JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1975, p. 359-362

Vol. 1, No. 4
Printed in U.S.A.

Evaluation of the Pathotec Rapid I-D System for Identification of *Enterobacteriaceae*

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Received for publication 30 January 1975

The PathoTec Rapid I-D System for identifying *Enterobacteriaceae* was evaluated with 471 cultures. In 4,910 individual test comparisons, 95.5% of the results agreed, with results of only two test strips, those for esculin hydrolysis and urease production, agreeing with conventional tests in less than 94% of the trials. The PathoTec system exhibited 94.3% accuracy in identifying these cultures in a double-blind study with conventional media and procedures as the alternate system. Two newly developed test strips, for *o*-nitrophenyl-β-D-galactopyranoside and ornithine decarboxylase, were found to be highly reliable.

Reagent-impregnated paper strips have been used instead of conventional media or tests in diagnostic bacteriology for about 10 years. In many studies and evaluations of these products, their accuracy and reliability varied. In 1971, Martin et al. (3) reported that their studies verified those of Matsen and Sherris (4) in that cytochrome oxidase, phenylalanine, indole, and Voges-Proskauer (VP) strip tests were reasonably reliable, but urease, lysine, and citrate strip tests were not reliable, for the identification of *Enterobacteriaceae*. In 1973, Blazevic et al. (1) and Rosner (5) reported independently on evaluations of an improved set of strips, the PathoTec Rapid I-D System, but results in these two laboratories were substantially different in many respects. In this report additional data on the reliability of these reagent-impregnated strips are given, and results are compared to those reported in 1973.

MATERIALS AND METHODS

Reagent-impregnated strips of the PathoTec Rapid I-D System were furnished by the manufacturer, the General Diagnostics Division of Warner-Lambert Co., Morris Plains, N.J. Strips provided initially were for cytochrome oxidase, lysine decarboxylase, nitrate reduction, phenylalanine deaminase, indole production, H_2S production, malonate utilization, acetoin production (VP), esculin hydrolysis, and urease production. Near the completion of the study, newly developed strips for *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and ornithine decarboxylase tests were provided. All strips were stored at 4 C and in the dark in the sealed vials provided. The detailed instructions provided by the manufacturer were followed precisely.

Conventional tests performed routinely included reactions on triple sugar iron agar, H_2S production, urease, indole, methyl red, acetoin, citrate, lysine and ornithine decarboxylase, arginine dihydrolase, phenylalanine deaminase, growth in KCN, motility, ONPG, and acid production from glucose, lactose, arabinose, rhamnose, and raffinose. Additional biochemical tests were used as required, however, to make identifications.

Bacterial cultures used were either fresh isolates from clinical specimens or coded unknowns from the Center for Disease Control (CDC) culture collection. The types and numbers of cultures used are shown in Table 2. Each culture was streaked on a MacConkey agar plate to simulate a primary plating procedure, incubated 18 to 24 h at 35 C, and then identified by the PathoTec system and by our conventional procedures (1). All cultures suspected of being *Salmonella* or *Shigella* were confirmed serologically, as the manufacturer recommends. Results from all tests were compiled independently, so that identifications made by using either the PathoTec or conventional system remained unknown until all results were obtained. Repeat tests were conducted when results conflicted, and, when necessary, CDC's Enterobacteriology Branch was consulted as a reference laboratory. All conventionally prepared media and reagents were pretested with quality control cultures before being used in this study.

RESULTS

Results obtained with the various tests of the PathoTec system were compared with those obtained with corresponding conventional tests (Table 1). Agreement of tests ranged from 100% for cytochrome oxidase to 85.5% for urease tests. The average percentage of agreement was 95.5%. Analysis of the discrepancies revealed several interesting points. All of the 67 urease tests which disagreed consisted of negative results with PathoTec strips and weakly positive results with conventional tests in Christensen urea agar. *Citrobacter freundii*

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and *C. diversus* were responsible for 27 of these differences, *Enterobacter cloacae* for 21, and *Serratia* for 10. The PathoTec urea strip functions quite well with strong urease producers, i.e., *Proteus* species, in the 4-h incubation period, but it generally will not detect weak or delayed urease activity. The 50 discrepancies in esculin results were about equally divided between false-positive (28) and false-negative (22) PathoTec results. *C. freundii* caused 13 of these discrepancies, *C. diversus* caused six, and *E. cloacae* caused eight. The 27 differences in VP results were mainly caused by *E. hafniae*, *Serratia*, and *Yersinia* strains (six each) and by four cultures of *Proteus mirabilis*. Of the 22 differences in malonate tests, five were found with cultures of *Arizona*, a significant result considering the importance of this test in differentiating *Arizona* from *Salmonella*. All of the 16 differences in indole reactions were due to false-negative PathoTec results, and 12 of these were encountered with *Providencia* strains. These particular discrepancies were of no consequence, however, in the identification of *Providencia* cultures, since other test reactions were characteristic.

The accuracy with which 471 "unknown" cultures were identified with the PathoTec system is shown in Table 2. These identifications were made without the results of the PathoTec ONPG and ornithine decarboxylase tests. An average of 94.3% of the identifications attempted were correct to the level of accuracy claimed by the manufacturer. We did not attempt to differentiate *P. rettgeri* and *P. morganii* or *Serratia liquefaciens* and *Serratia marcescens* with the PathoTec system; therefore, either identification within these pairs was considered correct. In addition, the manufacturer lists reactions for certain "difficult" members of the *Enterobacteriaceae*, namely, *E. agglomerans*, *C. diversus*, *Klebsiella ozaenae*, *K. rhinoscleromatis*, and *Yersinia* species, thereby suggesting that they can be identified by the PathoTec system. Several of these were among those organisms which were least accurately identified, thus tending to lower the average percentage of accurate results.

The misidentifications encountered with the PathoTec system, as well as the reasons for the errors and indications of which of these cultures were atypical (or aberrant) biochemically, are shown in Table 3. It is particularly notable that of the 27 misidentified cultures, 12 were not typical biochemically and could be identified conventionally only by using tests well beyond the range of the PathoTec system. Of the remaining 15 cultures which were misidentified because of erroneous PathoTec results, five produced errors in malonate tests and seven produced errors in esculin tests.

TABLE 1. *Agreement of biochemical reactions obtained with PathoTec strips and in conventional tests*

Test	No. in agreement/ no. tested	% Agreement
Cytochrome oxidase	471/471	100.0
Lysine decarboxylase	469/471	99.6
Nitrate reduction	465/471	98.7
Phenylalanine deaminase	464/471	98.5
Ornithine decarboxylase[a]	98/100	98.0
ONPG[a]	97/100	97.0
Indole production	455/471	96.6
H_2S production	451/471	95.8
Malonate utilization	449/471	95.3
Acetoin production (VP)	444/471	94.3
Esculin hydrolysis	421/471	89.4
Urease production	404/471	85.8

[a] Tested separately.

DISCUSSION

Other recent publications on the efficacy of the PathoTec system for identifying *Enterobacteriaceae* have either been extremely favorable (5) or somewhat equivocal (1). Rosner (5), reporting on tests with over 1,200 cultures, found all genera or species to be identified with

TABLE 2. *Accuracy of identification of unknown cultures by the PathoTec system (10 tests)*

Organism	No. correct/no. tested	% Correct
Arizona	28/28	100.0
Citrobacter diversus	10/10	100.0
Proteus vulgaris	11/11	100.0
Providencia	31/31	100.0
Shigella	19/19	100.0
Klebsiella pneumoniae	31/32	96.9
Enterobacter hafniae	27/28	96.4
Proteus mirabilis	26/27	96.3
Citrobacter freundii	24/25	96.0
Serratia liquefaciens/Serratia marcescens	47/49	95.9
Proteus rettgeri/P. morganii	34/36	94.4
Salmonella	30/32	93.8
Escherichia coli	28/30	93.3
Enterobacter cloacae	27/29	93.1
Yersinia	9/10	90.0
Edwardsiella	14/16	87.5
Enterobacter aerogenes	24/28	85.7
Enterobacter agglomerans	8/10	80.0
Klebsiella ozaenae	8/10	80.0
Klebsiella rhinoscleromatis	8/10	80.0

TABLE 3. *Errors of identification made with the PathoTec system (10 tests)*

Organism	Misidentification[a]	Reason
Salmonella	*E. hafniae*[b]	H_2S negative, VP negative
	Arizona[b]	Malonate positive
Escherichia coli	*E. hafniae* (2)[b]	Indole, esculin negative; urea weak
Edwardsiella tarda	*E. coli*	H_2S negative
	Salmonella[b]	Indole negative
Klebsiella rhinoscleromatis	*E. agglomerans*[b]	VP negative
	E. agglomerans	Malonate, esculin negative
Enterobacter agglomerans	*E. cloacae*	Malonate, urea negative
	K. ozaenae	Malonate, VP negative
Proteus mirabilis	*E. agglomerans*[b]	H_2S, NO_3 negative
Citrobacter freundii	*E. agglomerans*[b]	Indole positive, urea negative
Enterobacter aerogenes	*Serratia*[b]	Malonate negative
	Serratia	Malonate negative
	E. agglomerans	Lysine negative
	K. pneumoniae[b]	Nonmotile, urea negative
Enterobacter cloacae	*E. agglomerans*	VP negative, esculin positive
	E. hafniae	Lysine positive
Klebsiella pneumoniae	*Serratia*	Malonate negative
Klebsiella ozaenae	*E. agglomerans* (2)[b]	Esculin, VP negative
Enterobacter hafniae	*E. aerogenes*	Malonate, esculin positive
Proteus rettgeri	*Providencia* (2)	Urea negative
Yersinia enterocolitica	*E. agglomerans*[b]	Esculin positive, VP negative
Serratia	*E. hafniae* (2)	Esculin negative (2), VP negative (1)

[a] Number in parentheses indicates number of cultures.
[b] Atypical strain.

greater than 95% accuracy except for *Citrobacter* (94%), *Klebsiella* (93%), *Serratia* (90%), *P. vulgaris* (92%), and *P. morganii* (56%). However, he did not attempt to differentiate among *Citrobacter*, *Klebsiella*, or *Enterobacter* species, and he did not encounter *Yersinia* strains. Blazevic et al. (1) studied no more than 10 cultures of each genus or species, using 163 cultures of *Enterobacteriaceae*, and also did not differentiate between *E. hafniae*, *E. agglomerans*, or *S. liquefaciens*, or between *Klebsiella* species. She and her co-workers had considerable difficulty in identifying cultures of *C. freundii*, *Providencia*, *E. cloacae*, the two above-mentioned *Enterobacter* species, *Serratia*, and *Salmonella*, indicating that additional tests were needed to identify most of these cultures. More significantly, certain major differences appear in the results of Rosner and Blazevic: Rosner claimed 100% identification of *Providencia*, whereas Blazevic claimed 50%; Rosner claimed 100% identification of *P. rettgeri*, and Blazevic claimed only 10%; Rosner identified 56% of *P. morganii*, with Blazevic identifying 0%; and Rosner identified 96% of an unspeciated group of *Enterobacter*, whereas Blazevic identified 40% of the *E. cloacae* only. In the present study, we do not attempt to resolve these differences but rather to contribute additional data obtained both stock and freshly isolated cultures. In general, our results with the PathoTec system tend to agree more with those of Rosner than with those of Balazevic et al. For example, we accurately identified 24 of 25 cultures of *C. freundii* (96%), all of 31 *Providencia*, 27 of 29 *E. cloacae* (93%), and 34 of 36 *P. rettgeri* or *P. morganii* (94.4%). Adding the ONPG and ornithine decarboxylase strips to the PathoTec system should improve the overall accuracy of the system, as Rosner claimed, using experimental batches of these strips. In our laboratory, these new strips exhibited exceptionally good agreement with their conventional counterparts.

The major advantages of the PathoTec system over conventional methods, as discussed by both Blazevic et al. and Rosner, were also observed in this study. The ability of the PathoTec system to provide a highly accurate identification of *Enterobacteriaceae* within 24 to 30 h of receiving a clinical specimen is highly desirable in clinical laboratories. Even if some cultures should require additional tests, and thus another 24 to 48 h of incubation, there is still a gain of at least 24 h over conventional procedures. The other main advantage of this system is its versatility, i.e., any of the PathoTec strips can be used in lieu of the appropriate conventional tests. Thus, the user is not "locked in" to a specified system of biochemical tests and may retain whatever tests he normally uses. Understandably, however, the manufac-

turer would prefer to sell the strips as a total system, and most users will probably buy them as such. This places a responsibility on the manufacturer to provide complete and accurate guides to the use and interpretation of results with his product. The printed material originally provided us was somewhat lacking in this respect in two ways. (i) No information was given on what additional tests might be helpful in differentiating certain organisms, and (ii) Ewing's data were incompletely adapted to the "checkerboard" used for making identifications. These two faults have been recently corrected, however, and the presently available package inserts are more complete. For example, reactions are now given for *Shigella sonnei*, the three major species of *Salmonella*, the two species of *Providencia*, and three species of *Yersinia*. In addition, notes are more extensive, additional tests which might be required are indicated, and (most importantly) the percentages of positive reactions to be expected for the various tests are given. This latter information is highly useful, since with it the microbiologist can, if necessary, make decisions on the basis of the probable occurrence of atypical reactions. The system now available seems to be a highly acceptable alternate to conventional techniques for the identification of *Enterobacteriaceae* in the clinical laboratory.

LITERATURE CITED

1. Blazevic, D. J., P. C. Schreckenberger, and J. M. Matsen. 1973. Evaluation of the PathoTec "Rapid I-D System." Appl. Microbiol. **26**:886-889.
2. Ewing, W. H. 1973. Differentiation of *Enterobacteriaceae* by biochemical reactions. Department of Health, Education, and Welfare Publication no. (CDC) 74-8270, revised. Center for Disease Control, Atlanta, Ga.
3. Martin, W. J., S. F. Bartes, and M. M. Ball. 1971. Evaluation of reagent-impregnated strips for identification of *Enterobacteriaceae*. Am. J. Med. Technol. **37**:99-101.
4. Matsen, J. M., and J. C. Sherris. 1969. Comparative study of the efficacy of seven paper-reagent strips and conventional biochemical tests in identifying gram-negative organisms. Appl. Microbiol. **18**:452-457.
5. Rosner, R. 1973. Evaluation of the PathoTec "Rapid I-D System" and two additional experimental reagent-impregnated paper strips. Appl. Microbiol. **26**:890-893.