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# Interlaboratory Study Evaluating Quantitation of Antibodies to *Haemophilus influenzae* Type b Polysaccharide by Enzyme-Linked Immunosorbent Assay

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**An interlaboratory study was conducted to determine whether an enzyme-linked immunosorbent assay (ELISA) with an antigen preparation composed of various-sized fragments of *Haemophilus influenzae* type b polysaccharide conjugated to human serum albumin could be standardized across laboratories and whether the ELISA-derived results from different laboratories are equivalent to those obtained by the standard radioactive antigen binding assay (RABA) for quantitation of anti-*H. influenzae* type b polysaccharide antibodies. Twenty coded human serum samples were quantitated by ELISA in 11 laboratories and by RABA in 5 laboratories. The mean RABA-derived values served as the basis for all comparisons. While the overall correspondence of antibody values between the two methods was good, significant differences were found among some of the 11 ELISA data sets and among the mean RABA values. Seven laboratories generated higher ELISA antibody values for low-titered sera. Four laboratories generated antibody concentrations that were not statistically different between the two assay methods. The results therefore indicate that the ELISA can tolerate substantial variations in protocol, such as the use of different plates and different antibody reagents, without affecting the quantitation of serum antibodies. However, attention should be focused on low-titered sera, as some assay conditions may yield spurious results. This ELISA is a serologic assay which can serve as an alternative to the RABA for quantitation of antibodies to *H. influenzae* type b polysaccharide.**

Quantitation of antibodies specific for the capsule of *Haemophilus influenzae* type b (Hib) has been an active area of clinical research since the 1970s (6, 10, 11, 15, 17), continuing even after the introduction of Hib vaccines for infants in the United States in the 1990s (3). The radioactive antigen binding assay (RABA) for Hib polysaccharide (PS) antibodies was standardized following reports that data derived by RABA in different laboratories were quite variable (4, 5, 18). In fact, the need for reliable standardized assay methods is now paramount, as the availability of these vaccines creates new opportunities for immunization. The development of multiple Hib conjugate vaccines with various chemistries and immunogenicities has produced a situation in which the same Hib vaccine may not be used for an entire immunization sequence. Other changes in the presentation of the Hib vaccine, such as its use in combination with other vaccines (e.g., diphtheria, tetanus, and pertussis vaccines), require clinical testing to ensure that immunogenicity has not been adversely affected. Furthermore, the protective status of individuals who may be immunocompromised may need to be assessed to evaluate the benefits of immunization. Even with the demonstration of vaccine efficacy in one population, licensure expansion into other countries may depend on immunologic surrogates.

The Hib capsular PS, polyribosylribitol phosphate, is the primary immunogen in all Hib vaccines. Traditionally, the immune status and response to vaccination for antibodies specific to the Hib PS in human sera have been quantitated by RABA (5, 18). The antibody levels have provided a surrogate marker for predicting the minimum antibody concentrations associated with protection from invasive disease (9). However, quantitation by RABA requires the preparation of a radioactive antigen, can consume large amounts of serum, is cumbersome to carry out, and does not lend itself well to automation of either the assay or data analysis. Furthermore, RABA does not provide qualitative data, such as the isotype or subclass of antibody. Thus, several enzyme-linked immunosorbent assays (ELISAs) have been developed as alternatives to the RABA (2, 7).

An acceptable ELISA should yield antibody values that are comparable to those generated by the RABA and that are

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reproducible in different laboratories. Furthermore, the ELISA should be as sensitive as the RABA in the range in which minimum protective levels are assessed. On the basis of past efficacy trials with the Hib PS vaccine, levels above 0.15 and 1.0 μg/ml have been associated with short- and long-term protection, respectively (9). More recent studies suggest that different Hib conjugate vaccines elicit immunoglobulin G (IgG) antibodies with different avidities, and less high-avidity antibody is required for equivalent bactericidal killing, which is a correlate of protection from invasive disease (16).

An ELISA with an antigen preparation composed of various-sized oligosaccharide fragments of Hib PS conjugated to human serum albumin (HbO-HA) has been described as a substitute for the traditional RABA (12). An interlaboratory study was organized to determine whether this ELISA could be standardized across laboratories and whether the results obtained by this ELISA in different laboratories would be equivalent to those obtained by RABA. Twenty human serum samples were provided in coded form to 12 laboratories which had experience with quantitative Hib PS antibody assays. The data generated by the participating laboratories are described below, and they indicate that the HbO-HA ELISA generates antibody concentrations in different laboratories similar to those generated by RABA and can serve as an acceptable alternative to the RABA.

## MATERIALS AND METHODS

**Study design.** Participants in a workshop organized by C. Frasch at the Centers for Disease Control and Prevention (28 August 1992) were offered the opportunity to participate in an interlaboratory comparative study of the HbO-HA ELISA. Twelve laboratories participated, and some of the laboratories performed both the ELISA and the RABA. Data generated by each of these participating laboratories were coded and are referred to as coming from laboratories I to XII.

For this study, human sera were provided to the Center for Biologics Evaluation and Research (CBER) by G. Carlone, C. Frasch, B. Gray (University of Alabama), D. J. Herrmann (Connaught Laboratories), H. Käyhty, and G. Siber. These sera were from normal adults or from adults or children immunized with the Hib PS vaccine. Coded aliquots (0.2 ml each) of the 20 serum samples were redistributed to each of the participating laboratories. Lederle-Praxis Biologicals provided the HbO-HA antigen, the reference serum (lot 1983; 70 μg/ml; CBER), goat anti-human immunoglobulin specific alkaline phosphatase conjugate from Tago, and a protocol based upon the published assay (12). With the exception of the common antigen, the reference serum, and the coded test sera, each participating laboratory used its own source(s) of plates, reagents, and equipment, as well as its own method of data analysis.

**Assay methods.** The HbO-HA ELISA protocol evaluated in this study has been described (12). Briefly, each well of a microtiter plate was coated with 0.1 μg of HbO-HA antigen in 0.1 ml of sterile phosphate-buffered saline, pH 7.0 to 7.4 (PBS), for 90 min at 37°C. The coded sera were, according to protocol, serially diluted twofold, beginning with a 1:50 dilution, up to 1:64,000 in PBS–0.3% Tween–0.01 M EDTA. The initial dilution for the CBER reference serum was 1:400. Each of the antibody incubation steps lasted 1 h; all incubations and washes were carried out at room temperature.

While a standard protocol was provided to all participating laboratories, some of the laboratories deviated from this protocol. Laboratory X heat inactivated (56°C, 30 min) one set of sera and chemically inactivated [0.3% tri(*n*-butyl)phosphate and 0.2% sodium cholate (final concentrations)] a second set of sera prior to use in ELISA. In place of the polyvalent anti-human immunoglobulin conjugate, laboratory VI used an equimolar mixture of murine monoclonal antibodies specific for binding human IgG (HP6017), IgM (HP6083), and IgA (HP6123) that had been developed at the Centers for Disease Control and Prevention. Laboratory XI used not the CBER reference serum but an internal reference of a human serum containing 65.47 μg of anti-Hib PS antibody per ml, as previously quantitated against the CBER reference serum (lot 1983).

In order to establish a baseline for comparison of the ELISA results, five laboratories (III, VIII, IX, X, and XII) quantitated the 20 coded serum samples by the standardized RABA (4, 19). The RABA quantitates the total amount of antibody that specifically binds to extrinsically or intrinsically labeled Hib PS (1, 15). Antigen specifications conform to those established by C. Frasch in 1987 (19). Fractions of capsular PS of Hib strain Eagan with a distribution coefficient (Kd) between 0.3 and 0.7 on a Sepharose CL-2B column (Pharmacia) were pooled. Serially diluted test sera and the CBER reference standard serum (lot 1983, with 70 μg of anti-Hib PS antibody per ml) were incubated with the labeled Hib PS (10 ng/ml in 0.1 M phosphate buffer, pH 7.4). Antibody was quantitated (in micrograms per milliliter) by comparing the binding of dilutions of sera with that of the reference serum.

TABLE 1. Antibody concentrations obtained by RABA[a]

| Sample | Antibody concn (μg/ml) | | CV[c] |
|---|---|---|---|
| | Mean[b] | Range | |
| A | 0.100 | 0.100–0.100 | NA[d] |
| B | 1.138 | 0.850–2.120 | 48.4 |
| C | 0.100 | 0.100–0.100 | NA |
| D | 200.160 | 133.800–242.000 | 20.0 |
| E | 165.320 | 143.000–213.000 | 17.8 |
| F | 74.208 | 58.800–86.240 | 16.8 |
| G | 1.538 | 1.120–1.970 | 20.2 |
| H | 3.058 | 2.100–4.310 | 33.3 |
| I | 0.100 | 0.100–0.100 | NA |
| J | 1.067 | 0.687–1.400 | 28.5 |
| K | 0.216 | 0.100–0.350 | 51.8 |
| L | 1.234 | 0.780–1.910 | 34.9 |
| M | 0.130 | 0.100–0.250 | 51.6 |
| N | 28.056 | 19.000–39.900 | 29.0 |
| O | 0.140 | 0.100–0.300 | 63.9 |
| P | 145.680 | 98.400–225.000 | 36.6 |
| Q | 76.664 | 63.500–89.920 | 14.7 |
| R | 0.100 | 0.100–0.100 | NA |
| S | 6.766 | 4.390–9.040 | 25.6 |
| T | 2.472 | 1.660–3.160 | 22.2 |

[a] Five laboratories (III, VIII, IX, X, and XII) evaluated all samples. Lower limit of RABA, 0.2 μg/ml.
[b] Arithmetic mean.
[c] Obtained by the following formula: (mean/standard deviation) × 100.
[d] NA, not applicable because of undetectable antibody levels under the assay conditions used.

**Statistical methods.** Study participants analyzed their primary data by their own standard interpolation procedures and provided their final anti-Hib PS antibody estimates (in micrograms per milliliter) for the coded specimens to the organizing laboratory (CBER). For the analyses described herein, the least sensitive lower limit was used as the lower limit for all laboratories; the adjusted lower limits for the ELISA and RABA were 0.14 and 0.2 μg/ml, respectively. For calculations, all values below these lower limits were adjusted to one-half the lower-limit value (i.e., 0.07 and 0.1 μg/ml, respectively). All sera were diluted to determine an endpoint.

The antibody values provided by each of the participating laboratories were further analyzed by using a statistical graphics package, STATVIEW 4.01. For statistical analyses, the antibody concentrations were log transformed to normalize the data. For comparisons of the data generated by both ELISA and RABA, the least sensitive lower limit was applied to both data sets (i.e., 0.2 μg/ml).

## RESULTS

All 12 participating laboratories were provided with 20 coded serum samples and were asked to analyze the samples by HbO-HA ELISA and/or RABA. As RABA has been the traditional assay method and has been previously standardized (5, 18), the data obtained by this method served as the baseline for comparison with the data generated by the new ELISA method (12). The RABA was performed by five participating laboratories. The arithmetic mean RABA antibody concentrations, the minimum and maximum values observed for each sample, and the coefficients of variation (CVs) are shown in Table 1. These RABA-derived antibody concentrations were assigned to the samples coded A to T and were used for further evaluation of the HbO-HA ELISA.

These 20 samples were analyzed by 11 laboratories by the HbO-HA ELISA method, and antibody concentrations were determined for each sample. The anti-Hib PS antibody concentrations reported by the participating laboratories are shown in Table 2. One laboratory (X) tested the samples after heat inactivation or chemical inactivation, thus providing two

TABLE 2. Antibody concentrations obtained by HbO-HA ELISA as reported to CBER

| Serum sample | Antibody concn (μg/ml) reported by laboratory: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII | VIII | IX | Xa | Xb | XI |
| A | 0.53 | 0.10 | 1.51 | 0.14 | <0.10 | <0.14 | 0.15 | 1.67 | 0.14 | TL[a] | TL | 0.45 |
| B | 3.51 | 3.21 | 4.35 | 3.61 | 2.53 | 2.26 | 2.95 | 2.65 | 2.15 | 1.96 | 2.80 | 4.91 |
| C | 0.28 | 0.20 | 1.13 | 0.21 | 0.18 | 0.26 | 0.39 | 0.75 | 0.43 | 0.50 | NR[b] | 0.70 |
| D | 102.03 | 248.58 | 164.90 | 237.85 | 214.53 | 166.70 | 63.6 | 76.90 | 332.00 | 200.00 | 200.00 | 189.84 |
| E | 247.72 | 231.03 | 172.65 | 234.89 | 250.55 | 191.43 | 101.50 | 254.00 | 341.00 | 296.00 | 288.00 | 292.65 |
| F | 118.36 | 103.94 | 88.37 | 129.39 | 114.63 | 73.10 | 52.20 | 98.70 | 191.00 | 93.30 | 97.20 | 130.52 |
| G | 0.71 | 0.51 | 3.06 | 1.09 | 0.72 | 0.52 | 0.63 | 1.13 | 2.83 | 0.74 | 0.74 | 0.64 |
| H | 13.05 | 2.18 | 4.33 | 3.90 | 2.45 | 2.41 | 21.85 | 2.59 | 1.19 | 2.15 | 2.15 | 3.67 |
| I | 0.36 | 0.10 | 1.12 | 0.25 | 0.11 | <0.14 | 0.11 | 0.47 | 0.22 | TL | NR | 0.45 |
| J | 1.93 | 1.03 | 4.06 | 2.11 | 1.34 | 0.85 | 1.27 | 1.17 | 1.68 | 1.20 | 1.21 | 3.75 |
| K | 1.08 | 1.85 | 2.70 | 1.94 | 1.34 | 1.24 | 3.05 | 1.46 | 1.07 | 0.98 | 1.21 | 1.36 |
| L | 8.06 | 2.56 | 3.13 | 2.77 | 2.25 | 1.86 | 3.71 | 2.96 | 2.16 | 1.75 | 2.44 | 3.84 |
| M | 0.76 | 0.22 | 1.12 | 0.27 | 0.19 | <0.14 | 0.32 | 0.92 | 0.20 | TL | TL | 0.35 |
| N | 41.92 | 30.54 | 33.43 | 24.62 | 30.79 | 22.10 | 32.81 | 24.00 | 32.90 | 25.79 | 30.70 | 47.29 |
| O | 13.02 | 0.33 | 2.54 | 0.69 | 0.30 | <0.14 | 0.66 | 1.17 | 0.43 | 0.01 | 0.01 | 0.07 |
| P | 497.13 | 371.7 | 303.85 | 420.20 | 374.87 | 128.28 | 158.00 | NR | NR | TH[c] | TH | 175.62 |
| Q | 76.18 | 87.34 | 109.56 | 111.23 | 94.52 | 78.49 | 38.5 | 129.00 | 103.00 | 82.50 | 107.50 | 106.34 |
| R | 0.39 | 0.10 | 1.75 | 0.52 | 0.14 | 0.23 | 0.27 | 0.47 | 0.21 | TL | TL | 3.10 |
| S | 7.10 | 5.66 | 10.65 | 7.65 | 7.25 | 7.79 | 6.71 | 5.88 | 8.09 | 6.42 | 7.19 | 16.76 |
| T | 3.66 | 2.26 | 7.33 | 2.65 | 2.18 | 2.13 | 2.68 | 2.91 | 3.23 | 1.75 | 1.85 | 6.08 |

[a] TL, value too low; this value was excluded from the data analysis.
[b] NR, not reported.
[c] TH, value too high; this value was excluded from the data analysis.

sets of data. The samples yielded essentially the same anti-Hib PS antibody concentrations; thus, for the purposes of this interlaboratory evaluation, only one data set (heat inactivation) was used.

A preliminary review of the ELISA-generated data for each sample showed a good correspondence of values among the 11 laboratories that performed ELISA. However, there appeared to be occasional outliers and, for some laboratories, trends for higher values for samples which appeared to have antibody levels undetectable by RABA. The data were evaluated statistically to objectively address whether the differences between the laboratory's ELISA-derived data sets and the mean RABA values were significant.

In order to achieve normality, $\log_{10}$-transformed values were used for all analyses of ELISA- and RABA-derived antibody concentrations. A repeated-measures analysis of variance was used to test the null hypothesis that there were no differences between the mean $\log_{10}$ ELISA-derived values and the mean $\log_{10}$ RABA-derived values among the 11 data sets. This analysis concluded that there were differences ($P < 0.0001$).

In order to more clearly delineate where these differences occurred, each ELISA-derived data set was compared with the mean RABA-derived values by using the paired $t$ test. To account for the fact that 11 multiple comparisons were made, the $P$ value was set at 0.005 by the Bonferroni method (desired $P$ value, 0.05, divided by the number of pairwise comparisons, 11, as described in reference 8). The $P$ value for each laboratory's data set is shown in Table 3. By this paired $t$-test analysis of the HbO-HA ELISA data, we found that the derived antibody concentrations from five of the laboratories (II, V, VI, VII, and X) were not significantly different from the mean RABA-derived values.

To further compare the two assay methods for each laboratory, regression analysis was used. The regression analysis for each set of ELISA data in comparison with the assigned RABA-generated data is summarized in Table 3. Overall, each laboratory's ELISA-generated datum points exhibited a good correlation with the RABA-derived values, with each set of data having an $r^2$ of >0.82. However, the slopes varied considerably, from 0.648 to 0.999, making the $r^2$ values difficult to interpret (13).

An ideal ELISA should have a slope of 1, indicating that antibody values across all concentrations correlate to the values obtained by RABA. The ELISA-derived data sets were classified into one of two categories on the basis of having both a regression slope of >0.9 and a nonsignificant paired $t$-test result. Four laboratories (II, V, VI, and X), designated subset 4, met these criteria and seven (I, III, IV, VII, VIII, IX, and XI), designated subset 7, did not. Typical examples from each

TABLE 3. Comparison of antibody concentrations ($\log_{10}$) generated by RABA and HbO-HA ELISA

| Laboratory | No. of samples | Paired $t$ test ($P$) | Regression analysis | | |
|---|---|---|---|---|---|
| | | | $r^2$ | Slope | $y$ axis intercept |
| Subset 4 | | | | | |
| II | 20 | 0.0368 | 0.942 | 0.958 | 0.165 |
| V | 20 | 0.0348 | 0.963 | 0.999 | 0.124 |
| VI | 20 | 0.3143 | 0.959 | 0.928 | 0.085 |
| X | 15 | 0.1843 | 0.939 | 0.915 | 0.152 |
| Subset 7 | | | | | |
| I | 20 | 0.0012[a] | 0.827 | 0.756 | 0.532 |
| III | 20 | <0.0001[a] | 0.939 | 0.648 | 0.694 |
| IV | 20 | <0.0003[a] | 0.945 | 0.890 | 0.334 |
| VII | 20 | 0.0632 | 0.884 | 0.779 | 0.276 |
| VIII | 19 | 0.0032[a] | 0.889 | 0.690 | 0.449 |
| IX | 19 | 0.0003[a] | 0.956 | 0.934 | 0.272 |
| XI | 20 | 0.0003[a] | 0.902 | 0.814 | 0.471 |
| Mean | 20 | | 0.943 | 0.786 | 0.459 |

[a] $P$ values of <0.005 are significant on the basis of the Bonferroni method for paired $t$ test.

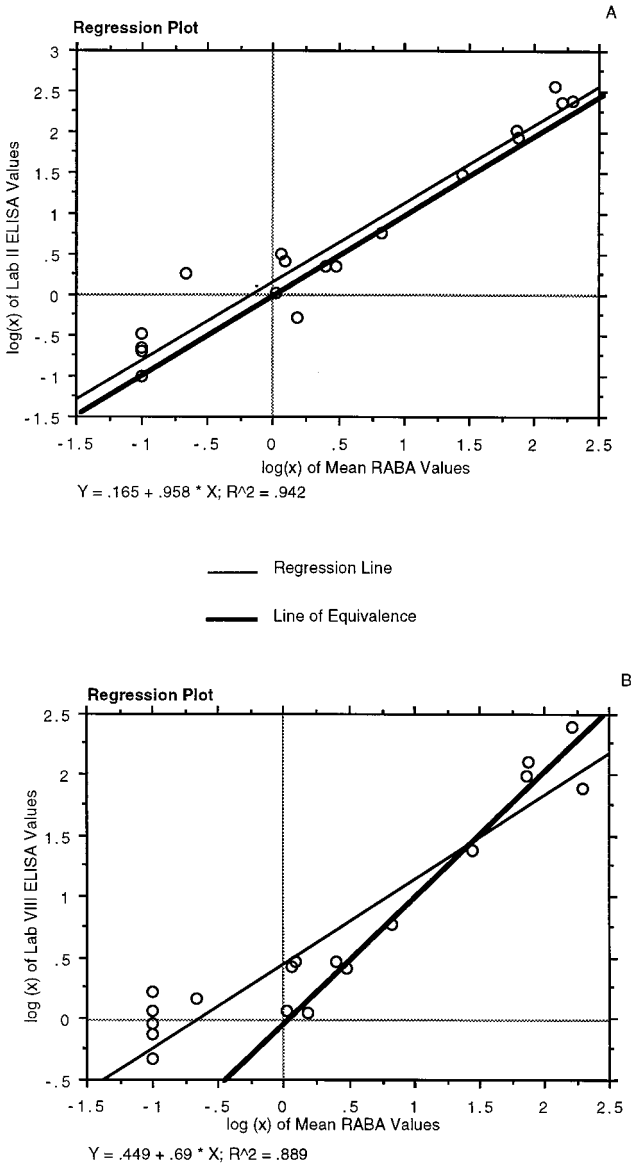


FIG. 1. The 20 anti-Hib PS antibody values obtained by ELISA by laboratory II (A) and laboratory VIII (B) were compared with the arithmetic mean RABA-derived values by regression analysis. The vertical and horizontal lines indicate the 1-μg/ml anti-Hib PS antibody concentration (0 when log transformed).

category are shown in Fig. 1A and B, respectively. The antibody concentrations obtained by the HbO-HA ELISA by the subset 4 laboratories were similar to those obtained by the RABA for both low- and high-titered sera. However, the subset 7 laboratories obtained similar values for higher-titered sera by RABA and HbO-HA ELISA but appeared to obtain greater values for low-titered samples by HbO-HA ELISA than by RABA. Greater values for low-titered samples skewed the equivalence line, resulting in a flatter slope (<0.9). Similarly, the intercept on the *y* axis indicates that laboratories in subset 7 obtained values by ELISA that were apparently greater than the mean RABA values for low-titered samples.

Among the subset 4 laboratories, two deviated procedurally from the HbO-HA protocol. Laboratory VI used a panel of monoclonal antibodies as a secondary enzyme conjugate in place of a polyclonal conjugate. Laboratory X inactivated the

TABLE 4. Antibody concentrations obtained by HbO-HA ELISA[a]

| Sample | $n^b$ | Antibody concn (μg/ml) | | CV[d] |
|---|---|---|---|---|
| | | Mean[c] | Range | |
| A | 3 | 0.070 | 0.070–0.070 | NA[e] |
| B | 4 | 2.449 | 1.960–3.210 | 51.7 |
| C | 4 | 0.262 | 0.180–0.500 | 51.7 |
| D | 4 | 205.343 | 166.700–248.580 | 16.4 |
| E | 4 | 239.313 | 191.430–296.000 | 17.9 |
| F | 4 | 94.945 | 73.100–114.630 | 18.4 |
| G | 4 | 0.613 | 0.510–0.740 | 20.0 |
| H | 4 | 2.294 | 2.150–2.450 | 6.7 |
| I | 3 | 0.070 | 0.070–0.070 | NA |
| J | 4 | 1.089 | 0.850–1.340 | 19.2 |
| K | 4 | 1.317 | 0.980–1.850 | 27.0 |
| L | 4 | 2.081 | 1.750–2.560 | 17.6 |
| M | 3 | 0.143 | 0.070–0.220 | 49.6 |
| N | 4 | 27.057 | 22.100–30.790 | 15.2 |
| O | 4 | 0.148 | 0.070–0.330 | 73.8 |
| P | 3 | 291.463 | 128.280–374.870 | 48.5 |
| Q | 4 | 85.507 | 78.490–94.520 | 8.0 |
| R | 3 | 0.131 | 0.070–0.230 | 54.7 |
| S | 4 | 6.731 | 5.660–7.790 | 13.8 |
| T | 4 | 2.070 | 1.750–2.260 | 10.9 |

[a] Subset 4 laboratories (II, V, VI, and X) only.
[b] Number of values provided; lower limit of ELISA, 0.14 μg/ml.
[c] Arithmetic mean.
[d] Obtained by the following formula: (mean/standard deviation) × 100.
[e] NA, not applicable because of undetectable antibody levels under the assay conditions used.

coded serum samples with heat prior to assay. However, when compared with the antibody concentrations determined by RABA, these data show that such variations from the protocol did not adversely affect quantitation.

In Table 4, the ELISA-derived values obtained by the laboratories in subset 4 are summarized. For most of the samples, the anti-Hib PS antibody concentrations were very similar to those determined by the RABA method (Table 1). Fifteen of the 20 samples (75%) varied by less than twofold, four samples (B, C, G, and P) differed by approximately twofold, and the largest difference, sixfold, was seen with sample K. In four of the five cases (samples B, C, K, and P), the ELISA yielded higher values. Samples B, K, and P behaved inconsistently not only between the two assay methods but also among laboratories using the same method, as shown by the CVs. These higher CVs make it difficult to determine which assay method provided the more correct determination of antibody concentration. High CVs were also observed with some low-titered sera (M, O, and R). Thus, the degree of variation observed among laboratories for samples tested by ELISA appears to be similar to the interlaboratory variations that are observed with the previously standardized RABA.

## DISCUSSION

The HbO-HA ELISA used in this study was first described in 1990. On the basis of the analysis of 214 serum samples, the results obtained by this technique showed an excellent correlation to the anti-Hib PS antibody values obtained by RABA (12). The original study evaluated four antigen lots and indicated that the HbO-HA ELISA yielded consistent results over an 8-month time period. Competition assays with soluble Hib PS, HbO-HA, and HA indicated that this assay detects only Hib PS-specific antibodies in human serum. Three independent laboratories also assessed this HbO-HA ELISA with a small number of samples ($n$ = 9 or 10). Their studies indicated

that the ELISA results were reproducible; the largest difference between anti-Hib PS antibody concentrations for any one sample was 1.76-fold (87.01 versus 153.5 μg/ml) (12).

The study described here was undertaken to more rigorously evaluate the reproducibility of this ELISA method in a greater number of laboratories. The HbO-HA antigen was provided to all the participants; however, the other assay reagents, procedures, and data analysis methods could vary from the Lederle-Praxis protocol. The results shown here indicate that antibody binding to HbO-HA was not affected by heat or chemical inactivation of the sera (laboratory X). The data also demonstrated that the assay is tolerant of different polyclonal secondary anti-human immunoglobulin enzyme conjugates and of substitution of monoclonal anti-human IgG, IgM, or IgA enzyme conjugates.

Seven of the participating laboratories obtained higher values for low-titered sera by HbO-HA ELISA than were obtained by RABA. The factors contributing to this observation are not readily apparent. The quality of the water used in antigen coating has previously been found to impact the quality of data generated by ELISAs (12, 14), in that endotoxin contamination led to higher background and apparent preimmune values. Quality control sera of known low titer should be used to optimize and validate assay performance.

In comparing the RABA and ELISA methods, it is important to recognize that the antigens and the mode of their presentation to the antibodies may be different. In the RABA, the antigen is a large PS in solution. In the HbO-HA ELISA, the PS has been sized into 20- to 30-mer repeat units and is covalently bound to HA by reductive amination (12); the albumin serves to attach the antigen to the plastic plate and allow the saccharides to be oriented into the liquid phase of the well. In spite of these stereophysical differences in antigen presentation, the overall correspondence of antibody titers obtained by these two methods is very good.

All but three of the serum samples yielded anti-Hib PS antibody concentrations that were in the same range (minimum and maximum observed) by both the ELISA and RABA methods. Samples C, K, and G did not, suggesting that the antibodies were preferentially detected in one of the assays. Sample C yielded detectable anti-Hib PS antibodies in all 11 laboratories using the ELISA, while none of the 5 laboratories testing by RABA detected any activity. The largest consistent difference between the two assay methods was found with sample K; the ELISA-derived values (all data sets) ranged from 0.98 to 3.05 μg/ml, while the RABA yielded values of 0.1 to 0.35 μg/ml. This difference may reflect the low avidity of the antibody, which may be ineffective in precipitating the antigen. Alternatively, it may indicate that these antibodies recognize epitopes on the shorter chains of HbO that are not as readily accessible on the longer polymers of Hib PS used in the RABA. The opposite situation was observed with sample G, which consistently yielded higher values in the RABA than in the ELISA.

The results of this interlaboratory evaluation of the HbO-HA ELISA substantiate that this method can provide antibody quantitations that are comparable to those made by the RABA. This study also indicates that substantial changes in the protocol can be made without affecting the anti-Hib PS antibody concentration detected. The HbO-HA ELISA can serve as an alternative to the RABA for the quantitation of antibodies to the Hib PS.

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