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Evaluation of the Repliscan System for Identification of *Enterobacteriaceae*

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The Repliscan system, a semiautomated method for identifying gram-negative bacilli, was evaluated for its potential usefulness in clinical microbiology laboratories. A total of 1,877 isolates, including 1,712 fermentative and 165 nonfermentative organisms, were tested in parallel with the Repliscan and Enterotube methods of enteric identification. Discrepancies were retested in each system as well as with conventional methods. The Repliscan method correctly identified 91%, misidentified 2%, and failed to identify 7% of the fermentative organisms tested. The system consistently failed to recognize satisfactorily nonfermentative organisms. Of the genera under study, *Enterobacter* posed the greatest problem to the system in terms of overall identification rates. The Repliscan appears to be an efficient, economic, and effective laboratory tool for identification of *Enterobacteriaceae*.

The accurate identification of *Enterobacteriaceae* family members represents a considerable expense for a microbiology laboratory in terms of a technologist's time and material costs (6). During the past decade, a considerable amount of effort has been directed toward the development of a variety of kits, systems, methods, and procedures for identifying these organisms. Commercial kits now available have proven to be highly reliable (10) but are relatively costly. Inexpensive replicate-plating techniques have been used for many years for agar dilution antibiotic susceptibility testing (3, 8) and are now being used in a limited way for the identification of microorganisms (1, 5, 9). The recent computerization of these methods (7; B. Filburn, F. Houston, V. Shull, W. L. Krause, and P. Charache, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C165, p. 304) has led to more accurate identifications and a decrease in overall cost. The Repliscan system (Cathra International, Ontario, Canada) is a commercially available replica-plating method for the identification of *Enterobacteriaceae*. This report presents the results of an evaluation of the computerized system for its accuracy in identifying fermentative, gram-negative bacilli.

MATERIALS AND METHODS

Cultures tested. A total of 1,786 gram-negative bacilli, including 1,621 fermentative and 165 nonfermentative strains, were selected from clinical specimens submitted to a microbiology laboratory. An additional 91 stock organisms were utilized to provide species that are normally encountered infrequently. Procedures for isolation and identification of clinically important bacteria have been described (2, 4). All organisms were assigned a numerical code and identified blindly by the respective test methods. Nonfermentative organisms were included in this evaluation in an effort to exceed the stated capabilities of the system and to test for possible false identification as a fermentative species.

Identification methods. All organisms were identified in parallel by using the Enterotube identification system (Roche Diagnostic Div., Nutley, N.J.) and the Repliscan method. Discrepancies were retested in each system. The Enterotube test system was inoculated, incubated, and read in accordance with the manufacturer's instructions. Since the performance characteristics of the Enterotube have been firmly established (10), further evaluation of this product is beyond the scope of this publication. Classical tests (2) were used to identify all stock culture organisms, as well as to resolve discrepancies between the Enterotube and Repliscan systems.

Repliscan system. The Repliscan system consists of a variety of biochemical and antibiotic plated media (Table 1), a multiple-inoculum replicating device, a viewing table allowing visual inspection and electronic recording of individual reactions, and a computer terminal complete with hard-copy printout. The plated biochemical media were purchased from the manufacturer and stored as directed. Antibiotic-containing medium was prepared as recommended by the International Collaborative Study (ICS) (3) and as described in detail elsewhere by Washington and Barry (11) for agar dilution susceptibility testing. All plates were inoculated according to the manufacturer's instructions. One to three colonies of the bacterium to be identified were inoculated into 2 ml of Mueller-Hinton broth and grown to a turbidity equal to that of a 0.5 McFarland barium sulfate turbidity standard. One-

TABLE 1. *Basic test components of Repliscan system*

Identification	Susceptibility testing	
	Antibiotic	μg/ml
Mueller-Hinton (growth control)	Tetracycline	4
Citrate	Chloramphenicol	16
Lysine decarboxylase	Kanamycin	8
Ornithine decarboxylase	Cephalothin	8
Urea	Gentamicin	4
Deoxyribonuclease	Ampicillin	8
Colistin		
Cephalothin		
H_2S		
Bile esculin		
Arginine dihydrolase		
Glucose		
Lactose		
Sucrose		
Mannitol		
Inositol		
Arabinose		

half milliliter of this suspension was then placed in an individual well of the replicating device and inoculated onto each of the 23 biochemical and antibiotic plates. The replicating device allows the simultaneous inoculation of up to 36 organisms on a single agar plate. All plates were incubated aerobically at 35°C for 18 to 20 h. The presence of 3% agar in the biochemical medium effectively inhibited the swarming of *Proteus* spp., as well as limiting the diffusion of metabolic products. After incubation, the plates were arranged on a loading tray and inserted into the viewing table. The growth of a single isolate on each of the 23 types of media was observed by transmitted light through individual windows in the viewing table. Positive and negative reactions were recorded by using a pen light and photosensitive receptors located directly adjacent to the viewing window for each test plate. When the reactions on all test plates had been recorded, the computer analyzed the information entered and provided the identification of the organism. The limited antibiogram was used by the computer to confirm the biochemical identification of the organism. When a given isolate had been identified, the viewing table automatically advanced the plates to the next reading position, and the identification cycle was repeated for each of the 36 isolates per plate.

Because the formulation of some of the media used in the Repliscan system differs considerably from that of conventional media, certain tests produce results that differed from those reported by Edwards and Ewing (2) and Ewing and Martin (3). Since, however, the manufacturer's computerized data base was developed independently of the classical reactions of Edwards and Ewing (2), a test-by-test comparison of individual reactions was determined to be of limited value and thus was not performed. The evaluation was designed and conducted to determine the overall accuracy of identification by the experimental system.

Antibiotic susceptibility tests. The multiple inoculator used in the Repliscan system consists of conical stainless-steel rods which taper to a point 1 mm in diameter. Since the volume delivered by these rods differs from that of a Steers replicator (8) and dilution of the inoculum is not made as for the ICS agar dilution method (3, 11), a study was undertaken to compare the minimal inhibitory concentrations (MIC) of organisms tested in each system. The ICS agar dilution technique (3) served as the reference method. A total of 242 consecutive gram-negative isolates was tested by each method. The results of the two systems were expressed as an MIC ratio (ICS method/ Repliscan method). If the MIC value for each of the two methods were identical, the resulting ratio would be 1; MICs which were within ±1 doubling dilution produced ratios of 0.5 and 2; MICs within ±2 doubling dilutions produced ratios of 0.25 and 4.

RESULTS

Biochemical identification. The Repliscan system correctly identified 91% of the 1,712 fermentative organisms tested in this evaluation (Table 2). Thirty-five organisms (2%) were incorrectly identified by the system, and an additional 120 organisms (7%) could not be identified and produced inconclusive results.

Escherichia coli and *Klebsiella pneumoniae* represented the majority of strains tested, producing correct identification rates of 97.5 and 88.8%, respectively (Table 3). *Proteus rettgeri, Providencia stuartii, Enterobacter cloacae, Enterobacter agglomerans, Enterobacter hafniae, Edwardsiella tarda,* and *Yersinia enterocolitica* provided the greatest challenge to the system with fewer than 85% of the strains being correctly identified. The remaining 10 species tested produced identification rates of greater than 85%. *K. pneumoniae* presented the largest single species identification problem to the system in terms of identification errors. Thirteen of the 341 isolates of *K. pneumoniae* (3.8%) were misidentified as *Klebsiella ozaenae* (Table 4). These isolates represented 38% of the total identification errors and were the result of falsely negative citrate reactions. All of the strains tested proved to be citrate positive by the Enterotube method and on conventional media, although many were delayed positive reactions that required 48 h of incubation. The genus *Enterobacter* also represented 38% of the identification errors with no consistent explanation for the misidentifications. There were no trends established within a given

TABLE 2. *Organism identification summary*

Organism characteristic	No. tested	% Correct	% Error	% Inconclusive
Fermentative	1,712	91.0	2.0	7.0
Nonfermentative	165	73.9	1.2	24.8

TABLE 3. *Organisms tested*

Organism	No. tested	% Correct	% Error	% Inconclusive
Escherichia coli	640	97.5	0.3	2.2
Klebsiella pneumoniae	341	88.8	3.8	7.3
K. ozaenae	2	100	0	0
Proteus mirabilis	137	97.8	0	2.2
P. morganii	55	94.5	0	5.5
P. vulgaris	39	92.3	2.6	5.1
P. rettgeri	21	76.2	0	23.8
Providencia stuartii	15	60	7	33
Enterobacter cloacae	125	70.4	6.4	23.2
E. aerogenes	72	93.1	0	6.9
E. agglomerans	16	18.7	25.0	56.3
E. hafniae	6	17	33	50
Serratia marcescens	71	95.7	1.4	2.8
Citrobacter diversus	45	97.8	0	2.2
C. freundii	52	85.6	3.9	10.5
Salmonella	32	100	0	0
Shigella	30	93	0	7
Arizona hinshawii	7	86	0	14
Edwardsiella tarda	2	50	0	50
Yersinia enterocolitica	4	0	25	75
Pseudomonas aeruginosa	97	97.9	0	2.1
P. maltophilia	10	80	0	20
Acinetobacter calcoaceticus	34	11.7	2.9	85.3
Miscellaneous	24	62.5	4.2	33.3

species and no obvious cause for the inconclusive or wrong identifications; however, the number of strains tested was insufficient for an in-depth analysis of the identification errors.

Seven percent of the organisms studied produced inconclusive results and were not identified by the Repliscan's computerized datum base. The highest percentage of inconclusive biochemical patterns was from species within the genus *Enterobacter*. No explanation can be offered for the relatively poor performance in this area other than possible inadequacies within the datum base itself. The small number of strains studied prevented the recognition of specific trends or "biotypes."

Although oxidase-psoitive, nonfermentative organisms are specifically beyond the stated capabilities of the system, a limited number of these strains were included in the study. Recognition of the biochemical pattern as belonging to a nonfermentative species was recorded as a correct response since genus and species designations were beyond the scope of the instrument. Of the 165 nonfermentative organisms studied, 122 (73.9%) were recognized as not belonging to the family *Enterobacteriaceae* (Table 2). Two organisms, *Aeromonas hydrophila* and *Acinetobacter calcoaceticus*, were misidentified as *E. coli* (Table 4) and represented an error rate of 1.2%. The remaining isolates (24.8%) were not recognized by the system's computerized datum base.

Antibiotic susceptibilities. Comparison of the ICS and Repliscan methods of susceptibility testing revealed that 99.4% of the end points were within a range of ± 1 $\log_2$ dilution (Table 5). All but six end points (0.2%) fell with a range of 2 dilutions (data not shown). The greatest variation was noted for ampicillin, cephalothin,

TABLE 4. *Errors in identification by Repliscan system*

Organism (no.)	Incorrectly identified as:
Klebsiella pneumoniae (13)	*K. ozaenae*
Citrobacter freundii (2)	*E. coli*
Enterobacter cloacae (3)	*E. agglomerans*
E. cloacae (2)	*C. freundii*
E. cloacae (3)	*C. diversus* or *E. agglomerans*
E. agglomerans (1)	*E. coli*
E. agglomerans (1)	*C. freundii*
E. agglomerans (2)	*K. rhinoschleromatis*
E. hafniae (1)	*K. ozaenae*
E. hafniae (1)	*E. coli*
Serratia marcescens (1)	*S. liquefaciens*
Proteus vulgaris (1)	*P. mirabilis*
Escherichia coli (1)	Afermentative
E. coli (1)	*K. ozaenae*
Providencia stuartii (1)	*P. alcalifaciens*
Yersinia enterocolitica (1)	*E. coli*
Aeromonas hydrophila (1)	*E. coli*
A. calcoaceticus (1)	*E. coli*

TABLE 5. *Comparison of ICS and Repliscan methods for the quantitative susceptibility tests*

Antibiotic	MIC[a] ratio (% of strains)				
	≤0.25	0.5	1.0	2.0	≥4.0
Amikacin	0.0	0.0	99.6	0.4	0.0
Ampicillin	1.6	10.7	83.1	4.5	0.0
Carbenicillin	0.4	5.8	92.1	1.6	0.0
Cephalothin	1.6	14.9	77.3	5.8	0.4
Chloramphenicol	0.8	5.0	79.3	13.2	1.6
Gentamicin	0.0	4.1	94.6	1.2	0.0
Kanamycin	0.0	2.0	97.5	0.4	0.0
Nalidixic acid	0.0	1.6	98.3	0.0	0.0
Nitrofurantoin	0.0	3.7	94.6	1.6	0.0
Tetracycline	0.4	0.8	71.9	26.5	0.4
Tobramycin	0.0	1.2	96.3	2.5	0.0
Trimethoprim-sulfamethoxazole	0.0	0.4	98.8	0.8	0.0
% of all tests	0.4	4.2	90.3	4.9	0.2

[a] MIC ratio, ICS/Repliscan, see text.

chloramphenicol, and tetracycline. Repliscan MIC values were generally lower than ICS values for chloramphenicol and tetracycline but higher for ampicillin and cephalothin. End point disagreement was evenly distributed among all species tested. All remaining antibiotics produced end point agreement in greater than 92% of the strains tested.

DISCUSSION

The results of this evaluation showed a good correlation between the Repliscan and conventional identifications. Many of the identification errors could be eliminated by adding one or more confirmatory tests. When for example, an organism is identified by Repliscan as *K. ozaenae*, a Simmons citrate test could be inoculated and held for 48 h. The final identification should reflect the results of the additional test result.

The positive and negative biochemical reactions were easily identifiable in most instances. Ornithine decarboxylase and arginine dihydrolase occasionally produced ambiguous results. In many cases, these questionable reactions did not affect the final identification of the organism in question. In other instances, the ambiguous reactions led to inconclusive identifications, and the organisms required additional testing for us to achieve proper identification.

The turbidity of the inoculum in the ICS agar dilution method (3, 11) is usually adjusted to match that of one-half of a McFarland no. 1 barium sulfate standard and is diluted 1:20 before application on the agar surface, usually by means of a Steers replicator (8), each inoculating prong of which is 3 mm in diameter. The MIC is determined by examining the agar surface with reflected light. With Repliscan the adjusted but undiluted inoculum is applied with an inoculating prong 1 mm in diameter, and the MIC is determined by examining the agar with transmitted light. Despite these technical differences, MIC values obtained with each method did not differ significantly. Moreover, fine, barely visible hazes, ignored in the ICS method (3, 11), are not visible with transmitted light, so that end points in the Repliscan were more definite.

The Repliscan system was a simple, economic, and efficient method for the identification of fermentative, gram-negative bacilli. Inoculation of plates, reading, and recording of results can be accomplished in approximately 1.5 min per organism. Mixed cultures were easily recognized on the solid media.

The economy realized in using the system takes two forms. The primary savings is in the form of decreased media costs. Since up to 36 organisms can be inoculated on a single plate, the cost of performing a given test was nominal ($0.02). Assuming 100% utilization (36 organisms per plate), the material cost for the identification of *Enterobacteriaceae* was determined to be $0.46 per isolate. This figure represents less than 20% of the costs of the API 20E or conventional seven-tube sets (6). Identification of only seven organisms per plate would cost $2.37 per isolate, a figure which is roughly comparable to the cost of many commercial systems. No allowances were made for overhead expenses, instrumentation costs, or the technologist's time. A further savings was noted with the merging of the biochemical testing with an agar dilution susceptibility method. Combining these two methods has allowed efficient work organization and a decreased requirement for technologists' time.

Whereas the day-to-day operating expense of the system is quite low, the initial investment in instrumentation is rather substantial. For this reason, the feasibility studies of the system must be determined by individual laboratories on the basis of need and the total number of isolates tested on a daily basis. Laboratories identifying more than 18 organisms per day could expect the system to become cost-effective within 4 years based on material savings alone. A smaller, less expensive model, known as the Replireader (not evaluated in this study) could be expected to become cost-effective in approximately 2 years or less.

Replicate-plating methods provide a distinct advantage in terms of quality control of media used in the system. Four quality-control organisms can be inoculated on each type of medium and identified as usual along with 32 other isolates. *E. coli, Pseudomonas aeruginosa, Proteus mirabilis,* and *Serratia marcescens* were selected as quality-control organisms because of their ability to provide a positive and negative response for each reaction tested. Tabulation of the control reactions was facilitated by the use of hexadecimal code numbers provided by the computer which identified the reactions of all the biochemical tests.

The major limitation to the system was the fact that 7% of the fermentative organisms tested produced inconclusive identifications; however, this percentage does not exceed that which we have noted with other systems for identifying the *Enterobacteriaceae* (10). These organisms required identification by conventional methods, resulting in another 24 to 48 h delay in reporting. As more data are obtained, additional identification profiles can be entered into the computer datum base of the system and hopefully reduce the number of strains requiring additional testing. Until this can be accomplished, a suitable

back-up identification system must be maintained to identify accurately those organisms producing inconclusive results on the Repliscan system. This requirement poses no problem to larger clinical centers but may adversely affect small hospital laboratories.

The necessity for a back-up system, together with the initial instrumentation cost, places the Repliscan system beyond the limits of practicality for many smaller laboratories. However, the Replireader system (a method using the Repliscan datum base but less instrumentation) may be well within the grasp of laboratories identifying as few as 10 organisms per day.

In conclusion, the results of this study indicate that the Repliscan system for identification of *Enterobacteriaceae* compared favorably with conventional methods. The advantages of decreased media costs and technologist's time outweigh the present limitations and the initial expense of the system. This method of enteric identification promises to be a valuable adjunct to a clinical laboratory which identifies large numbers of isolates daily.

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