JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1980, p. 684-689
0095-1137/80/11-0684/06$02.00/0

Vol. 12, No. 5

Evaluation of the MS-2 System for Rapid Identification of *Enterobacteriaceae*

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The precision, accuracy, and other performance characteristics of the MS-2 (Abbott Laboratories, Diagnostic Division, Dallas, Tex.) system for the identification of *Enterobacteriaceae* were evaluated in a collaborative study involving three clinical laboratories. When identifying 150 unknown, coded organisms, the MS-2 system was 97%, 98%, and 93% accurate, respectively, in three laboratories. The system showed an overall accuracy of 94% when compared with conventional manual tube methods in identifying 1,154 clinical isolates of 26 species of *Enterobacteriaceae*. Discrepancies between automated and conventional methods were chiefly caused by biochemical variants, especially among *Enterobacter* species. The MS-2 system was rapid and simple to operate and produced printed results of bacterial identification in 5 h. The cost of disposable components compared favorably with commercial, visually read systems for identifying *Enterobacteriaceae*.

Automation has been successfully applied to most clinical microbiological methods, including antimicrobial susceptibility testing, blood culture monitoring, detecting urinary tract infection and, lately, identifying bacteria. Identification of gram-negative rods, especially *Enterobacteriaceae*, is a major function of the clinical microbiology laboratory and is performed both as a guide to antimicrobial treatment and for epidemiological purposes. A rapid, precise, and accurate automated method of identification could, therefore, have considerable value in diagnostic bacteriology.

Vannest and his colleagues (R. D. Vannest, D. Brunson, M. Cornell, H. Terk, B. Perry, and R. Wilborn, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C(H)86, p. 360) reported a preliminary study of rapid, automated identification of enteric bacteria using the MS-2 (Abbott Laboratories, Diagnostic Division, Dallas, Tex.) system. Variations of this system have also been applied to antimicrobial susceptibility testing (H. J. Spencer, P. Welaj, R. Vannest, V. Hargrove, and L. Williams, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C204, p. 310; L. R. McCarthy, J. C. Sherris, and J. P. Anhalt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C205, p. 310), screening for urinary tract infection (L. R. McCarthy, C. L. Corlett, and J. A. Robson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C(H)95, p. 362), and monitoring of blood cultures (R. L. Holland, N. G. P. Helgeson, A. W. McCracken, and B. H. Cooper, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C4, p. 310). The present paper describes a collaborative study at three medical centers (Baylor University Medical Center, UCLA Medical Center, and University of North Carolina) to evaluate the MS-2 system for rapid identification of *Enterobacteriaceae*.

MATERIALS AND METHODS

MS-2 system. The MS-2 system used in this study consisted of the following components: an analysis module, a control module, a bacterial identification cartridge, an actuator, and an inoculum dispenser.

(i) Analysis module. The analysis module is both an electro-optical scanning device and an incubator-shaker. The latter function is required for antimicrobial susceptibility testing and urine screening, both of which can be performed simultaneously with bacterial identification. The electro-optical scanning system consists of light-emitting diodes (which emit light at a wavelength of 665 nm) and matched photodetectors. The analysis module has eight positions. Position 8 is used for bacterial identification. Positions 1 through 7 are used for antimicrobial susceptibility testing and urine screening.

(ii) Control module. The control module contains a microprocessor and performs the following functions: (a) controls the operation of the analysis module, (b) stores data obtained from the analysis module, (c) identifies bacteria by applying data from the analysis

module to a probability matrix, and (d) prints out the results of bacterial identification.

(iii) Bacterial identification cartridge. This cartridge is a disposable plastic container consisting of 20 optically clear chambers. Seventeen chambers contain lyophilized biochemical substrates and, with one exception, indicators which change color in response to the metabolic reactions of the test organism. The exception is chamber 18 which is used to detect indole production and to which 0.05 ml of Kovacs reagent is added after incubation. Chamber 17 is left empty to reduce the risk of adding Kovacs reagent to the wrong chamber. Chambers 19 and 20 are also empty. The active substances in the identification cartridges and their reactions are listed in Table 1. The 17 reactions were selected for their ability to distinguish between pairs of bacterial species and to give definitive reactions within 5 h in the MS-2 system. Identification cartridges are stored at 2 to 8°C until used.

Before use, one cartridge from each shipment is tested by rehydration of chambers 9 and 16 (L-arabinose and D-xylose, respectively) with 0.2 ml of sterile, deionized water. The resultant colors are compared with a standard color chart. Significant differences from the chart indicate that the cartridges may have been damaged by overheating during shipping.

(iv) Actuator. The actuator is placed in position 8 of the analysis module where it stabilizes the cartridge and aligns it correctly for transmitted light readings to be taken.

(v) Dispenser. The dispenser uses a 5-ml disposable syringe calibrated to deliver 0.2 ml of a standardized suspension of bacteria to each functional chamber of the identification cartridge.

Method for bacterial identification by the MS-2 system. Eighteen- to twenty-four-hour cultures of test organisms are obtained from blood agar plates, and an oxidase test is performed on each. All oxidase-positive bacteria are excluded.

Representative colonies of the test organism are touched with a sterile loop, transferred to 5 ml of sterile, deionized water, and blended in a Vortex mixer. This step is repeated until the turbidity of the suspension visually matches a 0.5 McFarland barium sulfate standard. Within 30 min of preparation, 0.2 ml of the bacterial suspension is delivered into each chamber of the identification cartridges. Chambers 1, 2, and 3, containing glucose, lysine, and ornithine, respectively, are sealed with polyester film to provide conditions of reduced oxygen tension.

The cartridges are placed in the actuator in position 8 in the analysis module. The system is activated by a series of keyboard entries which are sequentially prompted by instructions in the print-out. Among the entries are information for specimen identification and date and time of testing.

Initial readings of transmitted light are taken automatically for each chamber, and the data for each organism are stored in the control module. The cartridge is then removed, placed in an incubator at 35 to 37°C for 5 h, and reinserted into the analysis module for the final reading. The print-out of the data includes: (i) the assignment of a positive or negative sign to each biochemical test; (ii) the identity of the organism (up to five organisms are listed in descending order of likelihood); and (iii) a percent likelihood value for each organism listed. Percent likelihood is an expression of the degree of separation between organisms as defined by the formula: probability of first choice organism/sum of probabilities of all organisms in data base × 100. When the likelihood of a correct identification is low, the print-out includes the following message: "Warning: low assurance of identification. Percent likelihood is less than 80%."

Evaluation of precision and accuracy. In the first phase of this study, the precision of the MS-2 system was assessed in the following way.

A total of 150 strains of *Enterobacteriaceae* were given to each laboratory as unknown, coded samples. The 150 organisms were identified in parallel by the MS-2 and by a series of conventional tube tests (Table 2). With the conventional tests, identification was made by reference to Edwards and Ewing (4). During this phase, for reasons discussed later, modifications were made to the identification algorithm of the MS-2.

In the second phase, the accuracy of the MS-2 was evaluated by testing 1,154 strains of *Enterobacteriaceae* obtained from clinical sources with the MS-2 and conventional methods as described for phase 1. Included were 26 species of *Enterobacteriaceae* in numbers that reflected their relative frequency of isolation from clinical specimens.

All organisms tested during this study were stored at −60°C in defibrinated blood for later use in clarifying inter- or intra-laboratory discrepancies. In the second phase, when there was disagreement between the MS-2 and conventional identification, both methods were repeated. If there was still disagreement, the organism was submitted to the manufacturer who

TABLE 1. *Active biochemicals in MS-2 identification cartridges and their reactions*

Chamber no.	Active substance	Test concentration (% wt/vol)	Reaction
1	Glucose	1.0	Fermentation[a]
2	Lysine hydrochloride	1.0	Decarboxylation[a]
3	Ornithine hydrochloride	1.0	Decarboxylation[a]
4	Sodium citrate	0.2	Utilization
5	Sodium malonate	0.5	Utilization
6	Esculin	0.125	Hydrolysis
7	Urea	2.0	Hydrolysis
8	Adonitol	1.0	Fermentation
9	L-Arabinose	2.0	Fermentation
10	I-Inositol	2.0	Fermentation
11	Lactose	2.0	Fermentation
12	Mannitol	2.0	Fermentation
13	L-Rhamnose	0.5	Fermentation
14	Sorbitol	2.0	Fermentation
15	Sucrose	2.0	Fermentation
16	D-Xylose	0.5	Fermentation
17	Empty		
18	L-Tryptophan	0.125	Indole production
19	Empty		
20	Empty		

[a] Sealed to provide reduced oxygen tension.

TABLE 2. *Reactions in conventional tubed media used for identification of Enterobacteriaceae (6)*[a]

Biochemical reaction	Medium
Fermentation of:	
Arabinose	Fermentation broth base with Andrade indicator plus carbohydrate substrate
Glucose	
Lactose	
Rhamnose	
Sucrose	
Xylose	
Adonitol	
Inositol	
Mannitol	
Sorbitol	
Dulcitol	
Control	Fermentation broth base with Andrade indicator
Decarboxylation of:	
Lysine	Moeller base plus amino acid substrate
Ornithine	
Control	Moeller base
Utilization of:	
Acetate	Acetate agar
Citrate	Simmons citrate agar
Malonate	Malonate broth
Other tests:	
Urea hydrolysis	Christensen urea agar
Voges-Proskauer reaction	Methyl red-Voges-Proskauer broth
Esculin reduction	Esculin broth
Phenylalanine deamination	Phenylalanine agar
Indole production	Indole test broth
Deoxyribonuclease production	DNase test medium
Dextrose, lactose, and sucrose fermentation and H_2S production	Triple sugar iron agar
Motility	Motility test medium
Oxidase production	Patho-Tec[b]

[a] Unless otherwise noted, the methods listed are based on those in reference 6.

[b] General Diagnostics, Warner-Lambert Co., Morris Plains, N.J.

recoded and reissued it as an unknown for repeat of conventional identification by all three laboratories. The final identification of a controversial organism was that on which at least two laboratories agreed. If all three still disagreed, a subculture of the organism was sent to a reference laboratory whose identification was accepted as final.

RESULTS

The accuracy of the MS-2 system and conventional identifications in all three laboratories is shown in Table 3. The MS-2 results were those obtained after modifications had been made to the identification algorithm. The precision of identification of 150 bacterial strains by MS-2 and by conventional methods is compared in Table 4. With the MS-2, all three laboratories correctly identified 135 (90%) organisms. Of the 150 strains, 148 (99%) were correctly identified by at least two of the three laboratories.

The results of identification by conventional methods were very similar to those obtained by MS-2. All three laboratories agreed on the correct identity of 136 (91%) of the 150 strains, and at least two of the three laboratories agreed on the identity of all 150 strains. Correct identification was achieved in 149 (99%) strains.

A comparison by species of automated and conventional identifications of 1,154 strains of *Enterobacteriaceae* isolated from clinical specimens in all three laboratories combined is shown in Table 5. In assessing accuracy, the correct identification was taken as that obtained by conventional methods on which at least two of

TABLE 3. *Accuracy of identification by MS-2 and conventional methods of 150 strains of Enterobacteriaceae in three laboratories*

Site	Method	No. of correct identifications (%)	No. of incorrect identifications (%)	No. not identified (%)
BUMC[a]	MS-2	146 (97)	4 (3)	0 (0)
	Conventional	145 (97)	4 (3)	1 (<1)
UNC[b]	MS-2	147 (98)	3 (2)	0 (0)
	Conventional	149 (99)	1 (<1)	0 (0)
UCLA[c]	MS-2	139 (93)	8 (5)	3 (2)
	Conventional	141 (94)	8 (5)	1 (<1)

[a] Baylor University Medical Center.

[b] University of North Carolina.

[c] UCLA Medical Center.

TABLE 4. *Precision of identification by MS-2 and conventional methods of 150 strains of Enterobacteriaceae in three laboratories*

Precision of identification	No. (%) identified by:	
	MS-2	Conventional method
Correctly identified in all three laboratories	135 (90)	136 (91)
Correctly identified in two of three laboratories	13 (9)	13 (9)
Incorrectly identified in two of three laboratories	1[a] (<1)	1[a] (<1)
Incorrectly identified in all three laboratories	1[a] (<1)	0 (0)

[a] Same first choice in both or all laboratories.

TABLE 5. *Accuracy of MS-2 identification with 1,154 clinical isolates*[a]

Conventional Identification / MS-2 Identification	*E. coli*	*Shigella sp*	*E. tarda*	*C. freundii*	*C. diversus*	*Arizona sp*	*S. cholerae-suis*	*S. typhi*	*S. enteritidis*	*K. pneumoniae*	*K. ozaenae*	*K. rhinoscleromatis*	*E. aerogenes*	*E. cloacae*	*H. alvei*	*E. agglomerans*	*S. liquefaciens*	*S. marcescens*	*S. rubidaea*	*P. vulgaris*	*P. mirabilis*	*P. morganii*	*P. rettgeri*	*P. alcalifaciens*	*P. stuartii*	*Y. enterocolitica*	*A. calcoaceticus*	No identification	
E. coli	298																												
Shigella sp		25			1			1	1																				
E. tarda			12																										
C. freundii	1			21										3		5													
C. diversus					13											1													
Arizona sp				1		6								3	1														
S. cholerae-suis							1																						
S. typhi								2																					
S. enteritidis		4							25																				
K. pneumoniae										156						2													
K. ozaenae									1		4					1					1		1						
K. rhinoscleromatis											1	3																	
E. aerogenes													53	1															
E. cloacae														62		2													
H. alvei	2								2						19														
E. agglomerans	2			1					1	1				1		16													
S. liquefaciens	1									1				1			12									1			
S. marcescens																		32											
S. rubidaea										4	1					1			4		1								
P. vulgaris																				62			1						
P. mirabilis																					150								
P. morganii																						38							
P. rettgeri																				3		2	17						
P. alcalifaciens																								5					
P. stuartii																								1	36				
Y. enterocolitica																										8			
A. calcoaceticus															1						2						0		
No identification	1									1	1		1	2		1		1				3						0	
TOTAL	305	29	12	23	14	6	1	3	30	163	7	3	54	73	21	29	12	33	4	65	154	43	19	6	36	9	0	0	1154
NO CORRECT	298	25	12	21	13	6	1	2	25	156	4	3	53	62	19	16	12	32	4	62	150	38	17	5	36	8	—	—	1080
ACCURACY (% CORRECT)	98	86	100	91	93	100	100	67	83	96	57	100	98	85	90	55	100	97	100	95	97	88	89	83	100	89	—	—	94

[a] Conventional results are assumed to be correct. Deviations from the diagonal line of correlating results represent MS-2 discrepancies.

the three laboratories agreed. With the MS-2, 1,080 (94%) isolates were correctly identified, and no identification was made in 11 (1%) instances. Of the 63 (5%) discrepancies, 40 were correctly identified by MS-2 as the second choice. Moreover, in 21 of the 40 second choices, the print-out contained a warning of low percent likelihood (less than 80%) of correct identification of the first choice, indicating a need for further testing. Of the 1,080 strains correctly identified by MS-2 as first choice, only 86 (8%) were accompanied by the warning statement and would have required further testing.

Table 6 shows the accuracy of the MS-2 system in each of the three laboratories.

DISCUSSION

In a preliminary report (R. D. Vannest, D. Brunson, M. Cornell, H. Terk, B. Perry, and R. Wilborn, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C(H)86, p. 360), when 296 strains of enteric bacteria were tested by MS-2 and by conventional methods of identification, there was 95% agreement between the two methods. In both methods, bacterial identification was based on the interpretative data of Edwards and Ewing (4) and other similar sources (1, 3, 5). The present collaborative study has expanded and confirmed the findings of the preliminary report. The precision of the MS-2 in identifying 150 strains of *Enterobacteriaceae* exceeded 90% in the three laboratories, and the accuracy ranged from 93 to 98% when the programming changes discussed below were made.

The MS-2 with modified software had an accuracy of 94% in identifying 1,154 strains of *Enterobacteriaceae*. This is similar to the accuracy reported for commercial identification systems which are read visually after 4 h (1, 2) or overnight incubation (7, 9, 10).

Only a few strains of several uncommon species, notably *Serratia rubidaea*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromatis*, and *Yersinia enterocolitica*, were tested. No examples of *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Yersinia pseudotuberculosis*, or *Pasteurella* species were included. Further studies are required to evaluate the performance of the MS-2 with these organisms.

TABLE 6. *Accuracy of MS-2 identification in three laboratories*

Site	No. of strains tested (%)	No. correct (%)	No. incorrect (%)	No. not identified (%)
BUMC[a]	371 (100)	350 (94)	21 (6)	0
UNC[b]	371 (100)	342 (92)	23 (6)	6 (2)
UCLA[c]	412 (100)	388 (94)	19 (5)	5 (1)

[a] Baylor University Medical Center.
[b] University of North Carolina.
[c] UCLA Medical Center.

Although not a member of the family *Enterobacteriaceae*, *Acinetobacter calcoaceticus* (formerly var. *anitratus*—this study was completed before the publication of the Approved Lists of Bacterial Names [8]) was included in the data base of the instrument because it is oxidase negative and gives a positive glucose reaction in the MS-2.

There were several reasons for adjusting the programming of the control module during the first phase of this study. First, the threshold of positivity for each biochemical reaction was adjusted so that the MS-2 interpretation closely matched that from conventional tubed media. Second, the program which compared the set of biochemical reactions of each organism to a matrix of the expected positive reactions was originally based on data from the Center for Disease Control, Atlanta, Ga. (3, 5). As data from the MS-2 became available, the reactivity of each species in the various biochemical tests was determined, and the data matrix was revised with these figures. Third, it was noted after the threshhold had been adjusted and the data matrix had been modified that a few strains of certain species (for example, *Klebsiella pneumoniae* and *Serratia rubidaea*) could not be distinguished from each other, largely because the test strains of these species gave one or two atypical biochemical reactions. In these instances, the computer algorithm was altered by weighting specific biochemical reactions to achieve the correct identification.

The reasons for discrepancies between MS-2 and conventional methods are currently being investigated. Many of the disagreements were among the various species of *Enterobacter* (4 of 11 non-identifications and 30 of 63 misidentifications) and may reflect the variability of biochemical reactions among members of this genus. In six instances, *Enterobacter cloacae* was misidentified as an H_2S-producing organism; thus, the lack of a test for H_2S production may account for some of these discrepancies. The four discrepancies with *Shigella* species involved *Shigella sonnei* that were misidentified as *Salmonella enteritidis* bioser. *paratyphi A*. Further MS-2 data on this organism must be accumulated so that the program can be adjusted to distinguish *S. sonnei* from *S. paratyphi A*.

The manual operations involved in MS-2 identification take less than 4 min and are at least as simple to perform as conventional methods. The initial method for inoculation of the

identification cartridge by a pipette-syringe assembly carried some risk of aerosol formation, contamination of the tape covering the chambers, and skin puncture from the hypodermic needle. These disadvantages were corrected by modifications to the dispenser system. These were: (i) machine perforation of the tape sealing the chambers of the cartridge, and (ii) dispensing the inoculum with an automatic pipette with blunt sterile disposable tips. These modifications also eliminated the need for a disposable syringe with each organism tested. The retail price for each disposable cartridge is $2.10, which is similar to that of cartridges used in commercial manual methods for identifying *Enterobacteriaceae*.

The availability of bacterial identification within 5 h of primary isolation has considerable value as a guide to antimicrobial therapy. In addition to a high degree of physician acceptance, rapid identification can benefit the clinical laboratory by eliminating the need for preparation and distribution of preliminary reports. The advantages appear to be greatest to both physicians and laboratories when rapid identification is accompanied by antimicrobial susceptibility tests also performed in 3 to 5 h. At least two systems, MS-2 and AutoBac I (Pfizer Diagnostics, New York), can perform susceptibility studies within this period of time. Entry of results into data processing systems is also simplified if bacterial identification and susceptibilities are available at the same time rather than a day apart.

ACKNOWLEDGMENTS

The technical expertise of Gene Marso (UCLA) and the secretarial skills of Laura Carroll (Baylor) are gratefully acknowledged.

This study was supported by a grant from the Abbott Corp.

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