ORIGINAL ARTICLE

Evaluation of the ID 32E for the identification of Gram-negative glucose-fermenting and glucose-non-fermenting bacilli

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Objective: To evaluate the ID 32E bacterial identification system for accuracy in the identification of members of the family Enterobacteriaceae, *Pseudomonas aeruginosa, Stenotrophomonas maltophilia,* and *Acinetobacter baumannii/lwoffii.*

Methods: Stock cultures of 497 Enterobacteriaceae and 27 commonly encountered non-enteric Gram-negative rods were tested in the ID 32E system. For each isolate, the resulting 11-digit profile number was converted to an identification using the APILAB Plus software (version 3.2.2). This identification was then compared to the reference identification obtained using conventional biochemicals.

Results: Of the 524 isolates tested, 405 (77.3%) were identified correctly; 52 (9.9%) were identified incorrectly. Sixty-seven (12.8%) identifications were either doubtful or unacceptable, and were not limited to any particular genus or species, with the exception of *Ewingella americana* and *Serratia plymuthica,* which did not grow well enough in the strip at 35°C to be correctly identified. All five isolates of *Acinetobacter lwoffii* were misidentified as *Alcaligenes* spp.

Conclusions: With this challenge set of organisms, the ID 32E correctly identified 77.3% of the isolates tested. For commonly encountered isolates, the accuracy approached 90%. We conclude that the ID 32E offers an alternative for the identification of common clinical isolates.

Key words: ID 32E, evaluation, Enterobacteriaceae identification

INTRODUCTION

Clinical microbiology laboratories continually search for bacterial identification products that will accomplish two goals: accuracy and speed of testing. Products that are available commercially for the identification of Enterobacteriaceae and other glucose-fermenting and non-glucose-fermenting Gram-negative rods have accuracies approaching 98% and incubation times ranging from 2 h to 24 h [1,2]. The ID 32E is a more recent product that incorporates both colorimetric and enzyme-based tests for identification of organisms. This evaluation investigated the accuracy of ID 32E kits for identification of bacterial isolates from human infections.

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MATERIALS AND METHODS

The ID 32E (bioMérieux SA, Marcy-l'Etoile, France) is a manual method for the identification of members of the family Enterobacteriaceae and other oxidase-negative and oxidase-positive glucose-fermenting and non-fermenting Gram-negative rods. It is designed to render an identification after a 24-h incubation period. The strip uses the following 32 biochemical tests in a 'cupule' format: indol production; production of lysine and ornithine decarboxylase, arginine dihydrolase, urease, lipase, and malonate; acidification of adonitol, L-arabinose, D-arabitol, L-arabitol, cellobiose, glucose, inositol, mannitol, maltose, palatinose, rhamnose, saccharose, sorbitol, trehalose and phenol red; galacturonate, 5-ketogluconate, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, β-galactosidase, α-glucosidase, α-galactosidase, α-maltosidase, and L-aspartic acid arylamidase.

Version 1.0 of the database incorporates 71 genera and species of Enterobacteriaceae, 11 genera and species of oxidase-positive glucose-fermenting bacilli, including Vibrionaceae, and 21 genera and species of oxidase-positive and oxidase-negative glucose-non-

fermenting bacilli from both human and environmental sources.

Stock cultures of 497 biochemically typical and atypical Enterobacteriaceae and 27 oxidase-negative and oxidase-positive glucose-fermenting and glucose-non-fermenting non-enteric clinical isolates were tested in this system. The collection contained strains that would be found in a clinical microbiology laboratory as well as genera and species not likely to be found in routine work, but which were contained in the database.

All isolates were taken from either room temperature (enteric organisms) or −70°C (non-enteric organisms) storage and were passed three times on trypticase soy agar with 5% defibrinated sheep blood (TSA II: Becton Dickinson Microbiology Systems, Sparks, Maryland, USA) before testing. A 0.5 McFarland suspension of each organism was prepared and the ID 32E strip inoculated by pipetting 55 µL of the suspension into each cupule. The turbidity may be estimated visually with a 0.5 McFarland standard or measured with an ATB 1550 Densitometer. Because this laboratory did not have an ATB densitometer, we used the Dade turbidity meter (Dade Behring, Inc., W. Sacramento, California, USA). The pipetting was accomplished with an ATB MicroPipette. After a 24-h incubation period at 35°C, James reagent was added to the indole well and the reaction of each cupule was read by comparing the color of each well to the reading table in the package insert.

The reactions were coded into an 11-digit numerical profile and entered into the APILAB Plus computerized software (version 3.2.2). The identification is accompanied by both the percentage of identification accuracy (%id), which is an estimate of how closely the profile corresponds to the stated taxon relative to all the other taxa in the database, and the T index, which is an estimate of how closely the profile corresponds to the most typical set of reactions for the stated taxon. The closer the T value approaches 1.0, the closer the reactions of the test strain are to a typical set of biochemical responses for that taxon. In other words, an identification can be highly accurate (99% id), but exhibit several abberant reactions causing the T value to fall as low as 0.5. There are also comments on the quality of identification derived from the %id (or sum of the %id) and the T index of the selected taxon. These comments appear as 'excellent ID' (%id>99.9 and T>0.75), 'very good ID' (%id>99.0 and T>0.5), 'good ID' (%id>90.0 and T>0.25), and 'acceptable ID' (%id>80.0 and T>0). Possible comments also include 'low discrimination' if two, three or four taxa belonging to different genera have been selected by the program, or 'not reliable' if the sum of the %id is less than 80%. If the comment 'presumptive' appears, it is suggested that the strain be sent to a reference center for supplementary identification. The profile is 'doubtful' if a taxon having several tests against the identification is present among those taxons selected. The profile is 'unacceptable' if the profile number is not close enough to any taxa of the database. There may be complementary off-line tests suggested to confirm an answer [3].

In this study, each strain identification was compared with that obtained by using reference biochemical tests, as performed at the Centers for Disease Control and Prevention (CDC) [4–6]. Commercial media were used whenever possible. Strains that were misidentified by the ID 32E on initial testing were retested in duplicate to ensure that technologist error was not the cause of the incorrect answer.

RESULTS

Tables 1 and 2 list each genus and species that was tested and the number of identifications that occurred in each category. All answers of 'acceptable' or better were classified as 'correct'. The category of 'error' included any identification that was incorrect and remained incorrect upon repeat testing. The 'low/doubtful/unacceptable' category in the table includes answers that were correct, but at levels of confidence of 'low', 'doubtful', or 'unacceptable'.

Of the 497 isolates of Enterobacteriaceae that were tested, 384 (77.3%) were correctly identified to species level and 67 (13.5%) were identified at low, doubtful or unacceptable confidence levels. There were 46 (9.3%) errors in identification. Of the 27 non-enteric organisms that were tested, 21 (77.8%) were identified correctly and 6 (22.2%) incorrectly. Collectively, 77.3% of the isolates in these two groups were correctly identified with an error rate of 9.9%.

The 67 identifications with low/unacceptable confidence levels were not limited to any particular genus or species, although six of 10 *Serratia liquefaciens*, five of 10 *Escherichia vulneris* and four of six *Yersinia pseudotuberculosis* isolates fell into this category. All five strains of *Acinetobacter lwoffii* were misidentified.

Of 10 *Ewingella americana* isolates, two were identified correctly, two were identified incorrectly, and six did not grow in the ID 32E strip. We encountered similar problems with 10 strains of *Serratia plymuthica*. Two were identified correctly, one was identified at a low probability level, and seven did not grow in the strip.

A selected subset of 138 organisms resembling the ratio of different genera and species found in routine microbiology laboratories were evaluated to assess the

Table 1 Accuracy of identification of Enterobacteriaceae between ID 32E and reference biochemicals

Reference identification by genus (no. of isolates)	Correct	Low/doubtful/ unacceptable	Error
Cedecea davisae (10)	9	1	
Cedecea lapagei (4)	4		
Cedecea neteri (3)	3		
Citrobacter amalonaticus (10)	10		
Citrobacter freundii (10)	7	1	2
Citrobacter koseri (diversus) (10)	10		
Edwardsiella tarda (10)	6	3	1
Enterobacter aerogenes (10)	8	2	
Enterobacter asburiae (10)	5	1	4
Enterobacter cancerogenus (taylorae) (10)	8		2
Enterobacter cloacae (10)	9		1
Enterobacter gergoviae (10)	9	1	
Enterobacter intermedium (1)	1		
Enterobacter sakazakii (10)	5	4	1
Escherichia coli (32)	28	4	
Escherichia fergusonii (10)	7	3	
Escherichia hermannii (10)	10		
Escherichia vulneris (10)	4	5	1
Ewingella americana (10)	2	2	6
Hafnia alvei (10)	10		
Klebsiella ornithinolytica (10)	10		
Klebsiella oxytoca (10)	7		3
Klebsiella ozaenae (10)	9	1	
Klebsiella planticola (5)	5		
Klebsiella pneumoniae (10)	8		2
Klebsiella rhinoscleromatis (10)	9		1
Klebsiella terrigena (3)	3		
Kluyvera ascorbata (5)	2	3	
Kluyvera cryocrescens (5)	3	1	1
Leclercia adecarboxylata (10)	10		
Moellerella wisconsensis (8)	6	2	
Morganella morganii (10)	9	1	
Pantoea agglomerans (10)	5	4	1
Proteus mirabilis (10)	10		
Proteus penneri (10)	6	3	1
Proteus vulgaris (11)	11		
Providencia alcalifaciens (7)	7		
Providencia rettgeri (8)	8		
Providencia rustigianii (3)	1	1	1
Providencia stuartii (14)	13	1	
Rahnella aquatilis (2)	2		
Salmonella arizonae (10)	7	3	
Salmonella choleraesuis (2)	1	1	
Salmonella paratyphi A (2)	2		
Salmonella species (12)	9	1	2
Salmonella typhi (2)	1	1	
Serratia ficaria (4)	4		
Serratia fonticola (10)	10		
Serratia liquefaciens (10)	3	6	1
Serratia marcescens (10)	9		1
Serratia odorifera (10)	8	2	
Serratia plymuthica (10)	2	1	7
Serratia rubidaea (10)	9		1
Shigella spp.1 *(boydii)* (2)	2		
Shigella spp.2 *(dysenteriae)* (2)	1		1
Shigella spp.3 *(flexneri)* (2)	1		1
Shigella sonnei (4)	3	1	
Yersinia enterocolitica (12)	6	3	3
Yersinia frederiksenii (2)	2		
Yersinia intermedia (2)	1		1
Yersinia kristensenii (2)	2		
Yersinia pseudotuberculosis (6)	2	4	
Total (497)	384	67	46
	77.30%	13.50%	9.30%

Table 2 Accuracy of identification of selected non-Enterobacteriaceae between ID32 E and reference biochemicals

Reference identification by genus (no. of isolates)	Correct	Low/doubtful/ unacceptable	Error
Acinetobacter baumannii (7)	6		1
Acinetobacter lwoffi (5) – *Acinetobacter/Moraxella*			5
Pseudomonas aeruginosa (10)	10		
Stenotrophomonas maltophilia (5)	5		
Total (27)	21		6
	77.8%		22.2%

Table 3 Accuracy of identification of a weighted assortment of clinical isolates

Reference identification by genus (no. of isolates)	Correct	Low/doubtful/ unacceptable	Error
Acinetobacter baumannii (6)	6		
Acinetobacter lwoffii (1)			1
Citrobacter freundii (6)	5		1
Citrobacter koseri (4)	4		
Enterobacter aerogenes (6)	5	1	
Enterobacter cloacae (10)	9		1
Escherichia coli (32)	28	4	
Klebsiella pneumoniae (10)	8		2
Klebsiella oxytoca (6)	3		3
Morganella morganii (4)	4		
Proteus mirabilis (10)	10		
Proteus vulgaris (10)	10		
Providencia stuartii (2)	2		
Pseudomonas aeruginosa (10)	10		
Salmonella spp. (4)	4		
Serratia marcescens (10)	9		1
Shigella spp. (2)	2		
Stenotrophomonas maltophilia (5)	5		
Totals (138)	124	5	9
	89.9%	3.6%	6.5%

clinical utility of the product (Table 3). Of these, 124 (89.9%) were identified correctly.

DISCUSSION

The ID 32E is designed to identify Gram-negative, glucose-fermenting and glucose-non-fermenting, oxidase-negative and oxidase-positive bacilli in a 24-h time frame. The APILAB identification program offers complementary off-line biochemical tests that may be performed to confirm or resolve an identification if it is at a low or doubtful confidence level. In this study, very few of the 67 isolates in this group had these additional tests offered. Where additional tests were suggested, they did not change the identification. Therefore, in Tables 1–3, we have no identification category called 'correct with additional testing'. Many of the additional tests are enzyme-based tests that are not easily performed in a routine clinical laboratory.

The one strain of *Morganella morganii*, doubtful by ID 32E, was actually a *Morganella morganii* biogroup 1 strain, which is lysine decarboxylase positive. The database incorporates a 0% positive reaction for lysine, thereby rendering this identification 'doubtful'.

Four of the *Escherichia vulneris* strains identified with 'low' probability had false-negative lysine decarboxylase reactions. The color in the lysine cupule was grey, not the blue–violet required to be positive.

In the APILAB software, *Acinetobacter lwoffii* is meant to be identified as *Acinetobacter/Moraxella*. The five isolates of *Acinetobacter lwoffii* that we tested were identified as *Alcaligenes* sp. (4) and *Weeksella* sp. (1). If

the fact that *Alcaligenes* and *Weeksella* are oxidase positive is taken into account, the five isolates then become unidentified.

We found it very unusual that many of the isolates of *Ewingella americana* and *Serratia plymuthica* did not grow in the ID 32E strip at 35°C. Before repeat testing was completed, many of these strains had been passed as many as six or seven times. When the testing was performed using an incubation temperature of 25°C, the identifications of all 13 isolates that had not grown at 35°C were correct.

Our weighted assortment was composed of strains in an array comparable to that found in Atlanta area hospitals. Of the four *Escherichia coli* strains with 'low' probability identifications, one was atypically urea positive and one was negative for lysine and ornithine decarboxylase and arginine dihydrolase, but the other two were typical strains.

In reading the indole cupule, one must be aware that the positive color of a *Providencia* isolate may be more of a 'rusty' red than a true 'cherry' red. This is similar to what may sometimes be observed in a conventional tube indole test using Kovacs' reagent.

We could not use the bioMérieux ampoules of 0.85% NaCl, because we did not have access to an ATB densitometer. We used in-house-prepared 0.85% saline in ½-dram vials (Wheaton Science Products, Millville, New Jersey, USA).

While the ID 32E is approved only for industrial use in the USA, it has been in use in European clinical microbiology laboratories for several years. The only other published evaluation, one by Monnet et al [7], differs from our study in that it utilized the API 20E or carbon substrate assimilation tests as the reference method against which ID 32E identifications were compared. Unlike Monnet et al, this study concentrated almost entirely on enteric species while including only limited numbers of non-enteric organisms that are routinely found in a clinical laboratory. We also did not include any reference strains in this study.

Evaluations by these authors of other commonly used test kits or machines appear in the literature. While the set of strains tested varies depending on the database of the particular system, the set is essentially the same from one evaluation to the next, with reported degrees of accuracy ranging from 87.6% to 96.4% [2,8].

Because efficiency is important in laboratories, we evaluated the time factor in using this product. An average of 18 min is required to gather supplies, make suspensions and inoculate 10 strips, and clean up the area. An average of 22 min was required on the following day to add reagents, read the same 10 strips, and enter the profile numbers into APILAB to obtain answers. These times are similar to those of both the API 20E (24 min to set up and 15 min to read) and the Crystal ID-E/NF (22 min to set up and 20 min to read) [2,8].

In conclusion, the ID 32E is an acceptable alternative for the identification of enteric organisms routinely found in clinical and public-health laboratories as long as the limitations of accuracy are understood. In our hands, the product is more accurate with common isolates (89.9%) than with groups of isolates with atypical or unusual characteristics (77.3%).

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