with a monovalent meningococcal serogroup A polysaccharide vaccine and subsequently with a monovalent serogroup C polysaccharide vaccine (14). Total meningococcal serogroup A and C anticapsular weight-based antibody units (in micrograms per milliliter) were assigned to ECG by a quantitative precipitation assay (15). PB-2 was prepared in 1987 from a single donor who had been immunized with a meningococcal quadrivalent (serogroups A, C, Y, and W-135) polysaccharide vaccine (8, 9). Arbitrary total meningococcal serogroup A and C anticapsular antibody units (antibody units per milliliter) were assigned to PB-2 by an ELISA procedure with an outer membrane protein reference material (8). Class-specific antibody concentrations have not been reported for these meningococcal reference sera. Both ECG and PB-2 are in limited supply, and therefore, neither is suitable as a primary meningococcal standard reference serum.

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There is no standard method for assigning antibody concentrations to a standard reference serum. Units assigned to a standard reference serum can be either arbitrary (e.g., ELISA units or antibody units per milliliter) or weight based (micrograms per milliliter). Assignment of weight-based units is preferred; however, achieving a consensus antibody level has proved difficult. This was illustrated by the difficulty that occurred in comparing antibody concentrations in the *Haemophilus influenzae* type b (Hib) standard reference serum (FDA 1983) that were derived by a variety of techniques (16–18, 33). The total Hib anticapsular antibody concentration measured by different methods ranged between 44 μg/ml (16) and 80 μg/ml (33). Although a total antibody concentration of 70 μg/ml was assigned to FDA 1983 after a multilaboratory study (7), this episode illustrates the lack of a standard method for antibody quantification. Total antibody concentrations in reference sera have been directly assigned by quantitative precipitation assays (15, 22, 23, 31, 32) or by a homologous or heterologous ELISA format (1, 2, 5, 17, 18, 21, 23, 33, 34, 38). Quantitative precipitation assays offer the advantage of direct conversion to weight-based units; however, they are not as sensitive as other techniques (26) and are influenced by the antibody class distribution. The two ELISA formats are more common and practical approaches for assigning antibody concentrations to sera.

Homologous and heterologous ELISAs have several key differences. A homologous ELISA format is one in which the solid-phase antigens for the reference serum and unknown serum samples are identical (1, 2, 33, 34). In contrast, a heterologous ELISA format uses two different solid-phase antigens on the same microtiter plate (5, 17, 23, 38). One solid-phase antigen binds the standard reference serum antibody, and the other binds the unknown serum sample antibody. In both the homologous and heterologous ELISA formats, the antibody concentration in the unknown serum sample is extrapolated from a multipoint curve of the standard reference serum. The heterologous ELISA format assumes that if equal amounts of antibody are bound to the two different solid-phase antigens, the optical density of the bound labeled secondary antibody will be similar regardless of the specificities of the antibodies being measured (23, 38). Assuming that the standard reference curve and unknown curve are parallel, this format allows a previously calibrated standard reference serum of unrelated antibody specificity to be used to assign antibody concentrations to essentially any designated reference serum. This method is particularly attractive when assigning class-specific antibody concentrations to a new reference serum. A homologous ELISA format is used in a standardized assay once antibody concentrations have been assigned to a designated reference serum.

In the study described here, we prepared a new reference serum designated CDC1992 and assigned total and class-specific meningococcal serogroup A and C anticapsular antibody concentrations to CDC1992. Total anticapsular antibody concentrations were assigned to CDC1992 by using a standardized homologous meningococcal ELISA format with ECG as the reference serum and a heterologous ELISA format with the Hib standard reference serum FDA 1983 as the reference serum. Class-specific anticapsular antibody concentrations were assigned to CDC1992 by a heterologous ELISA format. Antibody concentrations were determined with various enzyme-labeled mouse anti-human monoclonal antibody (MAb) conjugates either singularly or in combination. The weight-based total antibody concentrations assigned to meningococcal serogroup A and C antibodies for CDC1992, determined by both the standardized homologous and heterologous ELISAs, were in good agreement. Finally, we determined the total and class-specific meningococcal serogroup A and C anticapsular antibody concentrations in six adult quality control serum samples from the Centers for Disease Control and Prevention (CDC) using the standardized homologous ELISA format and CDC1992 as the standard reference serum.

MATERIALS AND METHODS

CDC1992 standard reference serum. Twenty healthy adult volunteers underwent plasmapheresis 4 to 12 weeks postimmunization with a single injection (50 μg of A, C, Y, W-135 polysaccharide per 0.5 ml) of meningococcal polysaccharide vaccine (Menomune; Connaught Laboratories, Inc., Swiftwater, Pa.). All volunteers were tested and found to be negative for hepatitis B virus surface and core antigens and antibodies to hepatitis C virus, human immunodeficiency virus, human T-cell leukemia virus type 1, and syphilis. The plasma units were stored at −70°C; before use they were thawed overnight at 4°C, and then each unit was clotted with 4.0 ml of sterile glass microbeads (B. Braun Instruments, Burlingame, Calif.) and suspended in 1.5 M $CaCl_2$–2.0 M ε-amino-caproic acid. All units were allowed to clot overnight at ambient temperature and were then centrifuged at 2,200 × *g* for 15 min. The serum from each unit was expressed into individual 1-liter sterile plastic containers prior to pooling. A small aliquot of serum from each unit was tested for relative total, immunoglobulin G (IgG), IgA, and IgM meningococcal serogroup A and C anticapsular antibody units per milliliter by a standardized ELISA (4, 10). Meningococcal serogroup C serum bactericidial activity was determined for all units (1); a serogroup A bactericidal assay was not available. Fourteen of the units with the highest titers were pooled in a sterile plastic 15-liter carboy to be dispensed into 500-ml aliquots. The pooled sera were stored at −70°C. Approximately 500 ml of the pooled and frozen sera were thawed overnight at 4°C and, with a calibrated Digiflex dispenser (ICN Biomedicals, Inc., Horsham, Pa.), was dispensed into 1-ml aliquots, lyophilized, sealed under argon gas, and stored at 4°C.

Reference sera. The U.S. Center for Biologic Evaluation and Research Hib standard reference serum (FDA 1983) was provided by Carl Frasch (U.S. Food and Drug Administration, Bethesda, Md.). The IgG (60.9 μg/ml), IgA (5.6 μg/ml), and IgM (3.5 μg/ml) antibody concentrations for FDA 1983 were assigned by Madore et al. (21). The meningococcal serogroup A and C reference serum ECG was provided by Bernard Danve (Pasteur Mèrieux Sèrums & Vaccins, Lyon, France) and was previously assigned total meningococcal serogroup A and C anticapsular antibody concentrations of 140 and 127 μg/ml, respectively (15). The meningococcal serogroup A and C reference serum PB-2 was provided by Carl Frasch. PB-2 previously was assigned arbitrary total meningococcal serogroup A and C anticapsular antibody levels of 4,800 (≈48 μg/ml) and 2,800 (≈28 μg/ml) antibody units per ml, respectively (4, 10).

Solid-phase reagents. Meningococcal serogroup A and C and Hib capsular polysaccharides were provided by Connaught Laboratories, Inc., and Porter Anderson (University of Rochester Medical Center, Rochester, N.Y.), respectively. Methylated human serum albumin (mHSA) was prepared at CDC by a modified method of Mandell and Hershey (25) as described by Gheesling et al. (10).

Enzyme conjugates. Murine ascitic fluids containing MAbs specific for human IgG (HP6043), IgA (HP6123), IgM (HP6083), κ light chain (HP6156), and λ light chain (HP6054) were produced at CDC as described previously (30). The MAbs were purified and labeled with alkaline phosphatase (American Qualex International, Inc., La Mirada, Calif.). The specificities of the MAbs were verified at CDC with a panel of purified human IgG and IgM myeloma proteins (Calbiochem, La Jolla, Calif.) and IgA myeloma protein provided by Robert Hamilton (Johns Hopkins University, Baltimore, Md.). Checkerboard titrations were used to determine the optimum concentration of each individual conjugate or conjugate pool.

Homologous ELISA. The homologous ELISA was performed as described by Gheesling et al. (10), except that enzyme-labeled mouse anti-human immunoglobulin MAbs (American Qualex International, Inc.) were used as the secondary antibody. Data analysis was performed as described previously (27, 29).

Heterologous ELISA. The heterologous ELISA consisted of coating a portion of an Immulon II microtiter plate (Dynatech Laboratories, Inc., Chantilly, Va.) with 5 μg each of mHSA and Hib polyribosylribitol phosphate (PRP). The solid-phase antigen, mHSA-PRP, was used to bind antibody in the Hib FDA 1983 reference serum and a quality control serum. The solid-phase antigen for meningococcal serogroup A or C (5 μg each of mHSA and serogroup A or C native polysaccharide per ml) was coated onto another portion of the same plate and was used for estimating antibody concentrations in reference sera CDC1992, ECG, and PB-2. All coating mixtures were made in 10 mM phosphate-buffered saline (pH 7.2 to 7.4). The plates were sealed with a pressure-sensitive film (Becton Dickinson and Co., Oxnard, Calif.) and were incubated at 4°C overnight. Once it was coated, a microtiter plate was processed as described previously (10).

On the mHSA-PRP portion of the plate, both the Hib FDA 1983 standard reference serum and a quality control serum were assayed in triplicate by using seven twofold dilutions, while on the mHSA-serogroup A and mHSA-serogroup C portion of the microtiter plate CDC1992, ECG, and PB-2 were assayed two or

TABLE 1. IgG, IgA, IgM, and total meningococcal serogroup A and C anticapsular antibody concentrations in CDC1992 determined by heterologous ELISA format with Hib FDA 1983 as the standard reference serum[a]

Antibody type	Concn (μg/ml)	
	Serogroup A	Serogroup C
Class specific		
IgG	91.8 ± 21.2	24.1 ± 2.4
IgA	20.1 ± 2.9	5.9 ± 0.5
IgM	23.9 ± 3.4	2.0 ± 0.2
IgG + IgA + IgM[b]	135.8	32.0
G/A/M conjugate pool[c]	107.1 ± 19.3	34.9 ± 5.4
Kappa/lambda conjugate pool[c]	128.4 ± 20.7	33.8 ± 3.9

[a] IgG, IgA, IgM, and total antibody concentrations represent the mean ± 1 standard deviation of at least three independent assays.
[b] Total antibody concentration determined by summation of mean class-specific antibody concentrations.
[c] Total antibody concentration determined with a pool of enzyme-labeled MAb conjugates.

more times by using seven twofold dilutions. A 1:1,000 dilution of each MAb conjugate was used to detect class-specific antibodies, while for detection of total anticapsular antibodies, one of two MAb conjugate pools was used: (i) anti-IgG at 1:4,000, anti-IgA at 1:4,000, and anti-IgM at 1:2,000 (G/A/M conjugate pool) or (ii) anti-κ at 1:500, plus anti-λ at 1:2,000 (kappa/lambda conjugate pool). After the addition of substrate, each microtiter plate was allowed to incubate at ambient temperature, and the optical density at 410 nm was subsequently read (reference filter at 630 nm) at approximately 15-min intervals until the optical density of the highest dilution of the reference serum (FDA 1983) reached approximately 2.0. Data analysis was performed as described previously (27, 29).

RESULTS

A homologous ELISA format with ECG as the standard reference serum and a G/A/M MAb conjugate pool were used to assign total meningococcal serogroup A and C anticapsular antibody concentrations to CDC1992 and PB-2. With ECG as the standard reference serum, meningococcal serogroup A anticapsular antibody concentrations assigned to CDC1992 and PB-2, were 126.3 ± 5.7 and 144.1 ± 13.7 μg/ml, respectively, and those for serogroup C were 24.5 ± 1.9 and 14.1 ± 0.1 μg/ml, respectively. The total anticapsular antibody concentrations were obtained from at least two independent assays. The assigned total anticapsular antibody concentrations of ECG were 140 and 127 μg/ml for serogroups A and C, respectively (15).

A heterologous ELISA with the Hib standard reference serum FDA 1983 was used to assign class-specific antibody levels to reference serum CDC1992 (Table 1). Total anticapsular antibody for serogroups A and C was estimated in three different ways: (i) respective class-specific antibody concentrations for serogroups A and C were determined and then summed (IgG + IgA + IgM), (ii) a G/A/M conjugate pool was used for total antibody measurement, and (iii) a kappa/lambda conjugate pool was used for total antibody measurement. The antibody concentration assigned by the IgG + IgA + IgM conjugate pool for serogroup A (135.8 μg/ml) and serogroup C (32.0 μg/ml) differed from the total antibody estimates assigned by using the G/A/M conjugate pool by −21% (107 μg/ml) and +8% (34.9 μg/ml) for serogroups A and C, respectively. Total antibody estimates derived by using the kappa/lambda conjugate pool differed by 5% for both serogroups A and C from the antibody concentrations assigned by using the G/A/M conjugate pool. Standard reference curves obtained by the homologous and heterologous assays were parallel to that for CDC1992 (data not shown). Parallelism was determined by the guidelines described by Plikaytis et al. (28).

The heterologous ELISA format also was used to assign class-specific antibody levels to ECG and PB-2. The IgG, IgA, and IgM antibody levels assigned to ECG for serogroup A were 117.3 ± 28.7, 36.0 ± 3.5, and 34.1 ± 1.8 μg/ml, respectively, and those for serogroup C were 167.4 ± 13.8, 48.7 ± 4.2, and 21.2 ± 0.7 μg/ml, respectively. The IgG, IgA, and IgM antibody levels assigned to PB-2 for serogroup A were 23.9 ± 6.3, 39.2 ± 6.0, and 84.6 ± 22.8 μg/ml, respectively, and those for serogroup C were 4.9 ± 1.4, 6.3 ± 0.3, and 13.7 ± 0.5 μg/ml, respectively. Total antibody concentration estimates assigned by using the G/A/M conjugate pool for serogroups A and C for ECG were 114.8 and 183.0 μg/ml, respectively, and those for PB-2 were 110.4 and 18.9 μg/ml, respectively. Total antibody levels assigned by summing the individual IgG, IgA, and IgM antibody concentrations (IgG + IgA + IgM) for serogroup A and serogroup C differed from total antibody estimates assigned by using the G/A/M conjugate pool for ECG by −39 and −23%, respectively, and those for PB-2 differed by −25 and −24%, respectively.

Using a standardized meningococcal homologous ELISA format (4, 10) and CDC1992 as the standard reference serum, we determined class-specific meningococcal serogroup A and C anticapsular antibody concentrations for six adult postvaccination serum samples from CDC (Table 2). Total antibody concentrations for serogroups A and C were determined by using the IgG + IgA + IgM or the G/A/M conjugate pool. The coefficients of variation for total serogroup A and C anticapsular antibody concentrations in a comparison of the two ways of estimating total antibody ranged between 1.2 and 26.8% for serogroup A and 2.1 and 25.5% for serogroup C.

DISCUSSION

A standard reference serum sample with assigned antibody concentrations enables different laboratories to have a similar reference point from which to estimate antibody concentrations in serum. The use of a standard reference serum in conjunction with a standard assay, reagents, and data analysis procedure reduces the between-laboratory variability associated with the quantitation of antibody concentrations in serum. The currently available meningococcal reference sera (ECG and PB-2), each from single donors, are in short supply. ECG was assigned weight-based total antibody concentrations (in micrograms per milliliter) by quantitative precipitation (15). PB-2 was assigned total antibody units per milliliter on the basis of an ELISA (8) that could approximate weight-based concentrations (in micrograms per milliliter) (4, 10). Class-specific antibody was not measured in either of these reference sera.

CDC1992 is a large volume (approximately 7 liters) of reference serum with sufficiently high levels of antibody for routine use as a primary standard in ELISA for evaluation of current and developing serogroup A and C meningococcal vaccines. Individual sera for the CDC1992 serum pool were selected on the basis of meningococcal serogroup C serum bactericidal activity and the binding of antibody to serogroup A and C native polysaccharide solid-phase ELISA antigens. Demonstration of "functional" antibody activity, as defined by the World Health Organization (36) and the U.S. Food and Drug Administration (6), is required for licensure of meningococcal vaccines. In addition, selection of a reference serum for ELISA that contains antibodies with characteristics (class distribution, avidity) similar to those of the unknown samples

TABLE 2. Quantitation of total and class-specific meningococcal serogroup A and C anticapsular antibody concentrations in six adult quality control immune serum samples from CDC by ELISA with CDC1992 as the standard reference serum

Serogroup and serum sample no.	Concn (μg/ml)					CV (%)[a]
	Class-specific antibody[b]			Total antibody		
	IgG	IgA	IgM	IgG + IgA + IgM[c]	G/A/M conjugate pool[d]	
Serogroup A						
900415	7.0 ± 1.1	2.4 ± 0.4	11.1 ± 2.4	20.5	25.4 ± 2.1	15.1
900268	39.6 ± 3.4	15.2 ± 0.5	18.6 ± 0.9	73.5	74.7 ± 4.4	1.2
900274	143.6 ± 16.3	7.4 ± 1.2	24.4 ± 4.7	175.4	206.2 ± 20.0	11.4
900414	49.8 ± 6.7	5.5 ± 1.4	12.6 ± 1.7	67.9	74.3 ± 8.8	6.4
900242	17.2 ± 1.1	50.2 ± 2.4	35.0 ± 1.3	102.4	95.1 ± 9.7	5.3
900306	1.5 ± 0.2	0.6 ± 0.1	1.5 ± 0.2	3.7	5.4 ± 0.2	26.8
Serogroup C						
900415	2.9 ± 0.4	1.0 ± 0.2	1.2 ± 0.4	5.1	5.5 ± 0.5	6.5
900268	38.0 ± 1.6	21.0 ± 3.6	2.3 ± 0.4	61.4	59.4 ± 8.6	2.3
900274	15.4 ± 2.3	37.9 ± 8.2	11.1 ± 2.3	64.4	44.7 ± 4.5	25.5
900414	26.5 ± 9.2	1.7 ± 0.2	1.0 ± 0.3	29.1	30.0 ± 2.0	2.1
900242	119.9 ± 21.0	101.9 ± 17.0	24.5 ± 2.5	246.3	192.2 ± 50.8	17.5
900306	74.1 ± 10.8	0.7 ± 0.2	0.3 ± 0.1	75.2	80.1 ± 12.3	4.5

[a] CV, coefficient of variation (standard deviation/mean × 100) between two antibody concentrations with different enzyme-labeled conjugate preparations (IgG + IgA + IgM and G/A/M).
[b] Antibody concentrations represent the means ± 1 standard deviations of at least three independent assays.
[c] Total antibody concentration determined by summation of mean class-specific antibody concentrations.
[d] Total antibody concentration determined with a pool of enzyme-labeled MAbs (G/A/M conjugate pool).

is critical for accurate measurement of antibody concentration. Sera with high levels of a particular class of antibody were not selected for the pool. When six adult quality control serum samples from CDC were analyzed, most had a class distribution similar to that of CDC1992. Of these six quality control serum samples from CDC, only one contained predominantly IgM class antibodies. In addition, more than 95% of the sera from adults and more than 90% of the sera from children (approximately 1 to 5 years of age) tested to date in our laboratory (data not shown) gave total antibody dilution curves that were parallel with the CDC1992 standard reference curve as judged by the guidelines of Plikaytis et al. (28). This suggests that the avidities of CDC1992 antibodies for the serogroup A or C meningococcal solid-phase antigens is similar to the avidities of the antibodies in the unknown serum samples. These results also suggest that CDC1992 can be used as a reference serum for the analysis of sera from both adults and children.

Unfortunately, there is no standard method for quantifying the antibody levels in serum to be used as a standard reference serum. The assignment of antibody concentration tends to be method dependent as well as assay and reagent dependent. As mentioned earlier the Hib standard reference serum (FDA 1983) was assigned different antibody concentrations by different methods. Quantitative precipitation and various ELISA formats are commonly used to assign antibody concentrations. However, quantitative precipitation is dependent on the class-specific antibody present in the serum. In addition, measurement of class-specific antibody by quantitative precipitation requires purification of each class-specific antibody prior to precipitation. The heterologous ELISA format allows the measurement of total and class-specific antibody levels in a new reference serum. Antibody binding to an antigen can be measured with a standard dilution curve generated either by a sandwich (capture) assay with anti-human immunoglobulin, immunoglobulin, and labeled anti-human immunoglobulin or by an indirect assay with solid-phase antigen, antibody, and labeled anti-human immunoglobulin (38). However, since more class-specific differences are observed when secondary antibody binds to immunoglobulin (capture assay) than when it binds to antigen-bound primary antibody (38), we used the assay with solid-phase antigen, antibody, and labeled anti-human immunoglobulin to generate the standard reference curve.

H. influenzae capsular polysaccharide and Hib reference serum (FDA 1983), with previously assigned class-specific antibody concentrations (21), were used to generate the standard curve used to measure total and class-specific antibody concentrations in CDC1992. In order for the heterologous ELISA format to measure total and class-specific antibodies in reference serum, several requirements must be met (23, 38). (i) Antigen must not be limiting. Optimal antibody binding was obtained when Hib PRP or meningococcal serogroup A or C polysaccharide was used at 5 μg/ml. (ii) Dilution curves of the two serum samples (reference and unknown) must be parallel. The dilution curve of CDC1992 was judged to be parallel to the FDA 1983 dilution curve by the guidelines of Plikaytis et al. (28). (iii) The standard and the unknown serum samples must be assayed under the same experimental conditions. Both the Hib PRP antigen and the meningococcal polysaccharide antigen were coated on the same ELISA plate. This ensured that all assay parameters, including the times of incubation, temperature, and buffers, were identical for both antigens.

Several other factors, in addition to adherence to the criteria required for the heterologous ELISA format, suggest that the assignments of meningococcal serogroup A and C antibody concentrations to CDC1992 are valid. The three methods (summation of IgG + IgM + IgA antibody concentrations, total antibody measured with a pool of anti-IgG, anti-IgA, and anti-IgM enzyme conjugates, and total antibody measured with a pool of the anti-κ and anti-λ light-chain enzyme conjugates) used to determine total antibody by the heterologous ELISA format were in agreement. The mean antibody concentrations by the three methods were 123.8 μg/ml (coefficient of variation, 12%) and 33.6 μg/ml (coefficient of variation, 4.5%) for

serogroups A and C, respectively. In addition, the antibody concentrations measured by the heterologous ELISA format agreed with the total antibody concentrations measured by the homologous ELISA format with ECG as the meningococcal reference serum. The differences in serogroup A and C antibody levels measured in CDC1992 by both the homologous and heterologous ELISA formats were less than or equal to the differences observed when antibody concentrations were measured in murine ascitic fluids containing MAbs (23). This suggests that the error associated with the measurement of antibody concentrations in polyclonal serum by the heterologous ELISA format is no greater than the error associated with the measurement of antibody concentrations in murine ascitic fluids. Finally, the ratio of total serogroup A antibody concentrations to total serogroup C antibody concentrations measured by the heterologous ELISA format (135.8 μg/ml/32.0 μg/ml = 4.2) is similar to the dilution ratio required to produce equivalent optical densities on standard dilution curves for both serogroups A and C (700/150 = 4.7).

In summary, we have assigned total and class-specific antibody concentrations for serogroups A and C to a new meningococcal standard reference serum designated CDC1992 using both homologous and heterologous ELISA formats. The class-specific antibody concentrations for the Hib standard (FDA 1983) were measured by a homologous ELISA format with class-specific standards calibrated by a radioantigen-binding assay (21). Since FDA 1983 antibody concentrations are weight based, the antibody concentrations of CDC1992 are assigned units of micrograms per milliliter. In addition, the antibody concentration (in micrograms per milliliter) assigned by the heterologous ELISA format agrees with the antibody concentration assigned by the homologous ELISA format calibrated with a meningococcal reference serum with an independently assigned antibody concentration (15). Since class-specific antibody measurements may be important in the evaluation of new meningococcal vaccines and the sum of the individual class-specific antibody concentrations is equal to the total antibody concentration, we recommend that the sum of the individual class-specific antibody concentrations (summation of IgG + IgM + IgA) of CDC1992 (135.8 and 32.0 μg/ml for serogroups A and C, respectively) be used to estimate total antibody concentrations in unknown serum samples. Between-laboratory variability should be reduced significantly if both the standardized meningococcal ELISA (4, 10) and the new meningococcal standard reference serum CDC1992 are used for quantification of meningococcal serogroup A and C antibody levels in unknown serum samples. In addition, the measurement of antibody concentrations in ECG and PB-2 provides a historical link to studies reported previously.

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REFERENCES

1. **Anderson, E. L., T. Bowers, C. M. Mink, D. Kennedy, R. B. Belshe, H. Harakeh, L. P. Pais, P. Holder, and G. M. Carlone.** 1994. Safety and immunogenicity of meningococcal A and C conjugate vaccine in adults. Infect. Immun. **62:**3391–3395.
2. **Arakere, G., and C. E. Frasch.** 1991. Specificity of antibodies to O-acetyl-positive and O-acetyl-negative group C meningococcal polysaccharides in sera from vaccinees and carriers. Infect. Immun. **59:**4349–4356.
3. **Band, J. D., M. E. Chamberland, T. Platt, R. E. Weaver, C. Thornsberry, and D. W. Fraser.** 1983. Trends in meningococcal disease in the United States, 1975–1980. J. Infect. Dis. **148:**754–758.
4. **Carlone, G. M., C. E. Frasch, G. R. Siber, S. Quataert, L. L. Gheesling, S. H. Turner, B. D. Plikaytis, L. O. Helsel, W. E. DeWitt, W. F. Bibb, B. Swaminathan, G. Arakere, C. Thompson, D. Phipps, D. Madore, and C. V. Broome.** 1992. Multicenter comparison of levels of antibody to the *Neisseria meningitidis* group A capsular polysaccharide measured by using an enzyme-linked immunosorbent assay. J. Clin. Microbiol. **30:**154–159.
5. **Fomsgaard, A., and B. Dinesen.** 1987. ELISA for human IgG and IgM anti-lipopolysaccharide antibodies with indirect standardization. J. Immunoassay **8:**333–350.
6. **Frasch, C. E.** 1990. Production and control of *Neisseria meningitidis* vaccines, p. 123–145. *In* Bacterial vaccines. Alan R. Liss, Inc., New York.
7. **Frasch, C. E. (U.S. Food and Drug Administration, Bethesda, Md.).** 1994. Personal communication.
8. **Frasch, C. E., and J. D. Robbins.** 1978. Protection against group B meningococcal disease. III. Immunogenicity of serotype 2 vaccines and specificity of protection in a guinea pig model. J. Exp. Med. **147:**629–644.
9. **Frasch, C. E., J. M. Zahradnik, L. Y. Wang, L. F. Mocca, and C. M. Tsai.** 1988. Antibody response of adults to an aluminum hydroxide-adsorbed *Neisseria meningitidis* serotype 2b protein group B-polysaccharide vaccine. J. Infect. Dis. **158:**700–708.
10. **Gheesling, L. L., G. M. Carlone, L. B. Pais, P. F. Holder, S. E. Maslanka, B. D. Plikaytis, M. Achtman, P. Densen, C. E. Frasch, H. Käyhty, J. P. Mays, L. Nencioni, C. Peeters, D. C. Phipps, J. T. Poolman, E. Rosenqvist, G. R. Siber, B. Thiesen, J. Tai, C. M. Thompson, P. P. Vella, and J. D. Wenger.** 1994. Multicenter comparison of *Neisseria meningitidis* serogroup C anticapsular polysaccharide antibody levels measured by a standardized enzyme-linked immunosorbent assay. J. Clin. Microbiol. **32:**1475–1482.
11. **Gold, R., and M. L. Lepow.** 1976. Present status of polysaccharide vaccines in the prevention of meningococcal disease. Adv. Pediatr. **23:**71–87.
12. **Gold, R., M. L. Lepow, I. Goldschneider, T. F. Draper, and E. C. Gotschlish.** 1979. Kinetics of antibody production to group A and group C meningococcal polysaccharide vaccines administered during the first six years of life: prospects for routine immunization of infants and children. J. Infect. Dis. **140:**690–697.
13. **Goldschneider, I., M. L. Lepow, E. C. Gotschlich, F. T. Mauck, R. Bachl, and M. Randolph.** 1973. Immunogenicity of group A and group C meningococcal polysaccharides in human infants. J. Infect. Dis. **128:**769–776.
14. **Gotschlich, E. C., I. Goldschneider, and M. S. Artenstein.** 1969. Human immunity to the meningococcus. IV. Immunogenicity of group A and group C meningococcal polysaccharides in human volunteers. J. Exp. Med. **129:**1367–1384.
15. **Gotschlich, E. C., M. Rey, R. Triau, and K. J. Sparks.** 1972. Quantitative determination of the human immune response to immunization with meningococcal vaccines. J. Clin. Invest. **51:**89–96.
16. **Granoff, D. M., S. J. Holmes, M. T. Osterholm, J. E. McHugh, A. H. Lucas, E. L. Anderson, R. B. Belshe, J. L. Jacobs, F. Medley, and T. V. Murphy.** 1993. Induction of immunologic memory in infants primed with *Haemophilus influenzae* type b conjugate vaccine. J. Infect. Dis. **168:**663–671.
17. **Hamilton, R. G.** 1991. Application of engineered chimeric antibodies to the calibration of human antibody standards. Ann. Biol. Clin. **49:**242–248.
18. **Herrmann, D. J., R. G. Hamilton, T. Barrington, C. E. Frasch, G. Arakere, O. Mäkelä, L. A. Mitchell, J. Nagel, G. T. Rijkers, B. Zegers, B. Danve, J. I. Ward, and C. S. Brown.** 1992. Quantitation of human IgG subclass antibodies to *Haemophilus influenzae* type b capsular polysaccharide. J. Immunol. Methods **148:**101–114.
19. **Käyhty, H., V. Karanko, H. Peltola, S. Sarna, and P. H. Mäkelä.** 1980. Serum antibodies to capsular polysaccharide vaccine of group A *Neisseria meningitidis* followed for three years in infants and children. J. Infect. Dis. **142:**861–868.
20. **Lepow, M. L., I. Goldschneider, R. Gold, M. Randolph, and E. C. Gotschlich.** 1977. Persistence of antibody following immunization of children with groups A and C meningococcal polysaccharide vaccines. Pediatrics **60:**673–680.
21. **Madore, D. V., C. L. Johnson, D. C. Phipps, Pennridge Pediatric Associates, M. G. Myers, R. Eby, and D. H. Smith.** 1990. Safety and immunogenicity of *Haemophilus influenzae* type b oligosaccharide-CRM_{197} conjugate vaccine in infants aged 15 to 23 months. Pediatrics **86:**527–534.
22. **Mäkelä, O., P. Mattila, N. Rautonen, I. Seppälä, J. Eskola, and H. Kayhty.** 1987. Isotype concentrations of human antibodies to *Haemophilus influenzae* type b polysaccharide (Hib) in young adults immunized with the polysaccharide as such or conjugated to a protein (diptheria toxoid). J. Immunol. **139:**1999–2004.
23. **Mäkelä, O., and F. Pèterfy.** 1983. Standard sera in solid-phase immunoassays. Eur. J. Immunol. **13:**815–819.
24. **Mäkelä, P. H., H. Peltola, H. Käyhty, H. Jousimies, O. Pettay, E. Ruoslahti, A. Sivonen, and O. V. Renkonen.** 1977. Polysaccharide vaccines of group A

Neisseria meningitidis and *Haemophilus influenzae* type b: a field trial in Finland. J. Infect. Dis. **136:**S43–S50.

25. **Mandell, J. D., and A. D. Hershey.** 1960. A fractionating column for analysis of nucleic acids. Anal. Biochem. **1:**66–77.
26. **Pèterfy, F., P. Kuusela, and O. Mäkelä.** 1983. Affinity requirements for antibody assays mapped by monoclonal antibodies. J. Immunol. **130:**1809–1813.
27. **Plikaytis, B. D., G. M. Carlone, S. H. Turner, L. L. Gheesling, and P. F. Holder.** 1992. ELISA users manual. Centers for Disease Control, Atlanta.
28. **Plikaytis, B. D., P. K. Holder, L. B. Pais, S. E. Maslanka, L. L. Gheesling, and G. M. Carlone.** 1994. Determination of parallelism and nonparallelism in bioassay dilution curves. J. Clin. Microbiol. **32:**2441–2447.
29. **Plikaytis, B. D., S. H. Turner, L. L. Gheesling, and G. M. Carlone.** 1991. Comparisons of standard curve-fitting methods to quantitate *Neisseria meningitidis* group A polysaccharide antibody levels by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **29:**1439–1446.
30. **Reimer, C. B., D. J. Phillips, C. H. Aloisio, D. D. Moore, G. G. Galland, T. W. Wells, C. M. Black, and J. S. McDougal.** 1984. Evaluation of thirty-one mouse monoclonal antibodies to human IgG epitopes. Hybridoma **3:**263–275.
31. **Robbins, J. B., J. C. Parke, R. Schneerson, and J. K. Whisnant.** 1973. Quantitative measurement of "natural" and immunization-induced *Haemophilus influenzae* type b capsular polysaccharide antibodies. Pediatr. Res. **7:**103–110.
32. **Seppälä, I., H. Sarvas, O. Mäkelä, P. Mattila, J. Eskola, and H. Käyhty.** 1988. Human antibody responses to two conjugate vaccines of *Haemophilus influenzae* type b saccharides and diphtheria toxin. Scand. J. Immunol. **28:** 471–479.
33. **Shackelford, P. G., D. M. Granoff, S. J. Nelson, M. G. Scott, D. S. Smith, and M. H. Nahm.** 1987. Subclass distribution of human antibodies to *Haemophilus influenzae* type b capsular polysaccharide. J. Immunol. **138:**587–592.
34. **Siber, G. R., D. M. Ambrosino, J. McIver, T. J. Ervin, G. Schiffman, S. Sallan, and G. F. Grady.** 1984. Preparation of human hyperimmune globulin to *Haemophilus influenzae* b, *Streptococcus pneumoniae*, and *Neisseria meningitidis*. Infect. Immun. **45:**248–254.
35. **Wenger, J. D., A. W. Hightower, R. R. Facklam, S. Gaventa, C. V. Broome, and the Bacterial Meningitidis Study Group.** 1990. Bacterial meningitis in the United States, 1986: report of a multistate surveillance study. J. Infect. Dis. **162:**1316–1323.
36. **World Health Organization Expert Committee on Biological Standardization.** 1976. Requirements for meningococcal polysaccharide vaccine. WHO Tech. Rep. Ser. **594:**50–75.
37. **Zangwill, K. M., R. W. Stout, G. M. Carlone, L. Pais, H. Harakeh, S. Mitchell, W. H. Wolfe, V. Blackwood, B. D. Plikaytis, and J. D. Wenger.** 1994. Duration of antibody response after meningococcal polysaccharide vaccination in US Air Force personnel. J. Infect. Dis. **169:**847–852.
38. **Zollinger, W. D., and J. W. Boslego.** 1981. A general approach to standardization of the solid-phase radioimmunoassay for quantitation of class-specific antibodies. J. Immunol. Methods **46:**129–140.