JOURNAL OF CLINICAL MICROBIOLOGY, June 1994, p. 1475–1482
0095-1137/94/$04.00+0

Vol. 32, No. 6

Multicenter Comparison of *Neisseria meningitidis* Serogroup C Anti-Capsular Polysaccharide Antibody Levels Measured by a Standardized Enzyme-Linked Immunosorbent Assay

LINDA L. GHEESLING,[1] GEORGE M. CARLONE,[1]* LORNA B. PAIS,[1] PATRICIA F. HOLDER,[1] SUSAN E. MASLANKA,[1] BRIAN D. PLIKAYTIS,[2] MARK ACHTMAN,[3] PETER DENSEN,[4] CARL E. FRASCH,[5] HELENA KÄYHTY,[6] JOHN P. MAYS,[7] LUCIANO NENCIONI,[8] CARLA PEETERS,[9] DONNA C. PHIPPS,[10] JAN T. POOLMAN,[9] EINAR ROSENQVIST,[11] GEORGE R. SIBER,[12] BURGHARD THIESEN,[3] JOSEPH TAI,[13] CLAUDETTE M. THOMPSON,[12] PHILIP P. VELLA,[14] AND JAY D. WENGER[1]

Childhood and Respiratory Diseases Branch[1] and Biostatistics and Information Management Branch,[2] National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333; Max-Planck-Institut für moleculare Genetik, Berlin, Germany[3]; The University of Iowa, Iowa City, Iowa 52242[4]; Food and Drug Administration, Bethesda, Maryland 20892[5]; National Public Health Institute, Helsinki, Finland[6]; Connaught Laboratories, Inc., Swiftwater, Pennsylvania 18370[7]; Biocine Sclavo S.p.A., Siena, Italy[8]; National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands[9]; Lederle-Praxis Biologicals, Inc., West Henrietta, New York 14586[10]; National Institute of Public Health, Oslo, Norway[11]; Dana-Farber Cancer Institute, Boston, Massachusetts 02115[12]; AMVAX, American Vaccine Corporation, Beltsville, Maryland 20705[13]; and Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19484[14]

Received 15 December 1993/Returned for modification 13 January 1994/Accepted 22 February 1994

A standardized enzyme-linked immunosorbent assay (ELISA) was used by 11 laboratories to measure levels of total serum antibody to *Neisseria meningitidis* serogroup C capsular polysaccharide in 16 unpaired pre- and postvaccination serum samples. Twelve serum samples were from adults, and four were from children aged 2, 3, 5, and 9. The between-laboratory coefficient of variation for pre- and postvaccination sera ranged from 16 to 59% and 11 to 21%, respectively. The average percent difference (absolute value) from the between-laboratory means for all prevaccination sera measured by each laboratory was 24%, whereas the average percent difference was 13% for all postvaccination sera. A postvaccination quality control serum was diluted three times to give optical densities on the high, middle, and low portions of the standard reference curve. The three dilutions were assayed by the 11 laboratories a total of 241 times and yielded an overall coefficient of variation of 20%. Antibody-binding inhibition curves showed that the standardized ELISA was specific for *N. meningitidis* serogroup C capsular polysaccharide antibody. Fifty percent inhibition of seven serum samples was obtained after reaction with an average concentration of 0.9 μg of meningococcal serogroup C polysaccharide per ml; an average of 93% inhibition was obtained with 50 μg of polysaccharide per ml. The acceptance and use of this standardized ELISA will reduce between-laboratory assay variability and ensure a more accurate and reproducible assessment of immunogenicity for vaccines under development.

Neisseria meningitidis causes disease with substantial morbidity and mortality. Epidemics in many areas of the world result in severe disruption of public health systems. Children under 5 years of age are most susceptible to disease, but young adults also experience high rates of disease (23), and military recruits have been particularly susceptible to infection (4, 9). *N. meningitidis* polysaccharide vaccines have been available for over 20 years (14), and serogroup C vaccines have been used to halt epidemics in older persons. However, these vaccines are poorly immunogenic in children less than 2 years of age and have a short duration of protection in young children (12, 13, 21). To address these problems, oligosaccharide-protein conjugate vaccines are being developed to elicit T-cell-dependent antibody responses of sufficient magnitude and duration to protect young children against invasive disease (8, 18). Evaluation of candidate vaccines and assessment of the protective levels of antibody require an accurate and reproducible measurement of the immune response by a standardized assay.

In the past, the lack of a standardized assay to measure antibody responses made it impossible to compare results between laboratories for vaccines against *Haemophilus influenzae* type b (1, 15, 32). To avoid similar difficulties in evaluating meningococcal conjugate vaccines, the development and validation of a meningococcal standard reference assay for quantifying total anticapsular antibody levels is essential. This requires the standardization of the protocol, reagents, materials, quality controls, and data analysis method carried out through a well-designed multilaboratory comparison (24, 27, 29). A standard assay will facilitate comparison of data among different laboratories by reducing the within- and between-laboratory variabilities. Currently there is no accepted standard reference assay for the measurement of total anticapsular antibody against *N. meningitidis* serogroup C. However, Carlone et al. (7) have proposed a standardized enzyme-linked

* Corresponding author. Mailing address: Childhood and Respiratory Diseases Branch, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Building 1, Room 1260, mailstop A36, 1600 Clifton Rd., Atlanta, GA 30333. Phone: (404) 639-3622. Fax: (404) 639-3970. Electronic mail address: INTERNET:GMC3@CIDDBD2.EM.CDC.GOV.

immunosorbent assay (ELISA) for meningococcal serogroup A antibody.

The purpose of this study is to examine the variability associated with a standardized ELISA for the quantitation of total anticapsular antibody to meningococcal serogroup C. We describe the standardized ELISA and establish that this assay is reproducible, sensitive, simple to perform, and specific for antibody to *N. meningitidis* serogroup C polysaccharide. The present report illustrates the usefulness and validity of a standardized ELISA for comparing antibody levels among laboratories, as is often needed during vaccine immunogenicity evaluation.

(Preliminary results of this study were presented at the Second International Workshop on Meningococcal Immunology, 17 to 18 June 1992, Atlanta, Ga.)

MATERIALS AND METHODS

Study design. The total anticapsular antibody levels in 16 serum samples (8 pre- and 8 postvaccination, unpaired) were measured by using a standardized ELISA. Antibody levels in samples from six unvaccinated (sera no. 2, 4, 8, 11, 12, and 15) and six vaccinated (sera no. 3, 6, 7, 10, 14, and 16) adult volunteers were measured. The vaccinated adults had received a subcutaneous single injection (50 μg of each polysaccharide per 0.5 ml) of *N. meningitidis* vaccine (MENOMUNE; Connaught Laboratories, Inc., Swiftwater, Pa.) containing the polysaccharides from groups A, C, Y, and W135. None of the adults had been previously vaccinated with a meningococcal vaccine, nor did they have a known history of invasive meningococcal disease. Antibody levels were also assayed in sera from two unvaccinated children, ages 3 and 2 years (sera no. 9 and 13, respectively) and from two children ages 9 and 5 years (sera no. 1 and 5, respectively) who had received a bivalent (serogroups A and C) meningococcal polysaccharide vaccine (BioMerieux, Lyons, France). All sera were stored at −70°C in coded vials. The eight pre- and eight postvaccination serum samples were divided into aliquots and distributed to the following 11 laboratories: AMVAX, American Vaccine Corporation, Beltsville, Md.; Biocine Sclavo, S.p.A., Siena, Italy; Immunology & Methods Development Laboratory, Centers for Disease Control (CDC), Atlanta, Ga.; Connaught Laboratories, Inc., Swiftwater, Pa.; Dana-Farber Cancer Institute, Boston, Mass.; Lederle-Praxis Biologicals, Inc., West Henrietta, N.Y.; Max-Planck-Institut für moleculare Genetik, Berlin, Germany; Merck Sharp & Dohme Research Laboratories, West Point, Pa.; National Public Health Institute, Helsinki, Finland; National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands; and Division of Infectious Diseases, University of Iowa, Iowa City. Laboratories were randomly assigned numbers for comparison. No laboratory personnel had any knowledge of the source of specimens or of results from other laboratories.

ELISA. A standardized ELISA protocol and all required reagents and materials were provided to each participating laboratory by CDC. The reagents supplied were (i) lyophilized *N. meningitidis* serogroup C native polysaccharide, (ii) lyophilized methylated human serum albumin, (iii) reference serum PB-2 (supplied by C. E. Frasch), (iv) 16 frozen unknown serum samples, (v) three separate dilutions of a single quality control serum, (vi) newborn bovine serum (ICN Flow, Costa Mesa, Calif.), (vii) Brij 35 detergent (Sigma Chemical Co., St. Louis, Mo.), (viii) *p*-nitrophenyl phosphate substrate tablets (Sigma Chemical Co.), (ix) goat affinity-purified anti-human immunoglobulin (immunoglobulins G, A, and M) alkaline-phosphatase labeled conjugate (TAGO, Inc., Burlingame, Calif.), and (x) Immulon 2 microtitration plates (Dynatech Laboratories, Chantilly, Va.).

The ELISA was performed as described by Arakere and Frasch (2) as modified by Carlone et al. (7), with the following exceptions: Immulon 2 plates were coated with antigen at 4°C overnight, a blocking step was included before the primary antibody was added, and the wash buffer was 10 mM phosphate-buffered saline (PBS) (pH 7.2) containing 0.1% Brij 35 detergent. The standardized ELISA procedure was as follows. Immulon 2 plates were incubated overnight (ca. 18 h) at 4°C with an equal mixture of 5 μg of each native meningococcal serogroup C polysaccharide per ml and methylated human serum albumin (the preparation method is described below). The next morning, the plates were washed and nonspecific protein binding sites were blocked with 200 μl of 10 mM PBS (pH 7.2) containing 5% newborn bovine serum, 0.1% Brij 35, and 0.05% sodium azide (designated serum-conjugate [S/C] buffer) for 1 h at ambient temperature. After blocking of nonspecific binding sites, eight twofold dilutions of the reference serum (starting dilution, 1:300) and test sera (starting dilution, 1:50) were made directly in the microtiter plate by well-to-well transfer with a multichannel pipette. The reference serum was assayed in triplicate, and test sera were assayed in duplicate. The reference serum PB-2 was assigned the arbitrary value of 2,800 antibody units (U) of total anticapsular antibody against meningococcal serogroup C polysaccharide per ml by one of the authors (C.E.F.) by using calibration procedures described previously (11). As an internal quality control, an antimeningococcal adult immune serum (CDC 900242) was diluted (1:3,200, 1:6,400, and 1:32,000) to yield optical densities approximately on the high, middle, and low portions of the reference curve.

After overnight incubation at 4°C, the plates were washed and received 100 μl of a 1:1,000 dilution (in S/C buffer) of polyclonal alkaline phosphatase-labeled affinity-purified goat antibody to human immunoglobulins G, A, and M per well. The plates were washed again after 2 h of incubation at ambient temperature, and 100 μl of substrate (1 mg of *p*-nitrophenyl phosphate per ml in 1 M Tris base [pH 9.8] containing 0.3 mM $MgCl_2 \cdot 6H_2O$) was added. The enzyme reaction was stopped with 100 μl of 0.4 M NaOH when the absorbance value of the most concentrated reference serum dilution reached an optical density of approximately 2.5 at 410 nm on an ELISA reader. The minimum detectable limit of the assay was approximately 10 U/ml. After each serum was independently assayed twice by each laboratory (laboratory 2 assayed each serum only once), the raw optical density values in an ASCII text file were sent to CDC for analysis by use of a public-domain software program developed at CDC (26).

Methylated human serum albumin. Five grams of human albumin (25% solution; American Red Cross, Washington, D.C.) was suspended in 500 ml of absolute methyl alcohol; 4.2 ml of 12 N HCl was then added. The mixture was allowed to stand in the dark for 3 days with occasional agitation. The precipitate was collected by centrifugation and washed twice with absolute methyl alcohol and twice with anhydrous ether in two glass 250-ml centrifuge bottles. Most of the ether was evaporated in a ventilated chemical hood, after which the precipitate was dried in a desiccator with KOH pellets. The powder was rehydrated with sterile endotoxin-free water to a concentration of 2 mg/ml, lyophilized, and stored at 4°C (22).

Inhibition assays. Inhibition assays were done with the reference serum (PB-2) and six of the eight postvaccination serum samples (two samples, no. 1 and 5, had insufficient volumes), using a single dilution of each serum with six different concentrations of polysaccharide. The final dilution of

TABLE 1. Between-laboratory comparison of a quality control serum diluted 1:3,200, 1:6,400, and 1:32,000 to obtain optical density values approximately on the high, middle, and low portions, respectively, of the reference curve

Portion of curve (*n*)	Antibody level (U/ml)[a]					CV (%)
	Mean	Minimum	Maximum	Range	SD	
High (80)	20,005	9,508	33,535	24,027	4,275	21
Middle (81)	18,801	11,423	25,419	13,996	3,655	19
Low (80)	17,613	11,436	26,845	15,409	3,018	17
All three dilutions (241)	18,806	9,508	33,535	24,027	3,798	20

[a] Antibody levels, interpolated from the standard curve, were calculated from optical density values obtained at each dilution.

each serum was chosen from previous experiments to yield an ELISA absorbance value of approximately 1.0 after 30 min of color development. Six fourfold dilutions of meningococcal serogroup C polysaccharide (diluted in S/C buffer) were prepared (the highest concentration was 100 μg/ml), and an equal volume (250 μl) was added to the diluted sera; 150 μl each of S/C buffer and prediluted serum was added to a tube as the negative control. The serum-polysaccharide mixtures reacted while tumbling overnight at 4°C. The mixture was added in duplicate to wells (100 μl per well) of a microtiter plate, and the ELISA was performed as described above.

Statistical methods. ASCII text files of raw optical density values from each of the 11 laboratories were analyzed by a public-domain software program developed at CDC (26). The four-parameter logistic-log function was used for forming the reference curve (27). The total antibody level (antibody units per milliliter) against *N. meningitidis* group C polysaccharide was obtained by averaging the values from all serum dilutions (in duplicate) that fell within the working range of the reference dilution curve. The mean antibody level, standard deviation (SD), and percent coefficient of variation (CV) (SD divided by the mean × 100) were calculated by averaging the mean antibody values obtained from each of the 11 laboratories for each of the 16 sera. The between-laboratory differences in antibody levels for each pre- and postvaccination serum were found to be significant by the Kruskal-Wallis test (3).

RESULTS

The total antibody levels (units per milliliter) obtained from a single quality control serum diluted three times to yield optical densities approximately on the high, middle, and low portions of the standard reference curve and assayed a total of 241 times in the 11 laboratories are shown in Table 1. The overall mean antibody level of the quality control serum was 18,806 U/ml, and antibody levels ranged from 9,508 to 33,535 U/ml. The SD of the overall mean for the three quality control serum dilutions was 3,798 U/ml. The between-laboratory CVs for the three dilutions of the quality control serum were 17, 19, and 21% for the high, middle, and low dilutions, respectively.

The average of the mean antibody levels for each of the eight prevaccination and eight postvaccination serum samples obtained from the 11 laboratories (between-laboratory mean), the SD, the CV, the minimum and maximum antibody levels, and the range (difference between the minimum and maximum antibody levels) are shown in Table 2. Antibody levels in individual prevaccination sera obtained from each of the 11 laboratories ranged from 8 to 295 U/ml, whereas the levels in postvaccination sera ranged from 265 to 7,795 U/ml. The overall average between-laboratory CV for prevaccination sera was 35%, with a range of 16 to 59%; the overall average CV for postvaccination sera was 17%, and it ranged from 11 to 21%.

The percent difference between the antibody level obtained in each laboratory and the overall between-laboratory mean antibody level is shown in Fig. 1. The ±50% bounds in Fig. 1 represent 50% above and 50% below the mean antibody level for each serum. The overall average percent difference (absolute value) from the within-laboratory mean calculated from 88 values for all prevaccination sera was 24% (Fig. 1A), and that for all postvaccination sera was 13% (Fig. 1B). The two prevaccination serum samples with the greatest percent differences in antibody levels from the overall mean (Fig. 1A) were serum 2 in laboratory 7 (157% difference) and serum 13 in laboratory 5 (156% difference). Eighty of the 88 calculated percent difference values for prevaccination sera were within the 50% difference from the overall mean. Laboratories 4 and 10 reported levels in all prevaccination sera that were lower than the mean. The greatest difference from the overall mean in postvaccination antibody levels, 41%, was for serum 5 in laboratory 6 (Fig. 1B). Laboratories 5 and 8 reported levels in all postvaccination sera that were lower than the between-laboratory mean antibody level; laboratory 6 reported levels in all postvaccination sera that were higher than the mean. The remaining eight laboratories reported levels that were both lower and higher than the overall mean antibody level for each serum.

The within-laboratory CVs for pre- and postvaccination sera are shown in Fig. 2. The overall average within-laboratory CV

TABLE 2. Between-laboratory comparison (n = 11) of levels of total antibody to *N. meningitidis* serogroup C polysaccharide in 16 unpaired pre- and postvaccination serum samples

Sample type and no.	Antibody level (U/ml)					CV (%)[b]
	Mean	SD[a]	Minimum	Maximum	Range	
Prevaccination						
2	41	24	13	106	93	59
4	43	20	21	92	71	46
8	37	10	23	54	31	28
9	163	26	107	210	103	16
11	212	46	144	295	151	22
12	27	6	16	38	22	22
13	19	11	8	49	41	59
15	139	45	75	251	176	32
Postvaccination						
1	2,248	412	1,507	2,970	1,463	18
3	3,035	446	2,555	3,964	1,409	15
5	1,230	241	884	1,731	847	20
6	5,940	983	4,792	7,795	3,003	17
7	1,119	119	950	1,311	361	11
10	1,684	251	1,334	2,027	693	15
14	1,482	282	947	1,915	968	19
16	379	78	265	495	230	21

[a] Calculated by using one value obtained from each of the 11 laboratories.
[b] SD divided by mean units per milliliter × 100%.

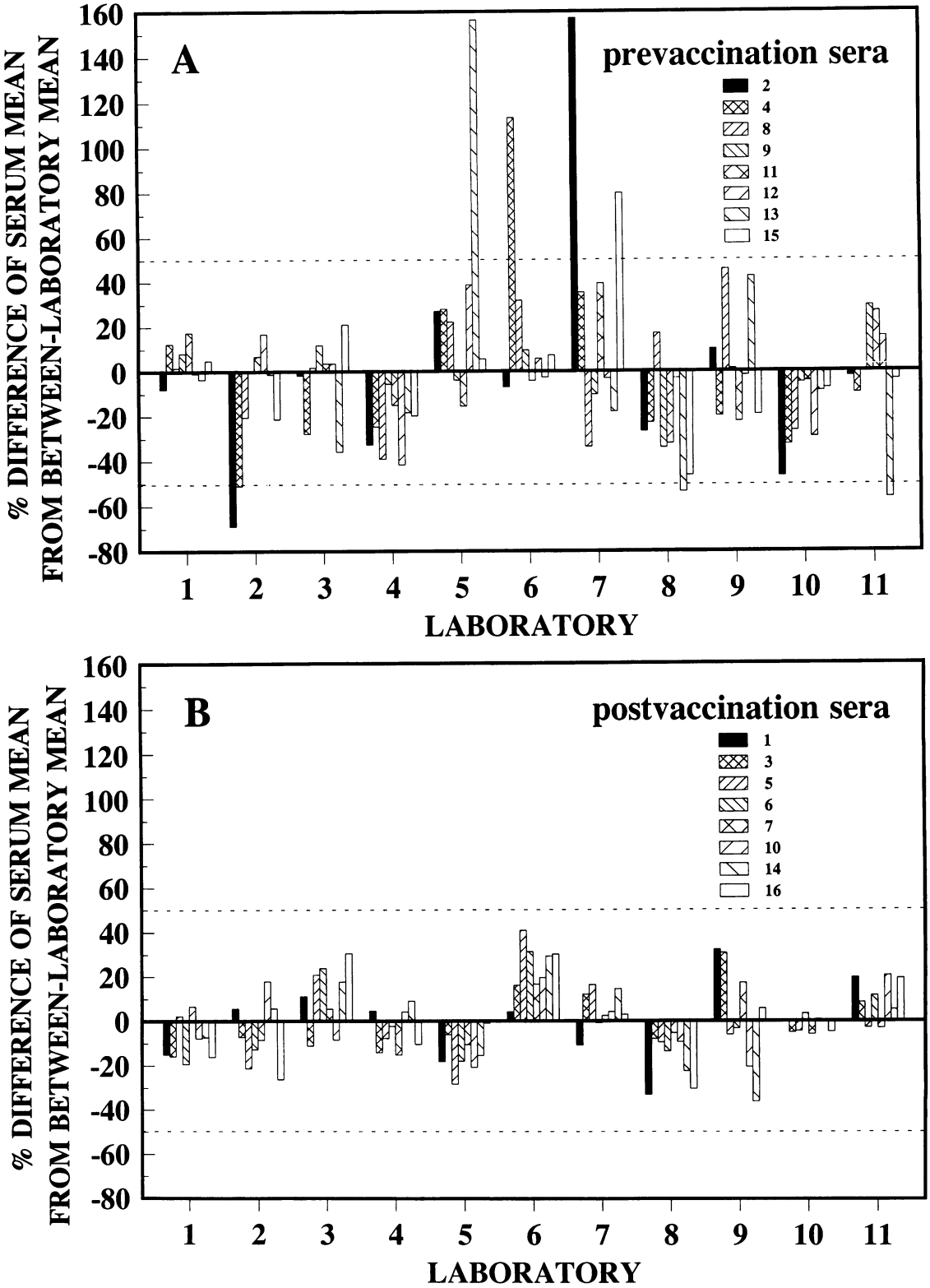

FIG. 1. Percent difference between the mean antibody level obtained by each individual laboratory and the overall between-laboratory mean for each prevaccination serum (A) and postvaccination serum (B). The dotted lines indicate bounds defined by the ±50% difference from the overall between-laboratory mean. Each laboratory was randomly assigned a number which does not correspond to the alphabetical listing.

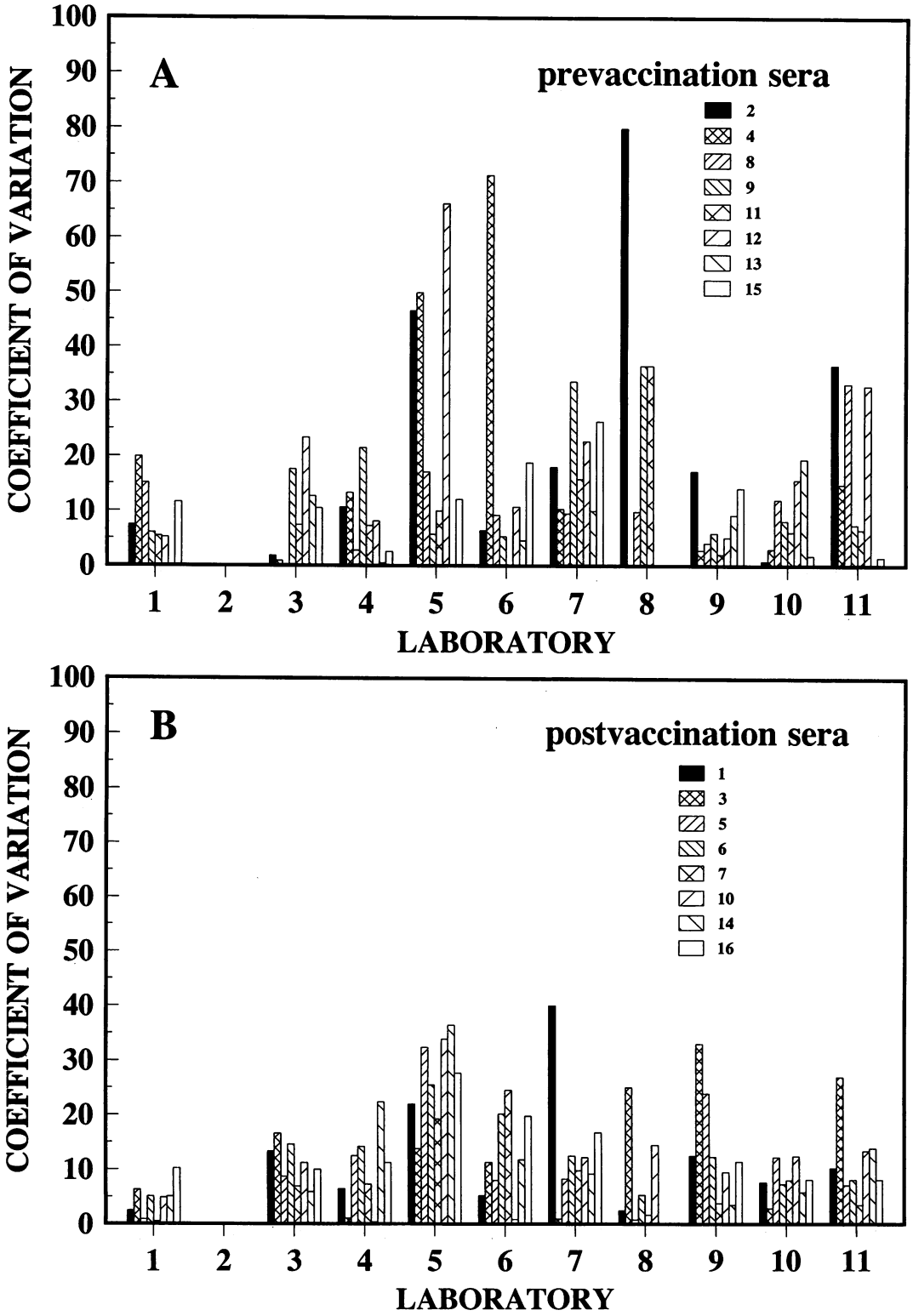

FIG. 2. Within-laboratory CV (percent) from two independent assays of each prevaccination serum (A) and postvaccination serum (B). (Laboratory 2 assayed each serum once.)

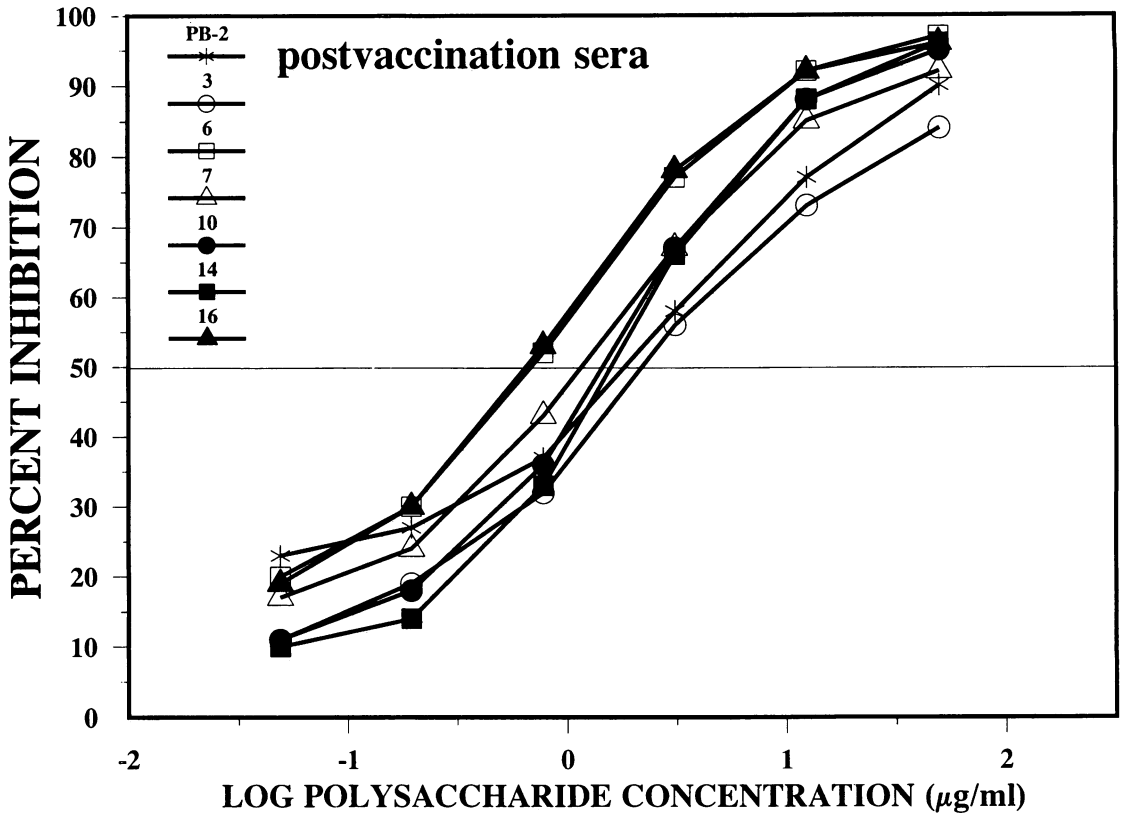

FIG. 3. Inhibition of antibody binding for the standard reference serum (PB-2) and six postvaccination serum samples (no. 3, 6, 7, 10, 14, and 16). Native polysaccharide was tested with diluted serum and assayed as described in Materials and Methods. The reciprocal of the final dilution of each serum was as follows: PB-2, 900; serum 3, 800; serum 6, 1,600; serum 7, 400; serum 10, 800; serum 14, 400; and serum 16, 100.

for prevaccination sera (Fig. 2A) was 15%, and it ranged from 0.01% (serum 11 in laboratory 6) to 80% (serum 2 in laboratory 8). Sixty-two of 73 within-laboratory CVs for the prevaccination sera were <30%, and 58 were <20% (Fig. 2A). The overall average within-laboratory CV for postvaccination sera (Fig. 2B) was 12%, and it ranged from 0.4% (serum 10 in laboratory 4) to 40% (serum 1 in laboratory 7). Seventy-three of the 78 within-laboratory CVs for postvaccination sera were <30%, and 64 were <20%.

The inhibition curves from competitive inhibition assays for the PB-2 reference serum and six postvaccination serum samples are shown in Fig. 3. One dilution of each serum was mixed with six fourfold dilutions of polysaccharide, with final concentrations ranging from 50 to 0.048 μg/ml. When the maximum final concentration (50 μg/ml) of meningococcal serogroup C polysaccharide was mixed with each serum, the percent inhibition was as follows: PB-2, 90%; serum 3, 84%; serum 6, 97%; serum 7, 92%; serum 10, 95%; serum 14, 96%; and serum 16, 96% (average inhibition was 93%). All sera were at least 90% inhibited except for serum 3, which was inhibited 84%. Serum 6 exhibited the highest percent inhibition (97%) with 50 μg of polysaccharide per ml and also had the highest antibody level (5,940 U/ml). The concentration (micrograms per milliliter) of meningococcal group C polysaccharide needed for 50% inhibition of each serum was as follows: PB-2, 1.03; serum 3, 1.77; serum 6, 0.41; serum 7, 0.83; serum 10, 0.94; serum 14, 0.93; and serum 16, 0.47. The average concentration needed for 50% inhibition of all seven sera was 0.9 μg/ml.

DISCUSSION

Reducing between-laboratory variability through assay standardization is essential in order to accurately compare antibody levels during vaccine immunogenicity studies. This study demonstrates good agreement between laboratories that used the standardized ELISA for quantitating *N. meningitidis* serogroup C total anticapsular antibody levels.

The ELISA format was chosen for standardization because it is simple, sensitive, reproducible, and specific for anticapsular antibody as shown by competitive inhibition curves. In the past, the radiolabeled-antigen binding assay has been used for quantitating levels of antibody to meningococcal polysaccharide (6, 10). However, unlike the radiolabeled-antigen binding assay, which is subject to inconsistencies in the method and intensity of antigen radiolabeling (1), the ELISA uses simple reagents accessible to most laboratories and is therefore easily standardized (29). Results with ELISA also have been shown to correlate well with those of the radiolabeled-antigen binding assay in studies of *H. influenzae* type b (5, 20, 25). In addition, the ELISA is easily modified with appropriate reagents to quantitate class-specific antibodies and immunoglobulin G subclasses, as demonstrated in assays with *H. influenzae* type b (5, 19, 31).

We evaluated a standardized quantitative ELISA in 11 laboratories by comparing variations in levels of total anticapsular antibody to *N. meningitidis* serogroup C. Variation was measured by comparing the CVs of each serum antibody level between laboratories and within each laboratory and by com-

paring the percent differences from the overall mean antibody level. Currently, there is not a fixed upper limit for an acceptable between-laboratory CV; the limit is now set by the investigator. A report of a previous study with meningococcal serogroup A proposed that a CV of approximately 30% was a reasonable upper limit, and an average CV of 23% was obtained for postvaccination sera (7). In this study, we compared the CVs from the mean antibody levels in three dilutions of a single quality control serum (Table 1) as one measure of assay variation. Each independent dilution had a between-laboratory CV that was nearly 10% lower than the suggested 30% limit. The overall average CV of 20% from the three dilutions in 241 assays demonstrates good agreement among the 11 laboratories. The variation that was observed may be attributed to inherent laboratory differences, such as technical expertise, pipetting, or quality of water.

Another measure of assay variation was the comparison of the CVs from the between-laboratory mean antibody levels of 16 unknown serum samples (Table 2). Although the present study obtained between-laboratory CVs from prevaccination sera that were higher than the previously suggested limit of 30% (7), lower CVs were obtained for all eight postvaccination serum samples. The overall average CV of the postvaccination sera was 17%. This low CV and the CV of 20% from the quality control serum indicate good between-laboratory agreement and reproducibility of the ELISA. In addition to the comparison of the between-laboratory CVs, each laboratory (except laboratory 2) performed two ELISAs on each serum, which allowed determination of the within-laboratory variation (Fig. 2). This comparison showed an overall average within-laboratory CV of 15% for prevaccination and 12% for postvaccination sera. The ELISA demonstrated good within-laboratory reproducibility, with CVs of less than 30% for 85% of the prevaccination sera and for 94% of the postvaccination sera.

The between-laboratory variation in the prevaccination sera, which had an overall average CV of 35%, was considerably higher than the 17% CV of postvaccination sera. This value is consistent with other studies that obtained between-laboratory CVs ranging from 35 to 50% for sera with low concentrations of antibody (7, 16). Variation can be influenced by several factors that affect the reproducible binding of antibody in prevaccination sera. The presence of lower-affinity antibodies may not be detected in some ELISAs, or the antibodies may be easily dissociated from the plate during washing (30). Moreover, interpolating the antibody level from a limited number of absorbance values on the lower portion of the reference curve, as with some prevaccination sera, may contribute to a higher between-laboratory CV. Table 2 shows that prevaccination sera 2 and 13 had the highest between-laboratory CV (59% each) and that both had antibody levels near the minimum level detectable in the assay. Generally, fewer than three of the eight dilutions from the lower portion of the reference curve were used to calculate the mean antibody levels in these two serum samples. This may partially explain why the CVs for some prevaccination sera were higher than expected.

When antibody levels from each laboratory were compared with the respective between-laboratory mean, results for prevaccination sera differed from the mean by an average of 24%, while those for postvaccination sera differed by only 13%. The percent differences from the overall mean for each serum (Fig. 1) are less than in a previous multicenter comparison of levels of antibody to *N. meningitidis* serogroup A capsular polysaccharide. In that study, results for prevaccination sera differed from the mean by an average of 37%, and those for postvaccination sera differed by 18% (7). In the present assay, lower between-laboratory variation may be attributed to further standardization, since the meningococcal serogroup A assay described by Carlone et al. (7) did not include a blocking step and used multiple methods for data analysis.

A blocking step can reduce variability by preventing unwanted nonspecific binding of serum and conjugate components (28). In contrast to the previous study of Carlone et al. (7), the present study incorporates a blocking step as a further step in standardization of the ELISA. Additionally, poor water quality has been shown to increase nonspecific binding (7); therefore, sterile, pyrogen-free distilled water should be used in all buffers, and reservoirs must be kept clean and free from contamination to keep nonspecific binding at a minimum.

It has been reported that about one-third of between-laboratory variation with CVs as high as 30% is due to differences in data analyses (17). One previous study compared several interpolation methods and found that the four-parameter logistic-log function was best for estimating the standard curve. This function uses a weighted robust regression analysis for the standard reference curve, which minimizes the effects of outlying points on the best fit of the curve (27). We therefore used only one curve-fitting technique, the four-parameter logistic-log function, to ensure the most reliable values over the widest range of serum dilutions (26) and to reduce variability between laboratories associated with differing methods of data analysis.

Assay variability can be influenced by polyclonal enzyme conjugates, which have lot-to-lot variations and differences in manufacturing techniques (28). The working dilution and incubation time, including assessment of the reproducibility of antibody levels in quality control sera, must be determined for each new reagent. In this study, variability due to the goat anti-human immunoglobulin conjugate was controlled by providing the same reagent to each laboratory. To achieve consistency in conjugates, we are currently investigating the use of monoclonal antibodies for total, class-specific, and immunoglobulin G isotype quantitation. The use of well-characterized monoclonal antibody enzyme-labeled conjugates should further reduce between-laboratory variability and will lead to more reproducible results over time.

The present study demonstrates that control of certain parameters (antigen, reference serum, plate, buffer, quality control serum, enzyme conjugate, detailed protocol, and data analysis method) reduces the variation of ELISA antibody measurements between laboratories. The results show that this standardized ELISA is sensitive and reproducible, allowing the between-laboratory comparison of levels of antibody to meningococcal serogroup C polysaccharide. A meningococcal standard reference serum (CDC 1992, replacing the interim reference serum PB-2), additional quality control sera, coating antigen, monoclonal antibodies, and a data-handling and data analysis program are available from CDC to aid in the development and use of a standard assay. We suggest that this assay be evaluated in other laboratories to determine total anticapsular antibody to *N. meningitidis* and that agreement on a standard reference assay evolve from these studies.

ACKNOWLEDGMENTS

This work was supported in part by a Merit Review grant from the Department of Veterans Affairs to Peter Densen, University of Iowa.

We greatly appreciate the technical assistance of Lucia Tagliaferri and Stefano Viti, Biocine Sclavo, S.p.A., Siena, Italy; Raana Anwaruddin, Dana-Farber Cancer Institute, Boston, Mass.; Susan L. Strohmeyer, Lederle-Praxis Biologicals, West Henrietta, N.Y.; Leena Saarinen, National Public Health Institute, Helsinki, Finland; and Leslie Saucedo, University of Iowa, Iowa City. We thank Connaught Laboratories, Inc., for providing purified meningococcal serogroup C native

polysaccharide. The World Health Organization Programme for Vaccine Development, Steering Committee on Encapsulated Bacteria, provided support and endorsement for development of a standardized assay. We also thank Claire V. Broome for her support and encouragement.

REFERENCES

1. **Anderson, P., R. A. Insel, S. Porcelli, and J. I. Ward.** 1987. Immunochemical variables affecting radioantigen-binding assays of antibody to *Haemophilus influenzae* type b capsular polysaccharide in childrens' sera. J. Infect. Dis. **156:**582–590.
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. **Armitage, P., and G. Berry.** 1987. Statistical methods in medical research. Blackwell Scientific Publications, Ltd., Oxford.
4. **Artenstein, M. S., R. Gold, J. G. Zimmerly, F. A. Wyle, H. Schneider, and C. Harkins.** 1970. Prevention of meningococcal disease by group C polysaccharide vaccine. N. Engl. J. Med. **282:**417–420.
5. **Barra, A., D. Schulz, P. Aucouturier, and J.-L. Preud'homme.** 1988. Measurement of anti-*Haemophilus influenzae* type b capsular polysaccharide antibodies by ELISA. J. Immunol. Methods **115:** 111–117.
6. **Brandt, B. L., F. A. Wyle, and M. S. Artenstein.** 1972. A radioactive antigen-binding assay for *Neisseria meningitidis* polysaccharide antibody. J. Immunol. **108:**913–920.
7. **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.
8. **Costantino, P., S. Viti, A. Podda, M. A. Velmonte, L. Nencioni, and R. Rappuoli.** 1992. Development and phase 1 clinical testing of a conjugate vaccine against meningococcus A and C. Vaccine **10:** 691–698.
9. **Evans, J. R., M. S. Artenstein, and D. H. Hunter.** 1968. Prevalence of meningococcal serogroups and description of three new groups. Am. J. Epidemiol. **87:**643–646.
10. **Farr, R. S.** 1958. A quantitative immunochemical measure of the primary interaction between I* BSA and antibody. J. Infect. Dis. **103:**239–262.
11. **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-absorbed *Neisseria meningitidis* serotype 2b protein group *B*-polysaccharide vaccine. J. Infect. Dis. **158:**710–718.
12. **Gold, R., M. L. Lepow, I. Goldschneider, T. F. Draper, and E. C. Gotschlich.** 1975. Clinical evaluation of group A and group C meningococcal polysaccharide vaccines in infants. J. Clin. Invest. **56:**1536–1547.
13. **Gold, R., M. L. Lepow, I. Goldschneider, T. F. Draper, and E. C. Gotschlich.** 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.
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. **Greenberg, D. P., J. I. Ward, K. Burkart, P. D. Christenson, L. Guravitz, and S. M. Marcy.** 1987. Factors influencing immunogenicity and safety of two *Haemophilus influenzae* type b polysaccharide vaccines in children 18 and 24 months of age. Pediatr. Infect. Dis. J. **6:**660–665.
16. **Herrmann, D. J., R. G. Hamilton, T. Barington, 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: results of an international collaborative study using enzyme immunoassay methodology. J. Immunol. Methods **148:**101–114.
17. **Jeffcoate, S. L., and R. E. G. Das.** 1977. Interlaboratory comparison of radioimmunoassay results: variation produced by different methods of calculation. Ann. Clin. Biochem. **14:**258–260.
18. **Jennings, H. J., and C. Lugowski.** 1981. Immunochemistry of groups A, B, and C meningococcal polysaccharide-tetanus toxoid conjugates. J. Immunol. **127:**1011–1018.
19. **Käyhty, H., O. Mäkelä, J. Eskola, L. Saarinen, and I. Seppälä.** 1988. Isotype distribution and bactericidal activity of antibodies after immunization with *Haemophilus influenzae* type b vaccines at 18–24 months of age. J. Infect. Dis. **158:**973–982.
20. **Lagergard, T., B. Trollfors, B. A. Claesson, R. Schneerson, and J. B. Robbins.** 1988. Comparison between radioimmunoassay and direct and indirect enzyme-linked immunosorbent assays for determination of antibodies against *Haemophilus influenzae* type b capsular polysaccharide. J. Clin. Microbiol. **26:**2554–2557.
21. **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.
22. **Mandell, J. D., and A. D. Hershey.** 1960. A fractionating column for analysis of nucleic acids. Anal. Biochem. **1:**66–77.
23. **Peltola, H.** 1983. Meningococcal disease: still with us. Rev. Infect. Dis. **5:**71–91.
24. **Peterman, J. H., and J. E. Butler.** 1989. Application of theoretical considerations to the analysis of ELISA data. BioTechniques **7:**608–615.
25. **Phipps, D. C., J. West, R. Eby, M. Koster, D. V. Madore, and S. A. Quataert.** 1990. An ELISA employing a *Haemophilus influenzae* type b oligosaccharide-human serum albumin conjugate correlates with the radioantigen binding assay. J. Immunol. Methods **135:** 121–128.
26. **Plikaytis, B. D., G. M. Carlone, S. H. Turner, L. L. Gheesling, and P. F. Holder.** 1993. Program **ELISA** user's manual. Centers for Disease Control and Prevention, Atlanta.
27. **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.
28. **Pruslin, F. H., S. E. To, R. Winston, and T. C. Rodman.** 1991. Caveats and suggestions for the ELISA. J. Immunol. Methods **137:**27–35.
29. **Siber, G. R., C. Priehs, and D. V. Madore.** 1989. Standardization of antibody assays for measuring the response to pneumococcal infection and immunization. Pediatr. Infect. Dis. J. **8:**S84–S91.
30. **Steward, M. W., and A. M. Lew.** 1985. The importance of antibody affinity in the performance of immunoassays for antibody. J. Immunol. Methods **78:**173–190.
31. **Trollfors, B., T. Lagergard, B. A. Claesson, E. Thornberg, J. Martinell, and R. Schneerson.** 1992. Characterization of the serum antibody response to the capsular polysaccharide of *Haemophilus influenzae* type b in children with invasive infections. J. Infect. Dis. **166:**1335–1339.
32. **Ward, J. I., D. P. Greenberg, P. W. Anderson, K. S. Burkart, P. D. Christenson, L. K. Gordon, H. Kayhty, J. S. C. Kuo, and P. Vella.** 1988. Variable quantitation of *Haemophilus influenzae* type b anticapsular antibody by radioantigen binding assay. J. Clin. Microbiol. **26:**72–78.