

## Comparison of Throat and Nasopharyngeal Swab Specimens for Culture Diagnosis of *Bordetella pertussis* Infection

MARIO J. MARCON,<sup>1,2\*</sup> AYSER C. HAMOUDI,<sup>1,2</sup> HAROLD J. CANNON,<sup>2</sup> AND MARILYN M. HRIBAR<sup>2</sup>

Department of Pathology, The Ohio State University,<sup>1</sup> and Department of Laboratory Medicine, Section of Clinical Microbiology, Children's Hospital,<sup>2</sup> Columbus, Ohio 43205

Received 22 December 1986/Accepted 17 February 1987

**During a 9-month period, we evaluated the relative sensitivity of throat and nasopharyngeal swab cultures for isolation of *Bordetella pertussis*. Of 38 pertussis cases, 36 (95%) had positive nasopharyngeal cultures, while only 16 of 36 (44%) had positive throat cultures. There were no cases of nasopharyngeal-negative, throat-positive cultures. The sensitivity of the direct fluorescent-antibody test was 70% when compared with culture.**

Laboratory diagnosis of *Bordetella pertussis* infection has challenged clinical microbiologists for many years. Factors which contribute to the difficulty in isolating the organism have been reviewed elsewhere (J. Regan, Clin. Microbiol. Newsl. 2:1-3, 1980). These factors include problems relating to specimen collection and transport as well as availability of appropriate culture media and culture techniques (6, 10-12). In addition, the misplaced emphasis on the use of the direct fluorescent-antibody test as a single laboratory diagnostic technique has been noted (5, 9; P. H. Gilligan, Clin. Microbiol. Newsl. 5:115-117, 1983).

Most experts recommend duplicate pernasal nasopharyngeal swab specimens or, alternatively, auger suction aspirates of the nasopharynx for the isolation of *B. pertussis* (1, 7, 8). The latter collection method is practical only in a hospital setting. Collection of throat swabs in addition to nasopharyngeal swabs has been reported to result in a higher rate of culture diagnoses but has not been recommended as a sole collection method (1).

Using duplicate nasopharyngeal swab specimens plated onto modified Regan-Lowe (charcoal-cephalexin-sheep blood; 9) agar, we have identified more than 70 cases of pertussis at our institution during a 2-year period from October 1984 through September 1986 (unpublished observations). In this communication, we report the comparative efficacy of throat versus nasopharyngeal swab specimens for the isolation of *B. pertussis*.

Dual nasopharyngeal and single throat calcium alginate swab (Transette I and III, respectively; Spectrum Labs, Inc., Houston, Tex.) specimens were collected from 200 patients during January through September of 1986. Specimens were separately plated onto two modified Regan-Lowe (prepared in-house and used within 8 weeks) agar plates (prewarmed to room temperature) at the bedside and subsequently quadrant streaked for isolation in the laboratory. The modified Regan-Lowe medium was prepared from charcoal agar (Oxoid U.S.A., Inc., Columbia, Md.) and supplemented with cephalexin (40 µg/ml) and 10% sheep blood. In addition, nasopharyngeal secretions were smeared in duplicate onto clean, ringed microscope slides for direct fluorescent-antibody tests (see below). The agar plates were incubated at 35°C in 5% CO<sub>2</sub> for 7 days and examined daily with a stereomicroscope for the presence of small, glistening colonies suggestive of *Bordetella* sp. All patient isolates

suggesting *Bordetella* sp. were Gram stain smeared, and gram-negative coccobacilli were fluorescent antibody stained with both *B. pertussis* and *Bordetella parapertussis* conjugates as described below to confirm the identification. Most organisms were detected within 4 days of incubation. The performance characteristics of Regan-Lowe agar containing cephalexin were monitored by demonstrating growth of laboratory isolates of *B. pertussis* and *B. parapertussis* and inhibition of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

The fluorescein-labeled anti-*B. pertussis* conjugate used in the direct fluorescent-antibody test and for culture confirmation was obtained from Difco Laboratories, Detroit, Mich., and prepared according to the instructions of the manufacturer. Slides were stained within 4 h of receipt or stored frozen at -70°C and stained within 24 h. Sensitivity and specificity of the conjugate were controlled with known laboratory isolates of *B. pertussis* and *B. parapertussis*. A direct fluorescent-antibody test for *B. pertussis* was considered positive when a minimum of 10 oval coccobacilli showing bright-green peripheral fluorescence were visualized under oil immersion.

A case definition of pertussis was a patient with a positive throat or nasopharyngeal culture or a positive direct fluorescent-antibody test of nasopharyngeal secretions or both and with clinical signs and symptoms of pertussis infection. Clinical presentation ranged from only mild upper respiratory tract manifestations (cough, congestion, and rhinorrhea) to the more severe presentation of recurrent paroxysmal coughing accompanied by cyanosis, whooping, and vomiting.

The results of the study showed that 36 (95%) of 38 laboratory-diagnosed pertussis patients had positive nasopharyngeal cultures for *B. pertussis*. Of the two patients with negative nasopharyngeal cultures, both were direct fluorescent-antibody test positive and throat culture negative, with clinical signs and symptoms consistent with pertussis. The nasopharyngeal culture of one of these patients was uninterpretable because of overgrowth of *Pseudomonas aeruginosa* and *Aspergillus* sp. The sensitivity of the throat swab culture was only 46% (16 of 35 pertussis patients were positive). Generally, throat cultures contained greater numbers of normal upper respiratory and transient colonizing organisms than did nasopharyngeal cultures; these organisms may have interfered with the growth and recognition of *Bordetella* colonies. The sensitivity of the direct fluores-

\* Corresponding author.

cent-antibody test for *B. pertussis* was 70% (25 of 36 pertussis patients were positive). No isolates of *B. parapertussis* were obtained during the study.

Although the incidence of pertussis has decreased dramatically in the United States with the widespread use of pertussis vaccine, sporadic cases and small outbreaks continue to occur. During the 10 years from 1975 to 1984, the annual number of cases reported to the Centers for Disease Control, Atlanta, Ga., ranged from a low of 1,010 to a high of 2,463 (2). More than 3,200 cases were reported in 1985, and more than 4,000 cases were reported in 1986 (3, 4). The number of cases of pertussis may continue to increase if concern about the safety of the whole-cell vaccine causes parents to withhold immunization. Because of the different case criteria used by individual states, as well as the technical problems associated with laboratory diagnosis, many cases are probably diagnosed and treated on clinical grounds alone and thus go unreported. This omission is particularly true of secondary cases once an index case is diagnosed.

Bordet-Gengou medium, with or without antibiotic supplementation, has been widely used for the isolation of *B. pertussis* from clinical specimens. Recently, however, several investigators reported on the efficacy of Regan-Lowe agar for recovery of *B. pertussis* from the nasopharynx (5, 9; F. Chan, E. Rossier, A. M. R. Mackenzie, and A. Camus, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C260, p. 355). We recently reviewed our *B. pertussis* nasopharyngeal culture results (unpublished data) obtained during two 18-month periods when Bordet-Gengou medium or modified Regan-Lowe medium was used exclusively in our laboratory. Interestingly, the rate of isolation of *B. pertussis* more than doubled during the period when Regan-Lowe agar was used, suggesting either an increased incidence in disease during that period or an increased isolation rate on Regan-Lowe agar or both.

Few comparative data are available on the relative efficacy of throat versus nasopharyngeal swab cultures for the isolation of *B. pertussis*, particularly with Regan-Lowe agar. The *Manual of Clinical Microbiology*, 4th ed., cites two references which address the higher rate of positive culture diagnoses when throat swabs are collected in addition to nasopharyngeal swabs (8). In our experience, throat swab

cultures performed as described in our study do not aid in the laboratory diagnosis of pertussis.

We thank Betsy Wilson-Mann for her secretarial expertise in preparing this manuscript.

#### LITERATURE CITED

1. Broome, C. V., D. W. Fraser, and W. J. English. 1979. Pertussis—diagnostic methods and surveillance, p. 19–22. In C. R. Manclark and J. C. Hill (ed.), *International Symposium on Pertussis*. U.S. Department of Health, Education, and Welfare, publication no. (NIH) 79-1830. U.S. Government Printing Office, Washington, D.C.
2. Centers for Disease Control. 1986. Annual summary 1984: reported morbidity and mortality in the United States. *Morbidity and Mortality Weekly Report* 33:42–44.
3. Centers for Disease Control. 1986. Cases of specified notifiable diseases, United States. *Morbidity and Mortality Weekly Report* 34:775–777.
4. Centers for Disease Control. 1987. Cases of specified notifiable disease, United States. *Morbidity and Mortality Weekly Report* 35:793–795.
5. Gilligan, P. H., and M. C. Fisher. 1984. Importance of culture in laboratory diagnosis of *Bordetella pertussis* infections. *J. Clin. Microbiol.* 20:891–893.
6. Jones, G. L., and P. L. Kendrick. 1969. Study of a blood free medium for transport and growth of *Bordetella pertussis*. *Health Lab. Sci.* 6:40–45.
7. Lautrop, H., and B. W. Lacey. 1960. Laboratory diagnosis of whooping-cough or *Bordetella* infections. *Bull. W.H.O.* 23:13–35.
8. Parker, C. D., and B. J. Payne. 1985. *Bordetella*, p. 394–399. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
9. Regan, J., and F. Lowe. 1977. Enrichment medium for the isolation of *Bordetella*. *J. Clin. Microbiol.* 6:303–309.
10. Ross, P. W., and C. G. Cumming. 1981. Isolation of *Bordetella pertussis* from swabs. *Br. Med. J.* 282:23–26.
11. Stauffer, L. R., D. R. Brown, and R. W. Sandstrom. 1983. Cephalixin-supplemented Jones-Kendrick charcoal agar for selective isolation of *Bordetella pertussis*: comparison with previously described media. *J. Clin. Microbiol.* 17:60–62.
12. Sutcliffe, E. M., and J. D. Abbott. 1972. Selective medium for the isolation of *Bordetella pertussis* and *parapertussis*. *J. Clin. Pathol.* 25:732–733.