JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1990, p. 32–38
0095-1137/90/ 010032-07$02.00/0

Vol. 28, No. 1

Use of a Chinese Hamster Ovary Cell Cytotoxicity Assay for the Rapid Diagnosis of Pertussis

S. A. HALPERIN,[1,2]* R. BORTOLUSSI,[1,2] A. KASINA,[1] AND A. J. WORT[2]

Departments of Pediatrics[1] and Microbiology,[2] Dalhousie University and The Izaak Walton Killam Hospital for Children, Halifax, Nova Scotia, Canada B3J 3G9

Received 23 June 1989/Accepted 25 September 1989

A cytotoxicity assay with Chinese hamster ovary cells (CHO) capable of detecting 750 pg of pertussis toxin was assessed for use as a rapid test for the diagnosis of pertussis and compared with direct immunofluorescence (DFA). With pure bacterial cultures and simulated clinical specimens, the CHO assay detected as few as two colonies of *Bordetella pertussis*; no cytotoxicity occurred with other respiratory tract microorganisms. Next, nasopharyngeal aspirate secretions and nasopharyngeal cultures harvested after 72 h of incubation from 57 culture-positive and 201 culture-negative patients were examined. The CHO assay with nasopharyngeal secretions was positive in 25 (45%) of 55 culture-positive cases; DFA was positive in 15 (26%) of 57 cases ($P = 0.05$). The CHO assay with 72-h culture washes was positive in 42 (75%) of 57 culture-positive cases ($P < 0.001$ compared with DFA). The CHO assay was more specific than DFA; all five CHO-positive, culture-negative cases were confirmed as true positives by serologic or toxin neutralization assays. In contrast, only 4 (36%) of 11 DFA-positive, culture-negative cases were confirmed as pertussis by serologic methods ($P = 0.03$). Combining the CHO assay with culture significantly decreased the delay in laboratory diagnosis of pertussis (3.30 versus 4.54 days; $P = 0.01$). The CHO assay is a sensitive and specific assay for the rapid diagnosis of pertussis.

Pertussis is an acute disease of the respiratory tract characterized by progressive, repetitive, paroxysmal coughing, mild systemic complaints, and lymphocytosis. The disease, caused by *Bordetella pertussis*, proceeds through a catarrhal and a paroxysmal phase of 1 to 2 weeks duration each before entering a prolonged convalescent phase. The laboratory diagnosis of pertussis has been problematic owing to the pathogenesis of the infection and the fastidious nature of the causative bacterium. Nasopharyngeal culture has been the standard for laboratory diagnosis, but the test lacks sensitivity, particularly later in the course of the disease (24). Isolation of *B. pertussis* can be achieved most consistently in the catarrhal or early paroxysmal phase, often before the diagnosis of pertussis is suspected (22). Even when positive, culture confirmation is often delayed owing to the slow growth of the bacteria in culture and to overgrowth of normal respiratory flora. Direct immunofluorescence (DFA) with nasopharyngeal secretions can provide rapid laboratory confirmation of pertussis, but the test lacks sensitivity and false-positives are common (2, 18). Problems with the commercially available reagents, nonspecific fluorescence, and interobserver variability are most often cited as the causes of the unreliability of the test.

Serologic methods have proved to be the most sensitive for the laboratory diagnosis of pertussis. Measurement of antibodies to the whole organism or its virulence factors in serum (1, 14, 17, 18, 23) or nasopharyngeal secretions (4, 11, 12) have permitted laboratory confirmation in up to 70% of cases. Optimal sensitivity, however, is only achieved with paired specimens taken several weeks apart; results on single acute specimens have not been as satisfactory (7, 15, 18, 31, 34).

Pertussis toxin (PT), a biologically active protein produced by *B. pertussis*, has been proposed as the primary cause of the harmful effects of pertussis (25) and as an important factor in immunity to whooping cough (29). Also known as lymphocytosis-promoting factor, histamine-sensitizing factor, and islet-activating factor, PT is a prototypic A-B toxin composed of an enzymatically active A monomer and a B oligomer essential for binding of the toxin to the cell and delivering the A subunit into the cell. The toxin, which catalyzes the ADP-ribosylation of a membrane-bound GTP-binding regulatory protein (32), induces a distinctive cytopathic effect in Chinese hamster ovary cells (CHO) (20). The assay is exquisitely sensitive detecting <1 ng of PT per ml (8, 9). This cytopathic effect can be neutralized with antibodies of PT and has been used to assess monoclonal antibodies to PT (28) and to measure antitoxin response following whooping cough (13, 33).

The purpose of the present investigation was to assess the usefulness of the CHO assay for a more rapid diagnosis of pertussis. Direct CHO assays with nasopharyngeal secretions and assays with cultures harvested after 3 days of incubation were compared with standard culture and DFA of nasopharyngeal secretions.

MATERIALS AND METHODS

Bacteria. *B. pertussis* Tohama and *B. pertussis* 165 were kindly provided by Charles Manclark at the Center for Drugs and Biologics, Food and Drug Administration, Bethesda, Md. *Bordetella parapertussis*, *Escherichia coli*, *Haemophilus influenzae* type b, *Streptococcus pyogenes* group A, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and viridans group streptococci were obtained from clinical upper respiratory tract specimens. *B. pertussis* was cultured on either Bordet-Gengou medium (Difco Laboratories, Detroit, Mich.) supplemented with 15% defibrinated horse blood or charcoal agar CM 119 (Oxoid Ltd., London, England) supplemented with 5% horse blood. Plates were incubated at 36°C in a humid environment. Bacteria to be

* Corresponding author.

tested were harvested by scraping the agar surface with a glass hockey stick with 1 ml of phosphate-buffered saline with 1% Casamino Acids (PBS-CAA) (Difco Laboratories).

Specimen collection and processing. Nasopharyngeal aspirate specimens (NPA) submitted for the diagnosis of pertussis were handled as previously described (18). Briefly, NPA were obtained by syringe aspiration through a fine flexible catheter passed through the nose into the nasopharynx, and a blood sample was obtained by fingerstick. Arrangements were made to collect a 3-week follow-up serum specimen. Blood and the NPA still in the catheter were immediately transported from the outpatient diagnostic laboratory to the microbiology laboratory of the Izaak Walton Killam Hospital for Children. There, serum was separated and stored at −20°C. Secretions were expelled onto duplicate sets of charcoal-horse blood agar plates with (C+) or without (C−) cephalexin (40 μg/ml) (27) and onto two clear glass slides for immunofluorescence. NPA remaining in the catheter were then collected by rinsing the catheter with 1 ml of PBS-CAA, and the solution was stored frozen at −70°C. Smears were processed by treatment with aprotinin (4 U/ml) (Sigma Chemical Co., St. Louis, Mo.) and were stored at 4°C until examination (19). Culture plates were incubated at 36°C in a humid environment; one set was visually examined daily for 7 days for colonies typical of *B. pertussis*. Colonies with typical morphology were examined by Gram stain, and organisms with characteristic gram-negative morphology were confirmed by agglutination as previously described (18). The second set of cultures was incubated at 36°C for 3 days, and an early culture wash (ECW) was obtained by flooding each plate (ECW [C+] or ECW [C−]) with 1 ml of PBS-CAA and scraping the agar surface with a glass hockey stick. ECW were stored frozen at −70°C until tested.

Simulated clinical specimens were prepared from NPA obtained from healthy laboratory personnel. Suspensions of *B. pertussis* or *B. parapertussis* were inoculated into the NPA obtained from these volunteers and processed as described above. ECW were also prepared from cultures of NPA from patients with normal respiratory flora including streptococci of the viridans group, *S. aureus* and coagulase-negative staphylococci, *Neisseria* species, *S. pyogenes* group A, *H. influenzae*, and *S. pneumoniae*.

CHO cell cytotoxicity assay. The CHO cell assay was performed by the method of Hewlett et al. (20). CHO cells (CCL61; American Type Culture Collection, Rockville, Md.) grown to confluence were trypsinized and diluted to 3×10^4 cells per ml in F-12 medium (Flow Laboratories, Mississauga, Ontario, Canada) supplemented with 10% fetal calf serum, 0.05% mercaptoethanol, and 1% penicillin and streptomycin (Sigma), and 250-μl samples were added to each well of a polystyrene microdilution plate (Nunc, Roskilde, Denmark). After 18 to 24 h at 37°C to permit cell attachment to the plates, 50 μl of the material to be tested was added to triplicate wells. NPA and ECW were first centrifuged at 8,800 × *g* for 15 min, heat inactivated at 56°C for 40 min, and recentrifuged for 15 min. PT purified by the method of Sekura et al. (30) was used as a positive control; PBS served as a negative control. For neutralization studies, equal volumes of the test material and a monoclonal antibody to the S1 subunit of PT were mixed at 22°C for 30 min, and 50 μl was added to the microdilution wells. After 20 h of incubation at 37°C in a CO_2 incubator, the culture medium was removed and the cells were fixed with methanol and stained with 2.5% Giemsa stain for 20 min. The plates were coded and read by a single observer who was unaware of the culture results. Cytotoxicity was scored as 0, ±, 1+, or 2+; values of 1+ or 2+ were considered positive. The assay was able to detect 750 pg of PT per ml.

Immunofluorescence and serology. Direct smears of NPA were examined as previously described (18). All slides were examined by a single observer who was unaware of whether the smear was from a patient or was a positive or negative control. Serum antibodies of PT and filamentous hemagglutinin were measured by an enzyme immunoassay (18). A fourfold rise in immunoglobulin G (IgG), IgA, or IgM or a fourfold fall in IgM was interpreted as a positive test.

Data analysis and ethical considerations. The protocol for this study was approved by the ethical review committee of the Izaak Walton Killam Hospital for Children. Sensitivity, specificity, and positive and negative predictive values were determined by standard methods (16, 26). Differences in proportions were tested by chi square with Yates' correction for contingency tables with 1 df or by Fisher's exact test. Differences in means were compared by Student's *t* test. All statistical calculations were performed with a computer-programmed statistics package (Stats Plus; Human Systems Dynamics, North Ridge, Calif.) on an Apple IIC personal computer (Apple Computers, Cupertino, Calif.); $P \leq 0.05$ was considered statistically significant.

RESULTS

CHO assay with pure bacterial cultures. Typical clustered morphology occurred only after incubation with *B. pertussis* (Fig. 1B) or with the PT control. No cytotoxicity occurred with undiluted supernatants from heavily inoculated cultures of *H. influenzae* (Fig. 1C), *B. parapertussis* (Fig. 1D), or other microorganisms commonly found in the upper respiratory tract (Table 1).

The sensitivity of the CHO assay was related to the number of bacteria present in the original specimen and the duration of bacterial growth before culture harvest. After 24 h of incubation, the CHO assay was unable to detect as many as 24 colonies (Table 2). However, after 48 and 72 h of incubation, an original inoculum of 9 to 14 and 2 bacteria (respectively) were all that were necessary for a positive reaction. Bacterial colonies were not visible when the cultures were harvested at 24 or 48 h; in heavily inoculated cultures, bacterial growth was faintly visible by 72 h. Serial dilutions were performed on the 48-h harvests to determine the number of bacteria present in 2, 9, or 24 colonies. These 48-h cultures contained 1.1×10^5, 1.6×10^6, and 1.1×10^7 CFU/ml respectively; the CHO assay demonstrated cytopathic morphology in the latter two specimens.

CHO assay on simulated clinical specimens. NPA and ECW from healthy volunteers induced no morphologic changes in the CHO cell assay. Positive cultures simulated by inoculating serial dilutions of *B. pertussis* into NPA from healthy volunteers demonstrated no loss of sensitivity in the CHO assay compared to pure *B. pertussis* cultures (Table 2). Despite the presence of contaminating normal respiratory flora, as few as two *B. pertussis* colonies were detected by the CHO assay after 48 or 72 h of incubation; no colonies of *B. pertussis* were visible at either time.

CHO assay with clinical specimens. Specimens from 57 *B. pertussis* culture-positive and 201 culture-negative patients were assayed by the CHO assay and DFA (Table 3). The CHO assay with NPA was positive in 25 of 55 culture-positive specimens (sensitivity, 45%) compared with 15 of 57 specimens assayed by DFA (sensitivity, 26%; $P = 0.05$). The CHO assay was positive in 42 ECW; the sensitivity of 75% was significantly higher than either DFA ($P < 0.001$) or CHO

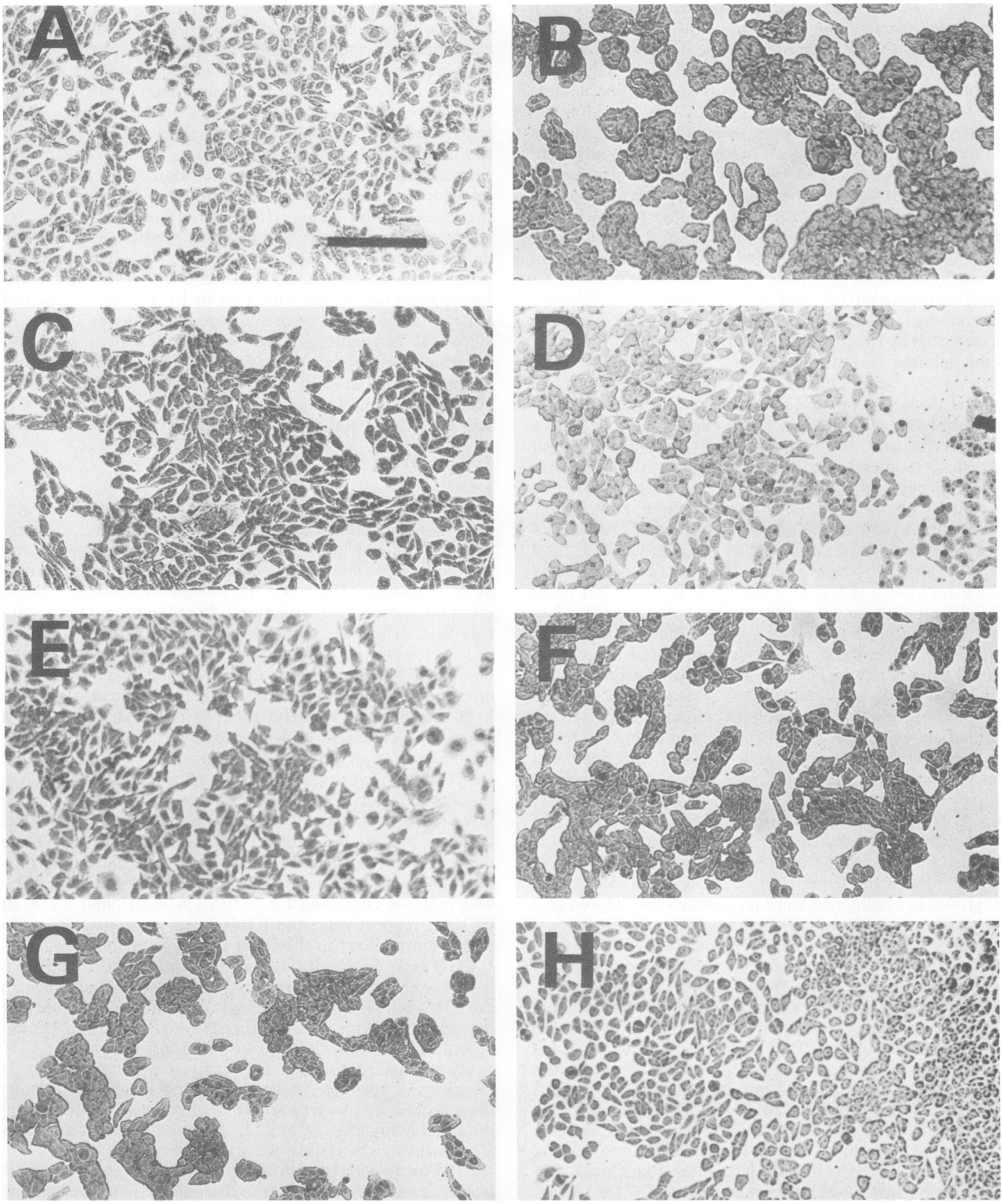

FIG. 1. Photomicrographs of CHO cell morphology after exposure for 20 h to a variety of material and fixation with 2.5% Giemsa. (A) Normal CHO cell monolayer. Supernatants of pure cultures of *B. pertussis* (B), *H. influenzae* (C), and *B. parapertussis* (D). NPA from a culture-negative (E) and culture-positive (F) patient. (G) Positive ECW. (H) Positive ECW neutralized by PT monoclonal antibody. Magnifications of ×190. Bar, 0.1 mm.

TABLE 1. Specificity of the CHO cell assay with culture supernatants of commonly isolated respiratory bacterial flora

Bacteria	Log CFU/ml[a]	CHO cytotoxicity
Streptococcus pneumoniae	6.51	−
Streptococcus pyogenes	9.60	−
Viridans group streptococci	6.51	−
Staphylococcus aureus	11.04	−
Coagulase-negative staphylococci	10.60	−
Neisseria spp.	7.97	−
Haemophilus influenzae	8.48	−
Escherichia coli	10.71	−
Bordetella parapertussis	9.81	−
Bordetella pertussis	5.04	−
	6.20	+
	7.04	+

[a] In 24-h or 48-h (*B. pertussis*) cultures harvested and titered by serial dilution. Undiluted culture supernatants were tested by the CHO cell assay.

with NPA ($P = 0.003$). CHO with ECW from culture medium containing cephalexin was superior to routine charcoal blood agar. All culture-positive specimens positive by CHO with NPA were also positive by CHO with ECW. All culture-positive specimens positive by CHO with ECW from medium without cephalexin were also positive from medium with cephalexin.

DFA was significantly less specific than either CHO with NPA ($P = 0.04$) or CHO with ECW ($P = 0.04$) (Table 3). DFA had a lower positive predictive value than CHO with either NPA ($P = 0.02$) or ECW ($P < 0.001$) and a lower negative predictive value than CHO with ECW ($P < 0.001$).

Paired serum specimens were available from 71% of patients with a positive test for pertussis. In 11 (52%) of 21 DFA-positive patients in whom paired sera were available, there was serologic evidence of pertussis by enzyme immunoassay. This was significantly less frequent than patients positive by NPA culture (33 [80%] of 41; $P < 0.001$), CHO with NPA (19 [95%] of 20; $P = 0.006$), or CHO with ECW (28 [88%] of 32; $P = 0.01$).

Using nasopharyngeal culture as the "gold standard," there were 13 "false-positive" specimens by DFA and 5 by CHO. Paired serum specimens were available in 11 of the 13 DFA false-positives; 4 (36%) of the 11 were confirmed as pertussis by demonstrating a significant change in antibody titer by enzyme immunoassay. All three of the CHO false-positives from whom sera were available demonstrated an antibody response diagnostic for pertussis. All five CHO-positive, culture-negative specimens were neutralized by a monoclonal PT antibody (Fig. 1H). One of the five patients who was culture negative and CHO positive had a repeat culture within 48 h which was culture and CHO positive.

In contrast to the culture-positive, CHO-positive specimens, all CHO assay types (NPA, ECW [C+], ECW [C−]) were required to detect the culture-negative, CHO-positive patients. Of the five culture-negative, CHO-positive patients, one was CHO positive only on NPA, while another was positive only by CHO with ECW. Two of the four culture-negative, CHO-ECW-positive patients had positive ECW with cephalexin-containing medium, while the other two were positive only on cephalexin-deficient medium.

The time to laboratory confirmation was significantly reduced with the DFA or CHO assay (Table 4). Combining the DFA or CHO assay with culture increased the rapidity of laboratory diagnosis without the loss of sensitivity which occurred by exclusive use of the rapid tests. All combinations of rapid tests and culture were significantly faster than culture alone. The combination of CHO assays and culture was significantly quicker than DFA and culture (3.30 versus 4.54 days; $P = 0.01$).

TABLE 2. Sensitivity of the CHO cell assay with pure cultures and simulated clinical specimens after 1 to 3 days of incubation

B. pertussis strain	No. of colonies[a]	CHO cell cytotoxicity results					
		Pure cultures assayed after:			Simulated clinical specimens assayed after:		
		24 h	48 h	72 h	24 h	48 h	72 h
165	200	NA[b]	+	+	NA	+	+
	109	NA	NA	NA	−	+	+
	22	NA	+	+	NA	+	+
	14	−	+	+	NA	NA	NA
	7	NA	NA	NA	−	+	+
	3	−	−	+	NA	NA	NA
	2	NA	−	+	NA	+	−
Tohama	350	NA	+	+	NA	+	+
	35	NA	+	+	NA	+	+
	24	−	+	+	NA	NA	NA
	9	NA	+	NA	NA	NA	NA
	2	−	−	+	NA	+	+

[a] Number of bacteria in original specimen as assessed in duplicate cultures, counted after 5 days of incubation.
[b] NA, Not assayed.

DISCUSSION

This study demonstrated that the CHO assay is a sensitive and specific test for the rapid diagnosis of pertussis. Cytotoxicity assays for the detection of bacterial toxins in clinical specimens have become standard laboratory tools. Assays have been developed to detect *Clostridium difficile* toxin in pseudomembranous colitis (6) and verotoxin produced by *E. coli* O157 in cases of hemolytic uremic syndrome (21). The exquisite sensitivity of CHO cell monolayers to PT and the distinctness of the morphologic changes produced make the CHO assay an attractive candidate for use as a pertussis diagnostic test. This study demonstrated that the CHO assay can be used to detect PT both in nasopharyngeal secretions and in early harvests of nasopharyngeal cultures.

The development of a rapid and reliable test for the laboratory diagnosis of pertussis has remained elusive. Nasopharyngeal culture, the standard by which all tests are judged owing to its absolute specificity, is slow and insensitive (24). DFA is the most frequently used rapid diagnostic test but also lacks sensitivity (2, 18). The specificity of DFA is also questioned; false-positive rates as high as 40% have been reported, presumably because of inadequate reagents, interobserver unreliability, and autofluorescence of respiratory tract mucus (2, 5, 10, 18).

CHO cell monolayers are sensitive to picogram quantities of PT (8, 9, 20). Using pure cultures, we demonstrated that sufficient PT is produced after 48 h by two colonies of *B. pertussis*, containing 1.6×10^6 CFU/ml, to induce morphologic changes in CHO cells. This effect was specific for *B. pertussis*; no cytopathic effect was induced by respiratory secretions or other respiratory tract microorganisms, nor did the presence of other organisms interfere with the cytotoxic effect produced by *B. pertussis* in mixed culture. The data also showed, for the first time, that sufficient free PT is present in the nasopharynx of patients with pertussis to induce the morphologic changes in CHO cells.

TABLE 3. Comparison of the CHO cell assay in nasopharyngeal secretions and ECW and direct immunofluorescence on nasopharyngeal secretions from 57 *B. pertussis* culture-positive and 201 culture-negative patients

Laboratory test and specimen	No. of NPA culture results		% Sensitivity	% Specificity	% predictive value		% False-positive	% False-negative
	Positive (n = 57)	Negative (n = 201)			Positive	Negative		
DFA-NPA								
Positive	15	13	26	94	54	82	6	74
Negative	42	188						
CHO-Total[a]								
Positive	42	5	74	98	89	93	2	26
Negative	15	196						
CHO-NPA								
Positive	25	4	45	98	86	87	2	55
Negative	30	197						
Not assayed	2	0						
CHO-ECW (total)[b]								
Positive	42	4	75	98	91	93	2	25
Negative	14	196						
Not assayed	1	1						
CHO-ECW (C+)								
Positive	42	2	75	99	95	93	1	25
Negative	14	198						
Not assayed	1	1						
CHO-ECW (C−)								
Positive	32	2	58	99	94	90	1	42
Negative	23	198						
Not assayed	2	1						

[a] Includes CHO assay with NPA secretions and ECW.
[b] Includes CHO assays with ECW from cultures with (ECW [C+]) and without (ECW [C−]) cephalexin.

The CHO assay proved superior to DFA in both ease of performance and interpretation. Specimen preparation only required heat sterilization and clarification by centrifugation. Filter sterilization, which would have been much quicker, caused unacceptable loss of sensitivity owing to binding of PT by the filters (data not shown). Difficulties with test interpretation were considerably less common with the CHO assay because the presence of small quantities of PT give rise to a full morphologic response in the CHO monolayer. The morphologic changes that occur are readily distinguishable from those caused by other toxins or nonspecific cellular damage (20) and were always neutralized by PT antibody. The CHO assay was also preferable to DFA because of its better sensitivity, specificity, and positive and negative predictive values. False-positives were more common with DFA and were less likely to be confirmed as true positives by serologic methods. In contrast, "false-positives" in the CHO assay were all derived from patients who had positive pertussis serology and CHO neutralization.

TABLE 4. Comparison of time to diagnosis in 57 *B. pertussis* culture-positive patients by laboratory method used

Laboratory test(s) used	No. of patients	Mean (range) days to diagnosis	Statistical significance (P) compared with culture alone[a]
Culture	57	5.84 (3–10)	
DFA	15	0.5	<0.001
CHO-NPA	26	1.0	<0.001
CHO-ECW	42	4.0	<0.001
Culture + DFA	57	4.54 (0.5–10)	0.002
Culture + CHO-NPA + CHO-ECW	57	3.30 (1–10)	<0.001
Culture + CHO-NPA	57	3.89 (1–10)	<0.001
Culture + CHO-ECW	57	4.58 (3–10)	<0.001

[a] Unpaired t test.

The use of the CHO assay in conjunction with nasopharyngeal culture reduced by 43% the delay in laboratory diagnosis of pertussis. The data obtained with pure cultures and simulated clinical specimens suggest that further reduction might be possible by harvesting cultures after 2 days; however, the effect on sensitivity will need to be examined. The CHO assay was unable to substitute for culture without significant loss of sensitivity; however, the use of both tests increased the number of laboratory-confirmed cases. The lower sensitivity of the CHO assay on NPA relative to culture may be related to the quality of the specimens available since material used for CHO was considerably more dilute than material used for culture. Catheters containing nasopharyngeal secretions were inoculated onto media for standard culture, then slides for DFA, then onto duplicate cultures, and finally rinsed with PBS-CAA. Thus, cultures used for ECW were secondary inoculations, and the CHO assay with NPA was performed on a rinse after all other procedures had been performed. The 75 and 45% sensitivities of the CHO assay on ECW and NPA, respectively, likely represent the minimum sensitivities of these assays.

One disadvantage of the CHO assay as a rapid diagnostic test is that, like DFA, bacteria must be present in the

nasopharynx. PT is an extracellular protein produced by virulent strains of *B. pertussis* (25) that binds to target cells by its B oligomer, allowing the enzymatically active A unit to exert its effect through ADP-ribosylation (32). Once bound, the toxin is unlikely to be detectable in secretions by the CHO assay, which requires the presence of the holotoxin (3). After clearance of the bacteria from the nasopharynx, the CHO assay is unlikely to aid in establishing the diagnosis. One potential advantage of the CHO assay is enabling the diagnosis of cases of pertussis that are culture negative, despite the presence of bacteria in the nasopharynx. This situation may occur because of previous immunization or antibiotic therapy, overgrowth in culture by normal respiratory flora, or difficulties with bacterial viability caused by specimen collection, transport, or culture (24). Symptoms characteristic of pertussis do not occur until the paroxysmal phase when the bacteria are less reliably recovered from the nasopharynx. Thus, specimens are often obtained for diagnosis after the organism has been eliminated. The CHO assay, like DFA and culture, depends on the presence of the bacteria and therefore will be useful only early in the clinical course. Other assays, such as enzyme immunoassays that detect antibody against PT or filamentous hemagglutinin in nasopharyngeal secretions, may be of greater benefit later in the course of infection (12).

The results of this study demonstrated that the CHO cell assay is a sensitive and specific test that can augment both the speed and reliability of the diagnosis of pertussis when used with nasopharyngeal culture. The CHO cell assay is a suitable alternative to DFA for the rapid diagnosis of pertussis. Improved specimen collection for use in the CHO cell assay may permit future substitution of this assay for nasopharyngeal culture, although further comparative studies are required. Diagnostic tests which are not dependent on the presence of the pathogen will be needed for the diagnosis of pertussis later in the course of the illness.

ACKNOWLEDGMENTS

This research was supported by National Health Research and Development grants 6603-1244-54 and 6603-1245-54 from Health and Welfare Canada.

We thank the staff of the Izaak Walton Killam Hospital for Children diagnostic test centre and clinical microbiology laboratory for the collection and processing of specimens and Nancy Steel for preparation of the manuscript.

LITERATURE CITED

1. **Askelöf, P., M. Granström, P. Gillenius, and A. A. Lindberg.** 1982. Purification and characterisation of a fimbrial haemagglutinin from *Bordetella pertussis* for use in an enzyme-linked immunosorbent assay. J. Med. Microbiol. **15**:73–83.
2. **Broome, C. V., D. W. Fraser, and W. J. English.** 1978. Pertussis—diagnostic methods and surveillance, p. 19–22. *In* C. R. Manclark and J. C. Hill (ed.), International Symposium on Pertussis. National Institutes of Health, Bethesda, Md.
3. **Burns, D. L., J. G. Kenimer, and C. R. Manclark.** 1987. Role of the A subunit of pertussis toxin in alteration of Chinese hamster ovary cell morphology. Infect. Immun. **55**:24–28.
4. **Campbell, P. B., P. L. Masters, and E. Rohwedder.** 1988. Whooping cough diagnosis: a clinical evaluation of complementing culture and immunofluorescence with enzyme-linked immunosorbent assay of pertussis immunoglobulin A in nasopharyngeal secretions. J. Med. Microbiol. **27**:247–254.
5. **Chalvardjian, N.** 1966. The laboratory diagnosis of whooping cough by fluorescent antibody and by culture methods. Can. Med. Assoc. J. **95**:263–266.
6. **Chang, T., M. Lauermann, and J. G. Bartlett.** 1979. Cytotoxicity assay in antibiotic-associated colitis. J. Infect. Dis. **140**: 765–770.
7. **Conway, S. P., A. H. Balfour, and H. Ross.** 1988. Serologic diagnosis of whooping cough by enzyme-linked immunosorbent assay. Pediatr. Infect. Dis. J. **7**:570–574.
8. **Fujiwara, H., and S. Iwasa.** 1989. The quantitative assay of the clustering activity of the lymphocytosis-promoting factor (pertussis toxin) of *Bordetella pertussis* on Chinese hamster ovary (CHO) cells. J. Biol. Stand. **17**:53–64.
9. **Gillenius, P., E. Jäätmaa, P. Askelöf, M. Granström, and M. Tiru.** 1985. The standardization of an assay for pertussis toxin and antitoxin in microplate culture of Chinese hamster ovary cells. J. Biol. Stand. **13**:61–66.
10. **Gilligan, P. H., and M. C. Fisher.** 1984. Importance of culture in laboratory diagnosis of *Bordetella pertussis* infections. J. Clin. Microbiol. **20**:891–893.
11. **Goodman, Y. E., A. J. Wort, and F. L. Jackson.** 1981. Enzyme-linked immunosorbent assay for detection of pertussis immunoglobulin A in nasopharyngeal secretions as an indicator of recent infection. J. Clin. Microbiol. **13**:286–292.
12. **Granström, G., P. Askelöf, and M. Granström.** 1988. Specific immunoglobulin A to *Bordetella pertussis* antigens in mucosal secretion for rapid diagnosis of whooping cough. J. Clin. Microbiol. **26**:869–874.
13. **Granström, M., G. Granström, P. Gillenius, and P. Askelöf.** 1985. Neutralizing antibodies to pertussis toxin in whooping cough. J. Infect. Dis. **151**:646–649.
14. **Granström, M., G. Granström, A. Lindfors, and P. Askelöf.** 1982. Serologic diagnosis of whooping cough by an enzyme-linked immunosorbent assay using fimbrial hemagglutinin as antigen. J. Infect. Dis. **146**:741–745.
15. **Granström, G., B. Wretlind, C.-R. Salenstedt, and M. Granström.** 1988. Evaluation of serologic assays for diagnosis of whooping cough. J. Clin. Microbiol. **26**:1818–1823.
16. **Griner, P. F., R. J. Mayewski, A. I. Mushlin, and P. Greenland.** 1981. Selection and interpretation of diagnostic tests and procedures. Ann. Intern. Med. **94**:553–600.
17. **Hakansson, S., C. G. Sundin, M. Granström, and B. Gastrin.** 1984. Diagnosis of whooping cough—a comparison of culture, immunofluorescence and serology with ELISA. Scand. J. Infect. Dis. **16**:281–284.
18. **Halperin, S. A., R. Bortolussi, and A. J. Wort.** 1989. Evaluation of culture, immunofluorescence, and serology for the diagnosis of pertussis. J. Clin. Microbiol. **27**:752–757.
19. **Harris, P. P., B. Thomason, and R. M. McKinney.** 1980. Preservation of nasopharyngeal smears for fluorescent antibody detection of *Bordetella pertussis*. J. Clin. Microbiol. **12**:799–801.
20. **Hewlett, E. L., K. T. Sauer, G. A. Myers, J. L. Cowell, and R. L. Guerrant.** 1983. Induction of a novel morphological response in Chinese hamster ovary cells by pertussis toxin. Infect. Immun. **40**:1198–1203.
21. **Karmali, M. A., M. Petric, C. Lim, P. C. Fleming, G. S. Arbus, and H. Lior.** 1985. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J. Infect. Dis. **151**:775–781.
22. **Manclark, C. R., and J. L. Cowell.** 1984. Pertussis, p. 69–106. *In* R. Germanier (ed.), Bacterial vaccines. Academic Press, Inc., Orlando, Fla.
23. **Mertsola, J., O. Ruuskanen, T. Kuronen, and M. K. Viljanen.** 1983. Serologic diagnosis of pertussis: comparison of enzyme-linked immunosorbent assay and bacterial agglutination. J. Infect. Dis. **147**:252–257.
24. **Onorato, I. M., and S. G. F. Wassilak.** 1987. Laboratory diagnosis of pertussis: the state of the art. Pediatr. Infect. Dis. J. **6**:145–151.
25. **Pittman, M.** 1979. Pertussis toxin: the cause of the harmful effects and prolonged immunity of whooping cough. A hypothesis. Rev. Infect. Dis. **1**:401–412.
26. **Radetsky, M., and J. K. Todd.** 1984. Criteria for the evaluation of new dignostic tests. Pediatr. Infect. Dis. J. **3**:461–466.
27. **Regan, J., and F. Lowe.** 1977. Enrichment medium for the isolation of *Bordetella*. J. Clin. Microbiol. **6**:303–309.
28. **Sato, H., Y. Sato, A. Ito, and I. Ohishi.** 1987. Effect of

monoclonal antibody to pertussis toxin on toxin activity. Infect. Immun. **55**:909–915.

29. **Sato, Y., K. Izumiya, H. Sato, J. L. Cowell, and C. R. Manclark.** 1981. Role of antibody to leukocytosis-promoting factor hemagglutinin and to filamentous hemagglutinin in immunity to pertussis. Infect. Immun. **31**:1223–1231.
30. **Sekura, R. D., F. Fish, C. R. Manclark, B. Meade, and Y. L. Zhang.** 1983. Pertussis toxin: affinity purification of a new ADP-ribosyltransferase. J. Biol. Chem. **258**:14647–14651.
31. **Steketee, R. W., D. G. Burstyn, S. G. F. Wassilak, W. N. Adkins, M. B. Polyak, J. P. Davis, and C. R. Manclark.** 1988. A comparison of laboratory and clinical methods for diagnosing pertussis in an outbreak in a facility for the developmentally disabled. J. Infect. Dis. **157**:441–449.
32. **Tamura, M., K. Nogimori, S. Murai, M. Yajima, K. Ito, T. Katada, M. Ui, and S. Ishii.** 1982. Subunit structure of islet-activating protein, pertussis toxin, in conformity with the A-B model. Biochemistry **21**:5516–5522.
33. **Trollfors, B., I. Krantz, N. Sigurs, J. Taranger, G. Zackrisson, and R. Roberson.** 1988. Toxin-neutralizing antibodies in patients with pertussis, as determined by an assay using Chinese hamster ovary cells. J. Infect. Dis. **158**:991–995.
34. **Viljanen, M. K., O. Ruuskanen, C. Granberg, and T. T. Salmi.** 1982. Serological diagnosis of pertussis: IgM, IgA, and IgG antibodies against *Bordetella pertussis* measured by enzyme-linked immunosorbent assay (ELISA). Scand. J. Infect. Dis. **14**:117–122.