JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1988, p. 1818–1823
0095-1137/88/091818-06$02.00/0

Vol. 26, No. 9

Evaluation of Serologic Assays for Diagnosis of Whooping Cough

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Received 1 February 1988/Accepted 3 June 1988

An enzyme-linked immunosorbent assay (ELISA) for the immunoglobulin G (IgG), IgM, and IgA response to *Bordetella pertussis* filamentous hemagglutinin (FHA) and pertussis toxin (PT) and a neutralization test (NT) in a microplate tissue culture assay for neutralizing antibodies to PT were evaluated in paired sera from 90 patients with culture-confirmed pertussis. Eighty patients were children (age, <15 years), and 6 of 80 children had been immunized with three doses of diphtheria-tetanus-pertussis vaccine as infants. A significant titer rise (≥twofold), determined by ELISA, of IgG, IgM, and IgA to FHA was recorded in 75 (83%), 28 (31%), and 47 (52%) of the patients, repectively. A significant titer rise to PT in IgG was found in 83 (92%), IgM in 29 (32%), and IgA in 44 (49%) of the patients. A significant titer rise to FHA or PT in IgG was found in 88 (98%) of the patients, in combination with a significant rise in the titer of IgA to FHA. These data were obtained in a single serum dilution of 1:500. Titrations performed later showed that the titer rise to FHA in IgG was a mean of 6.5-fold, which was significantly lower than the mean 67.0-fold rise in IgG to PT ($P < 0.001$). The mean titer of IgG to FHA in convalescent-phase serum was 270, which was also significantly lower than the mean PT titer of 2,943 ($P < 0.001$). A significant rise (≥fourfold) in PT titer by NT was found in 58 of 83 (70%) of the patients. The NT was significantly less sensitive than the ELISA for the determination of the IgG titer to PT ($P < 0.001$). Results showed that a 100% (90 of 90) sensitivity in terms of titer rises was achieved in the serologic diagnosis of pertussis by ELISA in a single-point determination of the IgG and IgA responses to FHA and of the IgG response to PT.

Serologic diagnosis of pertussis by enzyme-linked immunosorbent assay (ELISA) has been described in several studies in the past few years. A variety of antigens, e.g., whole bacteria (6), sonicate or extract of bacteria (15, 17, 19), and purified preparations of different antigens (3, 10, 11, 18, 20), have been used in patients with confirmed or suspected pertussis. In our first study (10), purified filamentous hemagglutinin (FHA) of *Bordetella pertussis* was evaluated in culture-confirmed cases. Purified preparations of FHA and pertussis toxin (PT) were used in a parallel line assay by Burstyn et al. (3). In the latter study, the use of both antigens as a diagnostic method was limited to an evaluation in two culture-confirmed cases of pertussis in adults.

We have shown previously (9) that neutralizing antibodies to PT, measured in a microplate tissue culture assay of Chinese hamster ovary (CHO) cells (7), developed during the acute phase of pertussis. The study was not designed for evaluation of the neutralization test (NT) as a diagnostic method. Such an evaluation of the NT has been presented recently (B. Trollfors, I. Krantz, N. Sigurs, J. Taranger, G. Zackrisson, R. Sekura, and R. Roberson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, E61, p. 113) in which 70% sensitivity was indicated. The NT was not compared with ELISA for immunoglobulin G (IgG) antibodies to PT in that study.

The aim of the present study was to make a systematic evaluation of antibody determination as a diagnostic tool using ELISA with highly purified preparations of FHA and PT and the NT in serum from patients with culture-confirmed pertussis.

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MATERIALS AND METHODS

Patients. The 90 patients with pertussis confirmed by culture of *B. pertussis* were seen in studies conducted at the Danderyd Hospital. The 80 children (ages, <15 years) had a median age of 3.8 years (mean, 4.3 years; range, 0.3 to 14.6 years), and the 10 adults had a median age of 35.5 years (mean, 38.5 years; range, 22.6 to 64.0 years). Of the children, 74 of 80 were unimmunized (median age, 3.6 years; range, 0.3 to 9.9 years), while 6 (ages, 6.1, 7.0, 8.4, 10.3, 13.8, and 14.6 years) had received three doses of diphtheria-tetanus-pertussis vaccine (DTP) as infants. Of 10 adults, 2 (ages, 22.6 and 27.5 years) also had a history of DTP immunization as infants, while immunization by vaccine was unknown or unlikely in the remaining adults.

Serum sampling. Serum samples from patients with pertussis were obtained with capillary blood samples from children, which were drawn in tubes (Microtainer; Beckton Dickinson Vacutainer Systems, Rutherford, N.J.), or by venous blood sampling in adults. At the first visit, a nasopharyngeal sample for culture and a blood sample were obtained. These acute-phase serum samples were drawn on mean day 10 (range, days 1 to 36) of disease. During a second visit, a convalescent-phase serum sample was taken on mean day 46 (range, days 17 to 90) after the first sample or on mean day 56 (range, days 24 to 112) after the onset of disease. The serum samples were kept at −70°C and analyzed in parallel at first by ELISA and then by NT.

Antigens for ELISA and NT. The FHA was purified by column chromatography on hydroxyapatite (HA-Ultrogel; LKB, Bromma, Sweden) essentially as described by Cowell et al. (5). The PT preparation was kindly provided by the Research Foundation for Microbiological Diseases (Biken, Osaka University, Osaka, Japan). The purity of the preparations was studied by sodium dodecyl sulfate-polyacryl-

amide gel electrophoresis and showed only the high-molecular-weight bands of FHA and the five subunits of PT, respectively. The toxin preparation used in NT was purchased from List Biological Laboratories (Campbell, Calif.). The lyophilized toxin (50 μg per vial) was constituted in 1 ml of glycerin-distilled water (1:1) and was used in a 1:1,500 dilution made in Parker 199 medium (National Bacteriological Laboratory, Stockholm, Sweden).

ELISA. Cobalt-irradiated 96-well polystyrene microplates (M129 B; Dynatech, Plochingen, Federal Republic of Germany) were coated overnight at room temperature (22°C) with FHA (2 μg/ml) and PT (1 μg/ml) diluted in phosphate-buffered saline (pH 7.2). Volumes of 100 μl were used throughout the test. The plates were washed four times between each step. Serum samples were screened by a single-point determination at a serum dilution of 1:500 and were analyzed in triplicate. Higher dilutions were made later for quantitative determination of titers by ELISA by repeated single-point determinations. The serum dilution(s) was incubated for 1 h at 22°C for IgG and IgA determinations and for 2 h at 37°C for the IgM determination (10, 13). Incubation with heavy-chain-specific alkaline phosphatase conjugates to human IgG, IgM, and IgA (Orion Diagnostica, Espoo, Finland), which were used at dilutions of 1:100, 1:50, and 1:25, respectively, was done at 22°C overnight. The substrate *p*-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, Mo.) was added at 22°C, and the reading of the microplates was done in a Titertek Multiscan apparatus (Flow Laboratories, Irvine, Scotland). The readings were made when the positive standard(s) reached a predetermined value(s); i.e., the assay was calibrated to a standard which gave substrate incubation times that varied between 45 and 65 min. The ELISA titers given represent the mean A_{405} value of the triplicate determinations of the sample multiplied by the serum dilution factor; e.g., a mean 0.4 absorbance value at a 1:500 dilution represents an ELISA titer of 200.

The intraassay coefficient of variation (CV; in percent) of the ELISA as a single-point measurement was established in 10 determinations on each sample as the standard deviation divided by the mean × 100. Two negative controls serum samples were used: a serum pool from unimmunized 6- to 8-month-old infants and an NT-negative serum sample from an adult, and an NT-positive standard was used. The CV was established to be 4, 8, and 7%, respectively. A significant rise in titer between two samples would therefore be any difference greater than 20%, but it was set at a twofold increase (100% difference) as a safety margin because of the possible oligoclonal stimulation that occurs in patients with an infectious disease and the higher expected CV in routine diagnostic assays. The interassay CVs were 28, 23, and 23% in the same samples, respectively.

NT for PT. NT was done essentially as previously described in detail (7, 9). The method was based on the observation that PT induces clustering of cells, which is inhibited by specific antibodies. Briefly, twofold dilutions of patient serum from a dilution of 1:2 were made, a predetermined 4 clustering units of PT was added, and the mixture was incubated for 3 h at 37°C. Thereafter, 10^4 CHO cells were added to each well and incubated for 48 h in a humidified CO_2 incubator. The last serum dilution giving a 100% neutralizing of the clustering effect of PT was taken as the NT titer, given as the reciprocal value. A significant rise was defined as at least a fourfold increase between two samples tested in parallel.

A modification made from the original description (7) of the assay was the use of Parker 199 medium with Hanks balanced salt solution (National Bacteriological Laboratory) during the NT, while the cells were grown in Eagle minimum essential medium with Earles salts (National Bacteriological Laboratory). The change from Eagles minimum essential medium considerably reduced the nonspecific toxicity for the cells in some serum samples, while the cells grew better on passages in Eagle minimum essential medium, possibly because of adaptation to this medium. Also, all titers were corrected against a positive standard, a pertussis immunoglobulin preparation, which was titrated in triplicate in each assay. The correction was used to decrease further the interassay variation and was made within only one titration step from the established NT titer of 2,560 of the standard. If the mean titer of the standard varied by more than one titration step from a titer of 2,560, the whole assay was repeated.

For three patients, the acute- or convalescent-phase sera were insufficient for testing by NT; and in four cases, a low-grade, nonspecific toxicity in one to three dilutions in either sample excluded a complete evaluation. A complete evaluation was thus made for 83 patients.

Statistical analysis. The mean titers and fold increases were calculated on logarithmic values of titers determined by ELISA and by NT and compared by Student's *t* test. Comparisons between groups were made by chi-square tabulation or by the Fisher exact test when appropriate.

TABLE 1. Significant titer rises (≥twofold) by ELISA in 90 patients with culture-confirmed *B. pertussis* infection

Antibody	No. (%) of patients positive by rise in titer to:	
	FHA	PT
IgG	75 (83)	83 (92)
IgM	28 (31)	29 (32)
IgA	47 (52)	44 (49)

RESULTS

Significant rise of titers by ELISA. The rate of positive samples determined by a significant rise in titer in the three antibody classes is shown in Table 1. The most sensitive method was determination of the PT titer in IgG, in which 92% (83 of 90) of the samples were positive. All patients that were determined to have positive samples by a rise in titer to PT in IgA and IgM were also found to be positive by a rise in IgG. A total of 21 of 90 (23%) patients showed rises in titer to PT in all three antibody classes. A significant rise in titer to FHA in IgG was found in 83% (75 of 90) of patients (Table 1). Of 28 patients positive by a rise in IgM to FHA, 1 was not positive by a titer rise in either IgG or IgA (but was positive by rises in titer to PT). Six patients had a significant titer rise to FHA in IgA but not in IgG. Two of these six patients had high, unchanging IgG titers and four had low (negative) titers. Sixteen (18%) patients showed rises in titer to FHA in all three antibody classes.

Antibody response by age and immunization status. Differences in the diagnostic sensitivities of the assays and antibody classes by immunization status are shown in Table 2. For the unimmunized children, a rise in IgG antibody to PT was found in 96%, versus 75% in adults and immunized children. Unimmunized children showed a significant rise of IgA to PT in 39 of 74 (53%) cases, with similar rates of 46 and 54% in children <2 years and 2 to 15 years of age, respec-

TABLE 2. Antibody responses by age and immunization status and by combination of assays and antibody classes

Immunization status and age	No. (%) of patients by rise in ELISA titer of:		
	FHA	PT	FHA or PT
Unimmunized children (n = 74)			
IgG alone	62 (84)	71 (96)	73 (99)
IgG or IgA	65 (88)	71 (96)	74 (100)
Immunized children and adults (n = 16)			
IgG alone	13 (81)	12 (75)	15 (94)
IgG or IgA	16 (100)	12 (75)	16 (100)
Totals (n = 90)			
IgG alone	75 (83)	83 (92)	88 (98)
IgG or IgA	81 (90)	83 (92)	90 (100)

tively. A rise in IgA to PT was seen in 5 of 16 (31%) immunized children and adults. A significant rise of IgA to FHA was found in 33 of 74 (45%) unimmunized children, with almost equal values of 46 and 44% in those <2 years and 2 to 15 years of age, respectively. A significant rise in IgA to FHA was seen in 14 of 16 (88%) immunized children and adults, which was significantly higher (P = 0.0032) than the 45% of unimmunized children. All 16 immunized children or adults showed significant rises in titers of FHA alone in either IgG or IgA. An IgM response in the unimmunized children of <2 years of age was found in 7 of 13 (54%) children to PT and in 4 of 13 (31%) children to FHA. In the unimmunized children of 2 to 15 years of age, the corresponding data were 20 of 61 (33%) children and 21 of 61 (34%) children, respectively. In immunized children and adults, IgM to PT was seen in 2 of 16 (13%) patients and to FHA in 3 of 16 (19%) patients.

Analysis of data by age, irrespective of immunization status, showed no significant differences. The three infants that were less than 6 months of age were positive by rises in titers to FHA and PT in IgG (data not shown). A diagnostic sensitivity of 100% by ELISA in all age groups and irrespective of immunization status was achieved by a combination of determination of IgG to PT and FHA and of IgA to FHA (Table 2).

Antibody responses in relation to disease duration. The majority of these culture-positive patients had a short disease duration (for 58% it was 1 week or less and for 87% it was 2 weeks or less) at the time of nasopharyngeal and acute-phase blood sampling (Table 3). Rises in titer were seen at all stages of disease. High, unchanging titers of IgG to PT were found earlier than they were to FHA. The two patients with high PT titers and a disease duration of 2 weeks or less were found among the 16 immunized children or adults, as was a third case with a disease duration of 22 days. The remaining patients with high PT titers were two unimmunized children with disease durations of 21 and 23 days. The two patients with high, unchanging FHA titers were immunized children with disease durations of 19 and 36 days. Of the 13 patients with low (negative) FHA titers, 12 were unimmunized children and 1 was the oldest adult (64 years). Two patients had no IgG response to PT.

TABLE 3. IgG antibody responses to FHA and PT by ELISA in relation to disease duration prior to first blood sampling

Disease duration (days)	No. of patients	No. (%) of IgG responses to[a]:					
		Sign rise	FHA		Sign rise	PT	
			High	Low		High	Low
1–7	52	44 (85)	0	8 (15)	50 (92)	1	1
8–14	25	22 (88)	0	3 (12)	24 (96)	1	0
15–21	8	5	1	2	6	1	1
22–28	3	3	0	0	1	2	0
29–35	0	0	0	0	0	0	0
36	2	1	1	0	2	0	0
Total		75 (83)	2 (2)	13 (14)	83 (92)	5 (6)	2 (2)

[a] A total of 77 (86%) patients responded to FHA, and 88 (98%) responded to PT.

TABLE 4. Antibody response in ELISA by fold rises in paired samples and by titers in convalescent-phase sera

Patient, antigen, and antibody class	Antibody responses as:	
	Fold rise (range)	Titer (range)
Unimmunized children		
FHA		
IgG	6.4 (2.0–71.0)	186 (25–5,600)
IgM	3.1 (2.0–30.0)	147 (35–655)
IgA	3.2 (2.0–9.2)	107 (35–2,760)
PT		
IgG	77.2 (3.6–2032)	2,809 (125–32,640)
IgM	3.5 (2.0–19.8)	129 (35–395)
IgA	3.3 (2.0–18.0)	113 (35–420)
Immunized children and adults		
FHA		
IgG	7.4 (2.3–139)	1,269 (25–14,420)
IgM	3.7 (3.3–4.2)	196 (100–520)
IgA	4.7 (2.0–34.1)	851 (10–5,200)
PT		
IgG	29.5 (2.1–1,984)	3,688 (630–13,600)
IgM	5.6 (5.4–5.8)	140 (135–145)
IgA	5.0 (2.1–11.6)	250 (80–520)
Totals		
FHA		
IgG	6.5 (2.0–139)	270 (25–14,420)
IgM	3.1 (2.0–30.0)	151 (35–655)
IgA	3.6 (2.0–34.1)	199 (10–5,200)
PT		
IgG	67.0 (2.1–2,032)	2,943 (125–32,640)
IgM	3.6 (2.0–19.8)	130 (35–395)
IgA	3.5 (2.0–18.0)	129 (35–520)

Height of antibody responses. Antibody responses by mean fold titer rises and ELISA titers in convalescent-phase sera are shown in Table 4. The mean fold rises were calculated for patients with significant rises in each antibody class (Table 1), while mean titers in convalescent-phase sera also included the patients with high titers (Table 3). The IgG responses to FHA were significantly lower than they were to PT in the unimmunized children, both in fold rises and titers ($P < 0.001$ for both). The mean titers of IgG to FHA were significantly lower in unimmunized children than in immunized children or adults ($P < 0.001$), while this difference in titers between the groups was not seen for IgG to PT.

IgM responses were low in fold and attained only low titers to both FHA and PT. The IgA response to PT was low

TABLE 5. Significant rise in titers by NT compared with ELISA for IgG to PT

Patients	No. of patients positive/ total no. (%) by:	
	ELISA for IgG	NT
Unimmunized children	71/74 (96)	48/68 (71)
Immunized children and adults	12/16 (75)	10/15 (67)
Total	83/90 (92)	58/83 (70)

in both groups, but the titers were significantly higher ($P < 0.001$) in the immunized group than in the unimmunized group. The mean titer of IgA to FHA and the fold rise were also significantly higher in immunized children and adults than in the unimmunized children ($P < 0.001$).

NT compared with ELISA for IgG to PT. The results of NT compared with ELISA for IgG to PT in unimmunized children and immunized children or adults are summarized in Table 5. The sensitivity of NT for the diagnosis of disease was 70%, versus 92% for IgG to PT by ELISA ($P < 0.001$). These results were obtained in the first blinded analysis of sera by NT. Of the convalescent-phase sera that scored negative (<2), many samples showed a pattern of partial neutralization in several dilution steps. On nonblinded retesting, these serum samples could be scored positive, but the titers showed wide interassay variations. If these 13 sera were counted as positive, the sensitivity of the NT test would increase to a total of 86% (71 of 83 samples), with 88% (60 of 68 samples) of the titer rises being in the unimmunized children.

Of the seven patients who were found to be entirely negative by NT, five showed significant rises in IgG titer to PT. Both titer rises (mean, 14-fold) and titers (mean, 426; range, 170 to 2,240) were lower than the corresponding mean values in NT-positive patients, although one NT-negative patient had a high titer of IgG to PT, 2,240, and also a high mean fold rise of 112. Five patients had high, unchanging titers; these were the same patients who had high titers of IgG to PT by ELISA (Table 3). Two patients, one unimmunized child (age, 2.5 years) from whom samples were taken on days 6 and 54 of disease and one adult (age 43.4 years) from whom samples were taken on days 20 and 55, were negative by NT. These patients also had low (negative) titers without a rise in IgG to PT.

The geometric mean titer by NT in the 63 convalescent-phase samples from 58 patients with titer rises and the 5 patients with high titers was 66 ± 6 (range, 4 to 8,092). The mean titer in sera from the 50 unimmunized children was 38 ± 4 (range, 4 to 1,024) and was significantly lower ($P < 0.001$) than the mean of 475 ± 5 (range, 32 to 8,092) in the 13 sera from immunized children and adults.

DISCUSSION

This study represents the first systematic evaluation of ELISA for the determination of IgG, IgM, and IgA responses to *B. pertussis* FHA and PT and of NT for antibodies to PT in a large patient population with culture-confirmed whooping cough.

The antibody response to FHA was markedly different in unimmunized children compared with that in immunized children and adults. The low IgG responses (Table 4) in unimmunized children seen in this study were also observed in our previous study in 1982 (10). At that time, it was unclear whether the difference reflected an age-related factor or a difference in immunization status, since all unimmunized children in that study were less than 2 years of age. As general immunization has been withheld in Sweden since 1979, the unimmunized children in the present study were up to school age, and we can now conclude that the difference in antibody response is a result of previous immunization, whether natural or by vaccine.

Determination of IgA to FHA was found to be an important diagnostic tool in our previous study (10), and this finding was confirmed and expanded in the present study. All 16 immunized children or adults could have been diagnosed by IgA and IgG determination to FHA alone. Although IgA responses were much lower in the unimmunized children, determination of this antibody class was of diagnostic value and necessary in order to obtain 100% sensitivity.

The IgG response to PT was the most sensitive diagnostic method in the unimmunized children. All but one of the unimmunized children with a disease duration of less than 3 weeks at the time of the first sample showed titer rises. In the immunized patients, rapid booster responses after 1 or 2 weeks of disease were noted. The results imply that diagnosis by rises in titer to PT may be difficult to obtain in a population immunized with DTP.

Antibodies of the IgM class were found in about one-third of the patients. This finding for FHA was lower than that in our previous study (10). The most likely explanation is that the IgM response measured in that study was, at least in part, directed to the small amounts of lipopolysaccharide that are present in the FHA preparation (1). IgM antibodies were most commonly found to PT in the youngest infants, but titers were low and in no case did the IgM determination increase the diagnostic sensitivity. One patient showed an IgM response to FHA without an IgG or IgA response. This child, however, was also positive by determination of the IgG response to PT. It can therefore be concluded that determination of the IgM response to PT and FHA is of little diagnostic value.

For the purposes of this study, an evaluation of the ELISA was made in patients with culture-confirmed pertussis, and titrations were made. When used in a diagnostic setting, the assay is run as a single-point determination at one dilution, i.e., 1:500. The values thus obtained do not allow for the quantitative determination of antibody responses in many convalescent-phase sera. The height of antibody responses is, on the other hand, of little interest to physicians who use serology to confirm or exclude a diagnosis of pertussis. The choice of the working dilution depends on the patient material. In our earlier study, we used a 1:1,000 dilution, since the majority of patients were previously immunized children or adults (10). Unimmunized children were also tested with a dilution of 1:500. When pertussis was seen mostly in unimmunized children, a lower working dilution was preferred. It is even likely that some of the unimmunized children with no rises in titer of IgG to FHA could have shown such a response at an even lower dilution, e.g., 1:100. This dilution would be too low for diagnosis in immunized children and adults, however.

The single-point determination at a low dilution is both practical and simple for routine diagnostic testing and ensures a high diagnostic sensitivity. ELISA measures not only changes in the amount of antibody but also in the affinity of the antibody during immunization, natural or experimental (4, 16). As shown by Lehtonen et al. (16), the change in affinity is more pronounced at low dilutions in serum than at high dilutions, e.g., endpoint titers. This gain in sensitivity

may be of little importance in DTP-immunized children or adults with high antibody responses to FHA. In unimmunized children with low fold increases and low titers, as shown in the present study, this difference may be crucial.

The NT was significantly less sensitive for the diagnosis of disease than the determination of the IgG response to PT by ELISA. Since the method is also more laborious, requires tissue culture facilities, and involves subjective readings, it is of little value as a diagnostic method. The partial neutralization and the low degree of reproducibility of determination in some convalescent-phase sera were difficulties that were unexpected based on results of earlier studies (2, 8, 9, 12). The difference in NT results between the present study and the earlier study on clinical pertussis (9) could be due to the immunization status of the children in the two studies, i.e., children who, for the most part, were immunized (9) and those who were unimmunized (present study). This conclusion is sustained by the significantly lower titers by NT in unimmunized children, a difference that was not noted in our previous study with a limited number of unimmunized children. An antitoxin response of any grade was observed in 76 of 83 (91%) patients in this study. In our previous study (9), we found an antitoxin response in 36 of 38 (95%) patients with culture-confirmed disease. These data now seem to firmly establish that not all patients develop measurable neutralizing antibodies after clinical and culture-confirmed whooping cough.

Determination of the IgG response to FHA and PT and of the IgA response to FHA gave a 100% sensitivity in terms of significant titer rises in patients with culture-confirmed disease. However, we want to warn against an extrapolation of these extremely favorable results to culture-negative patients when serology is needed. Any new method must be evaluated against a reference method, which, in the case of pertussis, is culturing of *B. pertussis* from nasopharyngeal samples. Culturing is highly specific but it has a decreasing sensitivity with disease duration (14). A short disease duration also yields the ideal early-acute-phase sample for serology. The combination of this favorable bias introduced by the reference method, the slow, primary antibody responses of unimmunized children, and the sampling of convalescent-phase sera taken a mean of 6 weeks after the acute-phase sample in this study must all have contributed to the high diagnostic sensitivity of the test.

In a routine diagnostic setting, a physician in charge of a patient suspected of having pertussis is often unwilling to wait many weeks to draw the convalescent-phase serum sample. Another diagnostic problem is represented by the unimmunized child with several weeks of disease and low titers of both FHA and PT. Such low-titer responses were seen in convalescent-phase sera from seven unimmunized children in this study. These patients could, without an early-acute-phase sample with titer rises, have been erroneously scored as negative, reducing the diagnostic sensitivity from 100 to 91%. The greatest diagnostic problem, however, can be expected in children and adults previously immunized with DTP, especially with the low awareness of pertussis in the immunized population. The rapid secondary antibody responses result in high titers in the acute-phase samples drawn after several weeks of disease (10). For serologic diagnosis in these patients, the upper limits of normal values must be established in a healthy age-matched population. The specificity can then be determined by the arbitrary choice of a cutoff limit, in which all gains in specificity are at the expense of losses in sensitivity. The sensitivity of a serologic assay with a reasonable specificity in such a setting can never be even close to 100%.

In conclusion, the combination of IgG determination to *B. pertussis* FHA and PT and of IgA determination to FHA by ELISA is sufficient for the serologic diagnosis of whooping cough. Under ideal conditions, the assay can reach a 100% diagnostic sensitivity. The NT for antibodies to PT was significantly less sensitive than determination of the IgG response to PT by ELISA and was found to be of little diagnostic value.

ACKNOWLEDGMENTS

This study was supported by contract no. 200-84-0752 from the Centers for Disease Control, Atlanta, Ga., for the development and evaluation of diagnostic methods for pertussis.

We thank Lorita Jansson, Marie Thorén, Ingrid Mattsson, and Eivor Bonin for technical assistance and Per Askelöf for the FHA preparation.

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