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Evaluation of the PathoTec "Rapid I-D System"

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The 10 biochemical test strips included in the PathoTec Rapid I-D System were evaluated for accuracy as compared to standard tests and for efficacy in identification of 193 gram-negative bacilli. The test agreement was 100% for oxidase and phenylalanine deaminase, 99% for indole, nitrate, and Voges-Proskauer, 98% for malonate, 97% for lysine decarboxylase, 90% for urease, 84% for H_2S, and 75% for esculin hydrolysis. Most of the commonly isolated *Enterobacteriaceae* were identified correctly within 4 h. Errors in identification of *Proteus morganii* and *P. rettgeri* occurred because of positive H_2S tests on the PathoTec strips with these organisms.

The PathoTec system consists of 10 reagent-impregnated strips designed to determine biochemical characteristics of the *Enterobacteriaceae* within 4 h. Previous performance tests evaluating some of these strips showed the accuracy of some and deficiencies of others (1, 5). Based on these evaluations, many of the strips have been redesigned and are now available either separately or in a kit of 10 for the identification of the *Enterobacteriaceae*. The present study was carried out to determine the accuracy of each individual strip and the efficacy of the system in identifying enteric organisms.

MATERIALS AND METHODS

The 193 strains of bacteria used in this study consisted of fresh clinical isolates where available and of stock cultures which had been maintained in our laboratory in Trypticase soy agar (BBL) deeps at room temperature in the dark. As far as possible, an effort was made to examine equal numbers of cultures from each species in the *Enterobacteriaceae*. All organisms were identified according to Edwards and Ewing (4). Prior to testing, the organisms were streaked to MacConkey agar plates to check for purity and as a source of inoculum for both the PathoTec and standard tests.

PathoTec strips (kindly supplied by the General Diagnostics Division of Warner-Lambert Co., Morris Plains, N.J.) were used following the manufacturer's directions; several colonies from the MacConkey plate were employed as inoculum. Standard tests were inoculated from the same MacConkey plate at the time the PathoTec tests were performed. The prepared standard media were obtained from Gibco Microbio Laboratory, Madison, Wis.; all media were tested for positive and negative reaction by using known organisms. Incubation for all standard tests was at 35 C. Procedures for cytochrome oxidase, nitrate reduction, phenylalanine deaminase, urease (Christensen urea), indole, H_2S (triple sugar iron agar [TSI]), lysine decarboxylase (Moeller method), Voges-Proskauer (VP), and malonate utilization were performed as described by Edwards and Ewing (4). For hydrolysis of esculin, esculin agar slants were used. When needed, motility tests were performed in MIO medium (3).

On the basis of results obtained with the PathoTec strips, organisms were identified according to the flow chart and the checkerboard identification chart in the PathoTec package insert.

RESULTS

The overall results obtained in the comparison of the PathoTec strips with standard tests are shown in Table 1. Seven of the tests showed 97% or greater agreement. There was a 100% correlation in the oxidase and phenylalanine deaminase tests. With indole, the correlation was 99%; 2 strains of *Proteus rettgeri* gave a negative indole test with the PathoTec strips. The nitrate test also showed 99% correlation, with 2 strains of *Serratia* negative by PathoTec. Likewise, there was 99% agreement in the VP test; 1 strain of *Enterobacter liquefaciens* gave a negative with PathoTec, and 1 *Proteus vulgaris* isolant showed a positive on the PathoTec strip. In the malonate determinations, the correlation was 98%; 2 strains of *Enterobacter agglomerans* and 1 *Pseudomonas aeruginosa* were positive with PathoTec and negative with the standard test. The lysine decarboxylase test showed a 97% correlation; 5 strains of *Enterobacter cloacae* were positive with PathoTec and negative with the Moeller method.

Tests for urease, H_2S, and esculin hydrolysis yielded a correlation of 90%, 84%, and 75%,

TABLE 1. *Results of PathoTec and standard tests*

Test	No. in agreement[a]	PathoTec +, standard −	PathoTec −, standard +	Agreement (%)[b]
Oxidase	193	0	0	100
PAD[c]	193	0	0	100
Indole	191	0	2	99
Nitrate	191	0	2	99
Voges-Proskauer	191	1	1	99
Malonate	190	3	0	98
Lysine	188	5	0	97
Urease	174	0	19	90
H_2S	163	27	3	84
Esculin	146	21	26	75

[a] Total of 193 organisms tested.
[b] Average, 94.3
[c] Phenylalanine deaminase.

respectively. With the urease test, the PathoTec method was negative in 19 cases (2 *Citrobacter freundii*, 10 *Citrobacter diversus*, 1 *E. liquefaciens*, 4 *E. cloacae*, 1 *P. vulgaris*, and 1 *P. rettgeri*) whereas the Christensen urea gave evidence of urease production in 24 h with these organisms. In the case of H_2S production, the PathoTec strips gave a positive test with 2 *Pseudomonas maltophilia*, 2 *P. rettgeri*, 10 *Proteus morganii*, 4 *Providencia* sp., and 2 *E. cloacae*; none of these organisms showed any blackening on TSI after 24 h. In contrast, with 2 strains of *Salmonella typhi* and 1 strain of *Salmonella typhimurium* the PathoTec strips were negative, whereas a small amount of blackening was seen on TSI within 24 h.

All of the above 9 tests were very easy to read as positive or negative, with the color reactions being very distinct. The test which showed the least agreement (75%), esculin hydrolysis, was very difficult to read on the PathoTec strips. Often there was a very dark greenish color which might easily be confused with the gray or black considered to be positive by the manufacturer. It was difficult to obtain agreement between more than 1 person on how to read these strips.

The accuracy of identification of the 193 organisms using the 10 PathoTec tests and the manufacturer's flow chart and checkerboard identification chart is shown in Table 2. *Arizona*, *C. diversus*, *Edwardsiella*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella*, *Proteus mirabilis*, *Shigella*, and *Yersinia* were all identified correctly. Nine out of 10 *P. vulgaris*, 7 out of 10 *C. freundii*, 6 out of 10 *Salmonella*, 4 out of 8 *Providencia*, 4 out of 10 *E. cloacae*, and 1 out of 10 of *P. rettgeri* were identified correctly; additional tests were necessary to identify 1 strain of *C. freundii*, 4 *Salmonella*, 4 *Providencia*, and 5 *E. cloacae*. None of the *P. morganii* were identified correctly. For the remaining organisms tested, *E. agglomerans*, *Serratia*, *Acinetobacter*, *P. aeruginosa*, and *P. maltophilia*, additional tests would be indicated as suggested by the manufacturer. Four stains of *P. maltophilia* were both oxidase negative and nitrate positive, which would indicate they were members of the *Enterobacteriaceae* according to the PathoTec flow chart. Further tests would also be indicated for these organisms. The overall accuracy of identification for those organisms with no additional tests indicated was 88%.

The discrepancies responsible for incorrect identification are noted in Table 3. The misidentified *C. freundii* were unusual in that both were H_2S negative, and 1 was indole positive with both standard and PathoTec tests. Our indole-negative *P. vulgaris* was identified as *P. mirabilis* on the basis of the PathoTec chart.

TABLE 2. *Accuracy of identification using the PathoTec "Rapid I-D System"*

Organism	No. correct/no. tested	Correct (%)
Arizona	9/9	100
Citrobacter diversus	10/10	100
Edwardsiella	7/7	100
Enterobacter aerogenes	10/10	100
Escherichia coli	10/10	100
Klebsiella	10/10	100
Proteus mirabilis	10/10	100
Shigella	10/10	100
Yersinia enterocolitica	2/2	100
Proteus vulgaris	9/10	90
Citrobacter freundii	7/10[a]	70
Salmonella	6/10[a]	60
Providencia	4/8[a]	50
Enterobacter cloacae	4/10[a]	40
Proteus rettgeri	1/10	10
Proteus morganii	0/10	0
Enterobacter[b]	?/7	Additional tests indicated
Serratia	?/10	Additional tests indicated
Acinetobacter	?/10	Additional tests indicated
Pseudomonas aeruginosa	?/10	Additional tests indicated
Pseudomonas maltophilia	?/10	Additional tests indicated

[a] Additional tests indicated for some strains.
[b] Four *E. liquefaciens*, one *E. hafniae*, and two *E. agglomerans*.

TABLE 3. *Errors in identification using the flow chart and checkerboard identification chart of PathoTec*

Organism	PathoTec identification	Reason
Citrobacter freundii	*C. diversus*	H_2S −,[a] indole −, urease +
C. freundii	*E. coli*	H_2S −,[a] indole +,[a] urease −
Enterobacter cloacae	*E. aerogenes*	lysine +
Proteus vulgaris	*P. mirabilis*	indole −[a]
P. morganii	*P. vulgaris* (10)	H_2S +
P. rettgeri	*P. mirabilis* (2)	H_2S +, indole −
P. rettgeri	*P. vulgaris* (7)	H_2S +

[a] Aberrant strain characteristic.

This organism was indole negative by both standard and PathoTec tests but had been confirmed as *P. vulgaris* because it was ornithine decarboxylase negative. The errors in identification of *P. morganii* and *P. rettgeri* were due to the fact that these organisms were H_2S positive on the PathoTec strips while negative for H_2S on TSI.

DISCUSSION

The PathoTec system differs in three ways from other kits now available for the identification of *Enterobacteriaceae*. First, it is designed to enable one to obtain answers within 4 h and thus identify or generate significant data from organisms on the same day they are isolated. Secondly, it is available as separate biochemical tests and provides a flexible system which allows the user to select individual tests as indicated. In addition, all substrate and detection reagents (except KOH for the VP test) are dry on the strips, resulting in prolonged stability and allowing test results to be read immediately after the incubation period is over.

As a total system, using all 10 strips, we were able to identify correctly the organisms most commonly isolated in the clinical laboratory within 1 day. Others, such as *Salmonella* or *Shigella*, could be rapidly confirmed by serological tests if a sufficient amount of growth is present. The rapidity of the PathoTec system is surely a great advantage in the microbiology laboratory where, heretofore, identification of gram-negative organisms has of necessity required at least 24 h. However, the misidentification of other organisms which are not rare (*P. morganii*, *P. rettgeri*, and *Providencia*) necessitates another look at the test responsible for these errors, namely the H_2S strip.

The H_2S strip uses sulfur-containing amino acids (probably cysteine) as substrate and lead acetate as an indicator. Because of the extreme sensitivity of lead acetate and the fact that most of the *Enterobacteriaceae* will produce H_2S from cysteine, the manufacturers have included an H_2S-adsorbent zone on the strip between the substrate and the indicator. This adsorbs out trace amounts of H_2S, allowing for detection of only larger amounts of H_2S so that results should compare with those obtained with either Kligler's or TSI, the standard tests which make H_2S production useful in the differentiation of *Enterobacteriaceae*. It is possible that the blackening obtained with the lead acetate is due to substances other than H_2S. Rodler et al. (6) have shown that volatile mercaptanes may be produced from cysteine by some organisms and that mercaptanes produce a black color in the presence of lead acetate. If this is true of *Proteus* and *Providencia*, it could account for the false-positive tests for H_2S.

It seems incongruous that false-negative results for H_2S would be obtained with organisms showing a definite (though slight) blackening on TSI after 24 h. This may be explained on the basis that TSI contains thiosulfate as a substrate for H_2S production (in addition to cysteine which may be present in the peptone base). Since different enzymes are responsible for H_2S production from thiosulfate and cysteine (2), this could account for a positive test on TSI and a negative test on the PathoTec strip. Although the manufacture indicates (personal communication) that thiosulfate has been added to the strip, the formulation of the substrate zone may vary enough from TSI to account for the different results. Perhaps it would either be possible to use thiosulfate as the strip substrate and eliminate the adsorption zone or to use a less sensitive indicator and one which would not give a black color with mercaptanes.

If the errors with the H_2S strips were corrected, this would eliminate the 19 misidentifications of *P. morganii* and *P. rettgeri*, thus resulting in an identification accuracy of almost 98%. Since these organisms were not spreaders, the astute microbiologist would probably have questioned their identification as *P. vulgaris* or *P. mirabilis*. Observations of colonial morphology along with the biochemical tests will improve the accuracy of any identification system.

It would be helpful if the PathoTec flow chart and checkerboard identification chart indicated that *C. freundii* can be H_2S negative. A suggestion that adonitol fermentation should be added to differentiate the H_2S-negative *Citrobacter* would also be helpful.

The discrepancies between the tests for urease are not surprising. Christensen urea is

known to be a very sensitive detector of urease activity, and evidently the PathoTec strip is not designed to this same level of sensitivity. This is not a serious problem, however, as no misidentifications occurred because of a urease reaction.

Although the 10 PathoTec tests do not allow for identification of all organisms and additional tests were indicated for some of the strains, the decreased time necessary for identifying common isolates is definitely an advantage to the laboratory.

The accuracy and ease of reading of the tests for oxidase, phenylalanine deaminase, indole, nitrate, VP, malonate, and lysine decarboxylase makes the use of these rapid tests very helpful in the clinical laboratory. Any of these rapid tests are extremely useful additions to any identification system, providing information when needed within 4 h. This enables the microbiologist to use a minimum and more economical number of tests for the identification of the common organisms, with the possibility of adding extra tests as required without delaying identification.

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