CULTURE AND FLUORESCENT-ANTIBODY METHODS IN DIAGNOSIS OF WHOOPING COUGH

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ABSTRACT

HOLWERDA, JACK (Michigan Department of Health, Grand Rapids) AND GRACE ELDERING. Culture and fluorescent-antibody methods in diagnosis of whooping cough. J. Bacteriol. **86:** 449–451. 1963.—Nasopharyngeal swabs from 517 suspected whooping-cough patients were examined by culture and by fluorescent-antibody (FA) staining procedures applied to direct slide preparations. A total of 138 were positive by both methods, 25 by culture only, and 25 by FA only. The FA technique was also used in the identification of young cultures. It was shown that a positive culture report could be speeded up by about 1 day by this method. Without FA, one-half the positive culture reports were made in 3 days; with FA, 75% were reported in a similar period.

The possible application of fluorescent-antibody (FA) procedures in the diagnosis of whooping cough has been recognized by several authors. Donaldson and Whitaker (1960) reported positive findings in 31 of 36 FA stained preparations of nasopharyngeal specimens from infants with symptoms of clinical whooping cough; no cultural results were given. Kendrick, Eldering, and Eveland (1961) reported the comparative results of culture and FA procedures applied to 130 nasopharyngeal specimens. *Bordetella pertussis* was demonstrated by both methods in 21 instances, by culture only in 4, and by FA only in 7. A total of 98 specimens were negative by both culture and FA staining. With respect to the FA method, Kendrick et al. (1961) stressed the importance of a thorough knowledge of the organism under study, strict control of technical factors related to all steps in the procedure, and use of a suitable antiserum. Certain *B. pertussis* antisera produced in rabbits cause fluorescence with staphylococci, an observation also reported by other workers in studying other organisms.

Subsequently, Eldering, Eveland, and Kendrick (1962) compared agglutination and FA reactions using antigens prepared with the three *Bordetella* species, *pertussis*, *parapertussis*, and *bronchiseptica*, and the antisera produced against them. Cross-reactions occurred between some of the antisera and heterologous antigens with both methods, but a positive agglutination test was not always correlated with a positive FA reaction, and the reverse was also true. Of particular interest to those doing diagnostic work with FA was the observation that none of the *B. pertussis* antisera tested caused fluorescence with *B. parapertussis*. Three of five *B. pertussis* antisera gave positive FA results with *B. bronchiseptica*, and cross-reactions occurred between some *B. parapertussis* and *B. bronchiseptica* antisera and the heterologous antigens of the genus. These findings emphasize the importance of knowing the characteristics of the particular antiserum used for FA tests.

An opportunity to study the diagnostic value of FA procedures in whooping cough occurred in 1962 when there was a marked increase in the incidence of this disease in the Grand Rapids area. For the calendar year 1961, only four cases of whooping cough were reported in Grand Rapids, a city of about 200,000. However, by mid-September of 1962, 63 cases had been reported in Grand Rapids, and 101 cases had been reported for Kent County outside of Grand Rapids.

MATERIALS AND METHODS

Specimens. Practically all the specimens examined for *B. pertussis* were collected at the Michigan Department of Health Laboratory from patients referred by their physicians. The nasopharyngeal swabs described by Bradford and Slavin (1940) were used to collect the exudate. Each slender swab was prepared with great care so that the ends of the wire were completely covered with cotton, and the cotton was secured firmly on the wire. When the patient was an in-

fant or a small child, he was usually held on his mother's lap while an assistant standing behind the child immobilized his head. The person taking the specimen inserted the swab through the nasal passage into the nasopharynx to a distance of about 3 in. If there was any resistance due to a deviated septum or some other obstacle, no pressure was used, and the other nostril was tried. Actually, it was usually possible to pass the swab readily through the nares.

Culture. The swab with the exudate was placed immediately in a small tube containing 0.2 ml of 1% Casamino Acids. Within 1 hr, the swab was streaked on two plates of Bordet-Gengou medium, one of which contained 0.5 unit of penicillin per ml of medium. The swab was then returned to the tube of Casamino Acids for rewashing before it was used to make a slide preparation. The plates were placed in the incubator at 35 to 37 C, and later examined by methods described elsewhere. The slides were air-dried and fixed by gentle heat for FA staining.

FA technique. The two *B. pertussis* antisera used in this study were produced in White Leghorn chickens; one antiserum was against a killed antigen and the other against a live suspension of *B. pertussis*. The chickens were bled 3 days after the last injection, and the serum was conjugated with fluorescein isothiocyanate according to the method of Marshall, Eveland, and Smith (1958).

The conjugated chicken sera, each in a 1:16 dilution, gave 4+ FA staining reactions with *B. pertussis*. No difference was observed in the results with the two sera. Many cultures of staphylococci and various organisms found in the respiratory tract were tested, and all gave negative FA results. In earlier work in which antisera produced in rabbits were used, excellent staining of *B. pertussis* also occurred, but some cross-reactions with certain strains of staphylococci were observed. Although the size and morphology of staphylococci differ from *B. pertussis*, positive identification was more difficult with the rabbit sera. Neither this cross-reaction nor any other nonspecific reaction has been encountered with these particular chicken antisera. Also, the degree of fluorescence appeared to be greater with these chicken antisera than with the particular rabbit antisera used.

RESULTS

Of the 517 specimens examined by culture and FA staining of direct smears, 138 were positive by both methods (Table 1). A total of 25 specimens were positive by culture and negative by FA; 25 were negative by culture and positive by FA; and 329 specimens were negative by both methods. The total number of specimens positive by either or both methods was 188, or 36.4%.

TABLE 1. *Results of FA staining and cultures for Bordetella pertussis*

Result	Number	Per cent
Positive (either or both methods)	188	36.4
Culture +, FA +	138	26.7
Culture +, FA −	25	4.8
Culture −, FA +	25	4.8
Negative (both methods)	329	63.6
Total examined	517	100.0

At first, positive reports were made only on the basis of the culture results, and the result of the FA slide was merely recorded. However, when more experience with the FA procedure proved its reliability, positive results based only on FA staining were reported. A difficulty recognized very early in the study was that adequate examination of the direct FA slide preparations was time-consuming. Some preliminary tests suggested that perhaps a combination of these methods, i.e., FA staining of young cultures, might be the diagnostic procedure of choice. This approach has been used successfully with certain other organisms, for example, hemolytic streptococci (Peebles, Spielman, and Moody, 1961). The procedure, if dependable, would greatly shorten the time required for identification of *B. pertussis* by the usual cultural procedures. The inoculated Bordet-Gengou plates, therefore, were examined early in the incubation period, and slide preparations were made either from very small colonies, visible only with a hand lens, or from confluent growth for FA staining. In many instances, the brightly stained *B. pertussis* organisms could be observed microscopically in these preparations, and reported immediately.

The time required for positive culture reports for the 76 positive cultures identified without FA during 1961 and until 15 June 1962 was compared with the time required for the 126 cultures received between 15 June and 15 September 1962, which were identified by FA (Table 2). Prior to using FA, positive culture reports were never made as early as 1 day after receipt. However, by use of the FA technique on the cultures, 18

TABLE 2. *Time required for positive culture report, without and with FA*

Time	No. of specimens	
	Without FA	With FA
days		
1	0	18
2	10	37
3	27	40
4	31	20
5	4	5
6	2	3
7 or 8	2	3
Total	76	126

TABLE 3. *Comparison of time required for positive culture report, without and with FA*

Method	No. of specimens	Cumulative percentages (by days)				
		1 day	2 days	3 days	4 days	5 to 8 days
Without FA.	76	0	13.2	48.7	89.5	100
With FA....	126	14.3	43.7	75.4	91.3	100

specimens were reported positive in 24 hr. There was also an increase in the proportion of positive reports on the second and third days when the FA technique was used. Without FA, 39 of 76 cultures, or more than half, required 4 or more days of incubation before a positive report was made. When FA was applied to the cultures, only 31 of 126 positive cultures (25%) required 4 or more days. The mean time required for a positive report was 3.6 days without FA and 2.9 days when FA was applied to the culture. In other words, it was possible to make a positive report 1 day earlier when the FA procedure was used.

Table 3 shows the cumulative positive reports by days, in percentages, comparing results without and with FA. Without FA, no positive reports were made in 24 hr, and with FA 14.3% of the positive results were reported in that time. On the third day, approximately one-half of the positive cultures examined without FA were reported; with FA, three-fourths were reported.

Discussion

The FA staining procedure was used in two ways in the diagnostic specimens for whooping cough. FA staining of slide preparations from nasopharyngeal swabs proved an effective method in comparison with the usual cultural procedure. As is to be expected whenever two methods are used, the percentage of total positive findings was increased. The 25 specimens positive by culture but not by FA might be explained by failure to find a very few organisms on the slide. The opposite discrepancy, the 25 specimens with positive slides and negative cultures, in some instances might be due to the overgrowth of the culture by organisms other than *B. pertussis*. A disadvantage of the method was the time required for examination of the slides when the organisms were few in number or absent.

The second method, the use of the FA staining procedure on young cultures, accomplishes several things. It shortens the time required for a positive report, and keeps the interpretation of the FA staining method on a firm basis. The advantage of the positive culture in the interpretation of FA results, particularly in the developmental stages of the procedure, cannot be overemphasized.

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