

Rapid Biotyping of *Haemophilus influenzae* and *Haemophilus parainfluenzae* with PathoTec Strips and Spot Biochemical Tests

BILLIE ANNE JUNI,* JEAN M. RYSAVY, AND DONNA J. BLAZEVIC

Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota 55455

Received 19 October 1981/Accepted 22 January 1982

PathoTec strips and spot biochemical tests were evaluated for the ability to biotype *Haemophilus influenzae* and *Haemophilus parainfluenzae*. Indole, urease, and ornithine decarboxylase reactions were tested. The results of PathoTec strips compared favorably with those conventional methods; the percent agreements were as follows: indole, 100; urease, 99.5; and ornithine, 97.5. Spot tests were simple and rapid, and the results also compared favorably with those of conventional tests; the percent agreements were as follows: indole, 99; urease, 100; and ornithine, 96.

Detailed biochemical characterization, or biotyping, of *Haemophilus* species yields valuable epidemiological information. Biotyping is especially helpful in the study of patients with recurrent infections caused by *Haemophilus* spp. In addition, specific biotypes have been associated with site of isolation, antigenic properties, and antibiotic resistance (5, 6). On the basis of indole, ornithine decarboxylase, and urease reactions (6), *Haemophilus influenzae* can be divided into five biotypes, and *H. parainfluenzae* can be divided into three. Kilian (6) used conventional methods which require 24 to 48 h. More recently, the use of the Micro-ID and Minitek systems has been described (2, 9). The present study was undertaken to determine whether the use of PathoTec test strips (General Diagnostics, Warner-Lambert Co., Morris Plains, N.Y.) and the observation of spot biochemical reactions are more rapid and less expensive methods for the determination of *H. influenzae* and *H. parainfluenzae* biotypes. PathoTec strips were developed for use with the *Enterobacteriaceae*, but the data given below show that they are useful for the detection of the enzymes of *Haemophilus* species as well.

A total of 200 strains of *H. influenzae* and 200 strains of *H. parainfluenzae* were tested. Of the 400 strains, 79% were from respiratory sources, and all had been freshly isolated from patients by the Diagnostic Microbiology Laboratory of the University of Minnesota Hospitals. The organisms were identified on the basis of typical colony morphology and hemolytic capacity on selective horse blood agar (3), ability to synthesize porphyrins from δ -amino-levulinic acid (8), and, in some instances, the requirement for X and V factors. The inoculum used for biotyping

was an 18- to 24-h culture from chocolate agar.

Conventional biochemical tests used were those recommended by Kilian (6). Indole production was detected by the National Collection of Type Cultures method of Cowan and Steel (1); the broth was dispensed in 0.5-ml amounts, the organism was incubated for 4 h at 37°C, and the reaction was read after Kovacs reagent had been added. Urease was detected by using a modification (7) of the method of Ferguson and Hook. The broth was dispensed in 0.5-ml amounts, and the reaction was read after 4 h at 37°C. If negative, the reaction was read again after 24 h of incubation. The method of Moeller, as described by Edwards and Ewing (4), modified by the addition of 10 μ g each of hemin and nicotinamide adenine dinucleotide (6) per ml (final concentrations), was used for ornithine decarboxylase determinations. Reactions were read at 4, 24, and 48 h of incubation at 37°C. PathoTec strips were used to test for indole, urease, and ornithine decarboxylase according to the manufacturer's instructions. Spot tests were performed by placing a dense filter paper such as Whatman no. 3 in a petri dish and saturating it with the urea solution or the ornithine solution which had been prepared for conventional tests. Test organisms were placed in spots on the saturated filter papers, held at room temperature, and observed for up to 1 h for the localized color changes, which were the same as those observed in conventional tests (pink for urease, purple for ornithine decarboxylase). Spot indole tests were done by saturating filter paper with Kovacs reagent, applying the organisms, and immediately determining whether a localized red area formed (10). Spot tests were performed before the results of conven-

tional determinations were known so that biased interpretation could be prevented. When disagreements occurred, both the conventional and the spot tests (or the PathoTec test) were repeated. When one test result disagreed with the other after both tests had been repeated, the reaction obtained by the conventional method was used to determine the biotype.

Overall agreement between the results of PathoTec strip tests and those of the conventional tests was 99% (Table 1). When initially tested, 28 strains (15 *H. influenzae*, 13 *H. parainfluenzae*) were ornithine positive by the conventional method (4) and ornithine negative by the PathoTec method. When the tests were repeated, 21 strains were ornithine positive by the PathoTec method, 2 strains were ornithine negative by the conventional method, and 5 strains (2 *H. influenzae*, 3 *H. parainfluenzae*) remained in disagreement. To check the reproducibility of the results of the PathoTec ornithine strip, we tested one strain of ornithine-positive *H. influenzae* and one strain of ornithine-positive *H. parainfluenzae* each with eight PathoTec ornithine strips. Of the 16 tests, 13 were strongly positive, 2 were weakly positive, and 1 had a defective water barrier. Edberg et al. also found that the greatest disagreement between the results of the Micro-ID test and those of conventional tests was in the ornithine decarboxylase reaction (2). Table 1 also compares the results of the spot test method with those of the conventional method. The overall agreement between the results of the two methods was 98%. The spot ornithine decarboxylase test results correlated 96% with the results of conventional test. Seven isolates of *H. influenzae* were ornithine decarboxylase positive by the spot method and ornithine decarboxylase negative by the conventional method. Two isolates of *H. influenzae* showed disparate indole reactions. The indole reaction was immediate, and the ornithine decarboxylase and urease spot tests were positive within 15 min. The pink of the positive urease reaction rapidly diffused in a large circle around the spot, and spots had to be placed at least 15 mm apart to prevent the reactions from overlapping.

Most of the *H. influenzae* isolates were of biotypes I, II, and III, and most of the *H. parainfluenzae* isolates were of biotypes I and II. A total of 29 isolates were unclassified. Of these 29, at least 22 were *H. segnis* or *H. paraphrophilus* (6). On the basis of the results of the porphyrin test, these organisms were not differentiated from *H. parainfluenzae*. The correlations of biotype with source were similar to those previously reported (2, 5, 6, 8).

Although organisms of the genus *Haemophilus* are usually considered fastidious, their en-

TABLE 1. Correlation of the results of conventional, PathoTec, and spot tests of 200 strains of *H. influenzae* and 200 strains of *H. parainfluenzae*^a

Method	No. of positive strains for:		
	Indole	Urease	Ornithine decarboxylase
Conventional	81	132	116
PathoTec	81	131	111
Conventional	81	157	113
Spot	81	157	119

^a We performed the conventional-PathoTec and conventional-spot test comparisons independently, using a different set of *H. influenzae* and *H. parainfluenzae* strains for each comparison. Percent agreements of the results of the conventional and PathoTec tests were as follows: indole, 100; urease, 99.5; and ornithine decarboxylase, 97.5. Percent agreements of the results of the conventional and spot tests were as follows: indole, 99 (one isolate was positive by conventional testing and negative by spot testing, and one isolate was negative by conventional testing and positive by spot testing; therefore, 100% agreement was not reached); urease, 100; and ornithine decarboxylase, 96.

zymes were easily detected by accurate, rapid, inexpensive methods. Both the PathoTec method and the spot test eliminated the need for supplementation of biochemical media with growth factors because growth is not required for the performance of these tests. The indole, ornithine, and urea spot tests were performed and interpreted easily. Their sensitivity was demonstrated by clear-cut results obtained in less than 15 min. This is particularly noteworthy in the case of the ornithine decarboxylase test; we are not aware of any previous report that this enzyme can be detected so rapidly. Positive and negative control organisms should be available for each spot test to aid in interpretation and to ensure the quality of the reagents. Control organisms should be of the *Haemophilus* genus because the ornithine and urea spot tests have not been evaluated for use with the *Enterobacteriaceae*.

Biotyping with PathoTec strips costs \$0.63 (list price). This is much less than the cost of a Micro-ID panel (\$1.89) or a Minitex system (minimum of \$1.16, depending on the number of organisms tested per plate). The strips also enable single tests to be selected and performed without an entire panel of tests being wasted. It is not possible to estimate accurately the cost of the spot test method because it is dependent on the cost of preparing the media. However, less technologist time is required to perform the spot tests than to perform the Micro-ID, Minitex panel, PathoTec strip, or conventional test. The

spot tests do provide an accurate, rapid, and biochemically specific method for the biotyping of *Haemophilus* spp.

LITERATURE CITED

1. Cowan, S. T., and K. J. Steel. 1970. The identification of medical bacteria. Cambridge University Press, Cambridge, England.
2. Edberg, S. C., E. Melton, and J. M. Singer. 1980. Rapid biochemical characterization of *Haemophilus* species by using the Micro-ID. J. Clin. Microbiol. 11:22-26.
3. Ederer, G. M., and M. L. Schurr. 1971. Selective medium for the isolation of *Haemophilus*. Am. J. Med. Tech. 37:304-305.
4. Edwards, P. R., and W. H. Ewing. 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis.
5. Goldberg, R., and J. A. Washington II. 1978. The taxonomy and antimicrobial susceptibility of *Haemophilus* species in clinical specimens. Am. J. Clin. Pathol. 70:899-904.
6. Kilian, M. 1976. A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. J. Gen. Microbiol. 93:9-62.
7. Lautrop, H., and B. W. Lacey. 1960. Laboratory diagnosis of whooping cough or *Bordetella* infections. Bull. W.H.O. 23:15-31.
8. Lund, M. E. 1981. Filter-paper porphyrin-production test for identification of *Haemophilus*. Clin. Microbiol. Newsl. 3:27-28.
9. Retter, M. E., and R. M. Bannatyne. 1981. Comparison of conventional and Minitek systems for biotyping *Haemophilus influenzae*. Am. J. Clin. Pathol. 75:827-829.
10. Vracko, R., and J. C. Sherris. 1963. Indole spot-test in bacteriology. Am. J. Clin. Pathol. 39:429-432.