APPLIED MICROBIOLOGY, Dec. 1973, p. 890-893
Vol. 26, No. 6

Printed in U.S.A.

Evaluation of the PathoTec "Rapid I-D System" and Two Additional Experimental Reagent-Impregnated Paper Strips

RICHARD ROSNER

St. Joseph's Hospital and Medical Center, Paterson, New Jersey 07503

Received for publication 1 August 1973

The PathoTec "Rapid I-D System" and two experimental test strips for ornithine decarboxylase and beta-galactosidase have been evaluated for accuracy and ability to identify 1,252 members of the *Enterobacteriaceae* obtained from fresh clinical specimens. Accuracy of identification with the commercially available test system was 94.7%; this level increased to 98.5% with the addition of the two experimental test strips. Average individual accuracy of the 12-strip test system on a side-by-side basis with similar conventional procedures was 98%. In addition, 103 gram-negative nonfermentors were accurately grouped. The PathoTec System was applicable to 95% of the primary isolation plates used and provided biochemical data within 4 h after inoculation. The conventional test procedures were applicable to 100% of the primary isolation plates used and produced data within 48 h.

A new reformulated PathoTec "Rapid I-D System" has been in routine use in this laboratory, first as an experimental product and later as a commercially available product, since October of 1971. Recently, we have also tested two additional experimental reagent-impregnated test strips, ornithine decarboxylase (OD) and beta-galactosidase (ONPG). This report deals with the evaluation of the commercially available 10-test system and the 12-test system in terms of accuracy of strip reactions and ability of the system to identify members of the family *Enterobacteriaceae*.

MATERIALS AND METHODS

All bacterial cultures were fresh gram-negative clinical isolates from specimens received in the bacteriology laboratory of St. Joseph's Hospital. Organisms were isolated on MacConkey agar, eosin methylene blue agar, salmonella-shigella agar, or Hektoen enteric agar plates. Cultures were identified by one technologist using conventional media representing the same tests as those available on the reagent-impregnated test strips plus other tests where required. A second technologist used the commercially available PathoTec strips plus the two experimental strips. Each technologist recorded results separately, and the identifications were compared by the author and the chief technologist. All conventional media were prepared in this laboratory from commercially available dehydrated stock (BBL, Difco, Pfizer). These media included nitrate broth (BBL), phenylalanine agar (Pfizer), Christensens urea agar modified (Difco), 1% tryptone broth (Difco), triple sugar iron agar (Difco), lysine decarboxylase broth (Moeller) (Difco), ornithine decarboxylase broth (Falkow) (Difco), MR-VP broth (BBL), malonate broth (Ewing modified) (Difco), esculin agar (Pfizer), and buffered ONPG prepared by Pfizer. The oxidase reaction was determined with freshly prepared tetramenthyl-*p*-phenylenediamine hydrochloride reagent. In addition, the following media were used for both the conventional and the paper strip systems: deoxyribonuclease agar (Difco) and arabinose broth (Difco). All identifications were made according to Edwards and Ewing (2).

All reagent-impregnated test strips were used according to the directions supplied by the manufacturer in the commercially available kit with the exception that we set up 12 tubes (13 by 100 mm) instead of 10 and used 3.2 ml of organism-saline suspension instead of 2.5 ml as described by the manufacturer. Experimental OD and ONPG strips were added to tubes containing approximately 0.3 ml of saline-organism suspension. A positive OD strip was read when the saline-organism suspension was blue or purple, and a negative reaction was recorded when this solution was yellow. The ONPG strip was interpreted as positive when the color of the saline-organism suspension was any shade of yellow and as negative when this solution remained colorless. All incubated strips, including the two experimental strips, were incubated for 4 h.

Test strips and conventional procedures were set up for all gram-negative isolates. These included 103 *Pseudomonadaceae* (oxidase-positive or nitrate-nega-

tive organisms) for which genus or species identification was not attempted, since the manufacturer defines the rapid system as only for the identification of the *Enterobacteriaceae*.

All organisms which were identified as members of either the genus *Salmonella* or the genus *Shigella* by either the PathoTec System or the conventional system were serologically typed. Any organism which was identified by either system as *Klebsiella*, *Enterobacter*, or *Serratia* (K-E-S group) and which was malonate negative was further tested on deoxyribonuclease agar and arabinose broth.

RESULTS

The 10-test system showed an overall identification accuracy of 95.1% with all 1,355 gram-negative bacilli tested. When the 103 members of the family *Pseudomonadaceae* were eliminated from the results, the 10-test system showed an identification accuracy of 94.7% for the *Enterobacteriaceae*. When the experimental OD and ONPG strips were added, the level of identification accuracy increased to 98.5% (Table 1). The use of the OD and ONPG strips allowed separation of *Klebsiella* from *Enterobacter* in those instances where the 10-strip system yielded an incorrect or inconclusive identification. The OD strip provided positive identification of *Proteus morganii*, where a false-positive H_2S test strip resulted in an identification of this organism as *Proteus vulgaris*. The ONPG strip was required for correct characterization of lactose-negative strains of *Escherichia coli* which were also lysine decarboxylase negative. With the addition of OD and ONPG test strips to the commercially available test strip system, we misidentified 19 of 1,252 organisms (1.5%) compared to 67 incorrect identifications (5.3%) when these two test strips were not included (Table 2).

Table 3 compares PathoTec test results to conventional procedures. Test strip data for cytochrome oxidase, phenylalanine deaminase, ornithine decarboxylase, and ONPG tests showed complete agreement. Correlation of strip tests with conventional procedures for malonate utilization, nitrate reduction, indole production, Voges-Proskauer reaction, lysine decarboxylase, H_2S production, and esculin hydrolysis were all above 98%. The urease reaction showed a correlation of 90.2% with all of the errors found in the genera *Klebsiella*, *Enterobacter*, and *Citrobacter*.

DISCUSSION

In the last several years, a number of commercially available kits have appeared on the market for the identification of the *Enterobacteriaceae*. All of these products have been shown to have a high level of accuracy (Roundtable on the *Enterobacteriaceae*, Session 23, 73rd Annu. Meet. Amer. Soc. Microbiol., 1973), and each has both advantages and disadvantages in the clinical laboratory. In our laboratory, reagent-impregnated strip tests have been found to have significant advantages over other approaches to identification of the strip tests; identification can usually be made on the same day that the primary isolation plates are examined. The strip system is completely flexible in that the laboratory can select

TABLE 1. *Strains tested and the percent agreement of identification between conventional media and the PathoTec "Rapid I-D System" with and without the additional strips*

Organism	No. of strains tested	PathoTec Rapid I-D system (no. correct/ no. tested)	Percent	PathoTec Rapid I-D System plus OD and ONPG (no correct/ no. tested)	Percent
Shigella	53	53/53	100	53/53	100
Edwardsiella	18	18/18	100	18/18	100
Arizona	24	24/24	100	24/24	100
Providencia	21	21/21	100	21/21	100
P. rettgeri	9	9/9	100	9/9	100
P. mirabilis	103	103/103	100	103/103	100
Pseudomonads	103	103/103	100	103/103	100
Escherichia	281	273/281	97.2	279/281	99.3
Salmonella	63	61/63	96.8	61/63	96.8
Enterobacter	258	249/258	96.5	258/258	100
Citrobacter	16	15/16	93.8	15/16	93.8
Klebsiella	266	247/266	92.9	263/266	98.9
P. vulgaris	66	61/66	92.4	66/66	100
Serratia	31	28/31	90.3	28/31	90.3
P. morganii	43	24/43	55.8	35/43	81.4

TABLE 2. *Organisms misidentified by PathoTec "Rapid I-D System" or PathoTec "Rapid I-D System" with the OD and ONPG strips added, or both*

No. of strains	Misidentification with PathoTec system	Reason for misidentification	Misidentification with PathoTec plus OD and ONPG
16	*Klebsiella* as *Enterobacter*	Incorrect motility determination	None
9	*Enterobacter* as *Klebsiella*	Incorrect motility	None
6	*Escherichia* as *Shigella*	False-negative lysine strip	None
1	*Escherichia* as *Klebsiella*	False-positive malonate strip	1
2	*Salmonella* not identified	False-negative H_2S strip	2
3	*Serratia* as *Escherichia*[a]	False-negative esculin strip	3
3	*Klebsiella* as *Escherichia*[b]	False-negative esculin strip	3
1	*Escherichia* as *Klebsiella*	False-positive esculin strip	1
3	*P. morganii* not identified	False-positive esculin strip	3
11	*P. morganii* as *P. vulgaris*	False-positive H_2S strip	None
1	*Citrobacter* not identified	False-positive esculin and indole strips	1
5	*P. vulgaris* as *P. mirabilis*	False-negative indole strips	None
5	*P. morganii* not identified	False-negative nitrate strips	

[a] VP-negative strains.
[b] VP- and malonate-negative strains.

TABLE 3. *Test result agreement between PathoTec and conventional biochemical procedures*

Test	No. agreement/no. tested	Agreement (%)	False-negative strip reactions	Organisms	False-positive strip reactions	Organisms
Cytochrome oxidase	1,252/1,252	100	None		None	
Phenylalanine deaminase	1,252/1,252	100	None		None	
Ornithine decarboxylase	1,252/1,252	100	None		None	
ONPG	1,252/1,252	100	None		None	
Malonate utilization	1,247/1,252	99.5	4	*Enterobacter*	1	*Escherichia*
Nitrate reduction	1,247/1,252	99.5	5	*P. morganii*	None	
Indole production	1,246/1,252	99.5	5	*P. vulgaris*	1	*Citrobacter*
Voges-Proskauer	1,244/1,252	99.4	6	*Klebsiella*	2	*Klebsiella*
Lysine decarboxylase	1,242/1,252	99.2	6	*Escherichia*	None	
			4	*Klebsiella*		
H_2S detection	1,234/1,252	98.6	5	*P. mirabilis*	11	*P. morganii*
			2	*Salmonella* sp.		
Esculin hydrolysis	1,230/1,252	98.2	4	*Enterobacter*	1	*Escherichia*
			3	*Serratia*	8	*Citrobacter*
			3	*Klebsiella*	3	*P. morganii*
Urease production	1,129/1,252	90.2	3	*Citrobacter*	20	*Klebsiella*
			83	*Klebsiella*		
			17	*Enterobacter*		

the number and types of biochemical tests required, based on the information deemed necessary by the examiner. The strip tests are stable for at least 2 years and require very little storage space.

Identification of microorganisms with strip tests differs in several parameters from conventional procedures and, as with all variations from the established procedures, these differences have generated controversy. In a previous publication (3), this investigator reported on the laboratory utility of the PathoTec concept and described the need for using several colonies from the primary isolation plate as the inoculum source. This is a requirement for the PathoTec System because the inoculum is used as a source of enzyme to rapidly measure a biochemical event and not as a measure of the ability of an organism to grow in a biochemical environment. A multiple colony inoculum is, however, different from the established isolation procedure where the top of one colony is inoculated to a growth support system to insure purity of the resultant subculture. Where time and/or availability of personnel are not factors in laboratory operations and where a number of

similar individually picked colonies from each plate are carried independently through an identification procedure, single colony picks are obviously highly satisfactory and even preferred. In the clinical laboratory, however, it is common practice for the bacteriologist to select a single colony as an inoculum source and assume that all other colonies having the same morphological appearance represent the same organism as is present in the selected colony. This leads the microbiologist to select one or two representative colonies of each visible morphological type colony present and assume that his selection is representative of the population present on the primary plate. Barry (1) indicated that, by working on the same assumption, the reverse is also valid, namely, that one can select several colonies which are morphologically identical and assume that they represent a single organism. It is the belief of this investigator that, with the proper use of at least two selective or differential primary isolation media, or both, this concept can be of significant advantage to the clinical laboratory. Primary plates must be examined with care, and it is essential that each colony chosen for strip reactions be well isolated. There must also be a sufficient number of these well-isolated colonies to allow preparations of an adequate inoculum. When isolation plates are properly streaked for isolation, we have found in this study, as well as in a previous study, that 95% of all plates examined were suitable for multiple pick inocula. In those cases where insufficient inoculum was available on the primary plates for strip tests, another commercially available product was used.

Few new approaches to technology can be used directly without an understanding or performance based on experience, and PathoTec is no exception. In the present study, there were 123 instances in which the strip urease reaction differed from the conventional method. This resulted in 25 errors in identification to genus (16 *Klebsiella* called *Enterobacter* and 9 *Enterobacter* called *Klebsiella*). Inclusion of the OD strip prevented this error. Incorrect urease reactions were also found in 3 of 16 *Citrobacter* sp., but these did not result in incorrect identification. In 11 *P. morganii* strains, the H_2S test strip was incorrectly positive. Although the manufacturer makes note of this characteristic in the identification chart supplied with the product, no provision is made for determining a false-positive from a true-positive reaction. Therefore, any organism which was phenylalanine deaminase, urease, indole, and H_2S positive had to be identified as a *P. vulgaris* by the 10-strip system. The inclusion of the OD experimental strip eliminated this error, since *P. vulgaris* is uniformly negative, whereas *P. morganii* is uniformly positive. Of the 11 strains of *P. morganii* which produced false-positive H_2S strip reactions, 9 eventually produced small amounts of H_2S in triple sugar iron (4 to 7 days).

There were six strains of *E. coli* which were lactose and lysine decarboxylase negative and were identified as serologically untypable members of the genus *Shigella*. The inclusion of the ONPG experimental test strip completely eliminated this identification error. Some problems were encountered with the esculin hydrolysis test strip. Based on the data of Wasilauskas (4), the rapid hydrolysis of esculin by members of the family *Enterobacteriaceae* is characteristic of the K-E-S group. Edwards and Ewing (2), however, indicate that some strains of *Proteus* and *Citrobacter* also rapidly hydrolize esculin. In this study there were three strains of *Klebsiella* and three strains of *Serratia* incorrectly identified as *E. coli* due to false-negative esculin reactions coupled with negative malonate and Voges-Proskauer reactions.

In summary, the number of cultures tested in this study was reasonably extensive and represents enteric organisms isolated in this laboratory over a 6-month period. The PathoTec Rapid I-D System allowed us to provide "same day" identification of 95% of the *Enterobacteriaceae* isolated with an accuracy of 94.7%. With the addition of experimental ornithine decarboxylase and ONPG test strips, correct identification was increased to 98.5%. Although the use of this system does require some modification of established procedures and some level of change in thinking in the laboratory, it also provides a highly useful system for identification of the bulk of the daily routine work and makes more time available for those organisms or specimens which require more extensive examination or biochemical analysis.

LITERATURE CITED

1. Barry, A. L. 1971. Letter to the editors: identifying enteric bacteria directly from primary isolation media. Amer. J. Clin. Pathol. **56**:250.
2. Edwards, P. R., and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
3. Rosner, R. F. 1970. Identification of enteric bacilli directly from primary isolation media using reagent-impregnated paper strips. Amer. J. Clin. Pathol. **54**:587-595.
4. Wasilauskas, B. L. 1971. Preliminary observations on the rapid differentiation of the K-E-S group on bile esculin agar. Appl. Microbiol. **21**:162-163.