JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1977, p. 458-464
Vol. 5, No. 4

Printed in U.S.A.

Comparative Study of Three Methods of Identification of *Enterobacteriaceae*

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Received for publication 2 September 1976

Three separate hospital clinical microbiology laboratories using three different identification systems participated in the identification of *Enterobacteriaceae* from a central pool of "unknown" clinical isolates. With conventional tubed media, API-20E (Analytab Products Inc.) and R/B tube (Corning Diagnostics) systems, there was a 91.1% agreement in the species designation. No significant differences at the 95% confidence level were found among the systems. Evaluation of individual tests within the systems used revealed lysine decarboxylase of the conventional and citrate of the API-20E system to be significantly different from the same test within the other two systems. The lysine decarboxylase of the conventional system had species relatedness, whereas the differences in citrate of the API-20E system were not related to a particular species. These individual test variations did not affect final organism identification. Reproducibility, evaluated as the system's ability to designate the same identification on two separate occasions, was 92 to 94% for each system. Exact duplication of selected sets of reactions was 60% for conventional, 45% for API-20E, and 61% for R/B. The variations in sets of reactions differed with the system and with the organism involved. The findings suggest equivalency among the three systems in ability to identify common clinical isolates of *Enterobacteriaceae* and point out the limited usefulness of these systems for biochemical biotyping.

The introduction of commercially prepared test systems or kits for the identification of *Enterobacteriaceae* has resulted in multiple comparative studies by various laboratories of these systems with each other or with conventional tubed media (2-7). The significance of the unequal experience with test systems or more familiarity with one or more of the systems being compared was often difficult to assess. Other variables such as number of operators involved, the time interval between testing and repeat testing, and the number of different media lots utilized were generally different and rarely resembled a routine use situation. The question of whether the high percentage of correct identifications previously reported for a variety of systems would occur in routine use was still unanswered. To approach this question, three separate hospitals using three different identification systems selected clinical isolates from the routine microbiology bench and sent these isolates along with their biochemical profile to a central pool where they were converted into "unknowns." Each unknown was then resubmitted to all participating laboratories for identification and submission of the biochemical data. This format had the advantage of requiring unprejudiced identification of each isolate by the routine system, allowing passage of time between the first and subsequent identifications, and involving multiple operators. The collected data were then analyzed by an outside party.

The participating laboratories and systems were: The Ohio State University Hospitals (OSU) with conventional (Conv.) tubed media, The Cleveland Clinic Foundation (CCF) with API-20E, and St. Elizabeth Medical Center (SEMC) with R/B 3 tube system (Table 1).

MATERIALS AND METHODS

Organisms. Each laboratory collected 130 organisms from clinical and study cultures to form a pool of 390 organisms belonging to the *Enterobacteriaceae*. The cultures were sent to an independent laboratory (Corning Diagnostics) on supplied Trypticase soy agar slants for coding. Separate computer-assigned codes for each laboratory were given to 301

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randomly selected species from the pool. A single colony of each of these selected organisms was used to prepare the three individually coded Trypticase soy agar slants to be sent to the laboratories. The organisms, in batches of 25, were sent simultaneously to the laboratories over a 5-month period. On receipt of these now coded unknowns, OSU and CCF prepared isolation plates on MacConkey agar and SEMC prepared isolation plates on eosin-methylene blue agar. From the isolation plates, colonies for identification were chosen according to the method of the participating laboratory.

Conv. method. The Conv. method used by OSU consisted of media prepared in the laboratory from dehydrated commercial media. The six tubes providing 11 test reactions (Table 1) were triple sugar iron agar (Difco Laboratories), motility-indole-ornithine medium (Difco), lysine decarboxylase (LDC; Falkow formulation) broth with 0.3% agar (Difco), phenylalanine deaminase agar (BBL), Simmons citrate agar (Difco), and purple broth base (Difco) with 1% L-rhamnose (Sigma Chemical Co.). When necessary, as determined by Edwards and Ewing charts, additional tests were peformed for identification of species. Test reactions were read at 18, 21, and 24 h, with the exception of indole (IND) and phenylalanine deaminase (PD) that were read at 24 h only. Occasional tests were interpreted at 48 h as well as at the other stated time intervals. After the identification of the organisms in a particular batch, data including interpretation of reactions at specified time intervals, additional tests performed, and identification were sent to the computer center. Identification was made at 24 h with an occasional final identification made at 48 h. Seven technologists participated in the project, with only five doing all the interpretation of results.

API-20E method. At CCF, the commercial microtube system API-20E and the API Analytical Profile Index (Analytab Products Inc., Plainview, N.Y.) were used for the identification of *Enterobacteriaceae*. This system has 20 test reactions (Table 1) that are read in groups of three to generate a number found in the Profile register to indicate identification of the organisms based on the observed biochemical reactions. The method of inoculation and interpretation was carried out according to manufacturer's instructions using a colony from the MacConkey isolation plate. Except for tryptophane deaminase (TDA), indole, and Voges-Proskauer reactions that require addition of reagents, the other 17 tests were read at 18, 21, and 24 h. Identification and results of biochemical reactions were returned to the computer center on completion of identification of all organisms within a batch. Only three technologists participated in this portion of the study.

R/B tube method. The R/B 3 tube (Corning Diagnostics), a commercial macrotube system composed of three tubes used by SEMC, provides 11 test reactions (Table 1). The tubes were inoculated and interpreted according to manufacturer instructions. All reactions were interpreted at 18, 21, and 24 h. According to the identification procedure used at SEMC, it was necessary to set up the fourth available commercially prepared tube "Soranase" (Corning Diagnostics) to provide an additional four test reactions for definitive identification in some instances. With the addition of the fourth tube, 15 possible test reactions were available. Identification was made by pattern recognition of reactions at 24 h. The observed reactions and identification were returned to the computer center on completion of organism identification within each batch. Five technologists participated in this study, with three technologists making the majority of reaction interpretations.

TABLE 1. *Test reactions of systems used by laboratories*

Test	Test reactions		
	Conv. (OSU)	API-20E (CCF)	R/B 3 tube (SEMC)
PD[a]	Yes	Tryptophane deaminase	Yes
GLU[a]	Yes	Yes	Yes
H_2S[a]	Yes	Yes	Yes
LDC[a]	Yes	Yes	Yes
IND[a]	Yes	Yes	Yes
ODC[a]	Yes	Yes	Yes
CIT[a]	Yes	Yes	Yes
RHA[a]	Yes	Yes	Yes
LAC	Yes	No	Yes
MOT	Yes	No	Yes
GAS	Yes	No	Yes
RAF	No	No	Yes[b]
DNase	No	No	Yes[b]
SOR	No	Yes	Yes[b]
ARA	No	Yes	Yes[b]
ONPG	No	Yes	No
URE	No	Yes	No
GEL	No	Yes	No
MAN	No	Yes	No
INO	No	Yes	No
SAC	No	Yes	No
MEL	No	Yes	No
AMY	No	Yes	No
ADH	No	Yes	No
V-P	No	Yes	No

[a] Tests common to all three systems. PD, Phenylalanine deaminase; GLU, glucose; H_2S, H_2S production; LDS, lysine decarboxylase; IND, indole production; ODC, ornithine decarboxylase; CIT, citrate utilization; RHA, rhamnose; LAC, lactose; MOT, motility; GAS, gas from glucose; RAF, raffinose; DNase, deoxyribonuclease; SOR, sorbitol; ARA, arabinose: ONPG, β-galactosidase; URE, urease; GEL, gelatinase; MAN, mannitol; INO, inositol; SAC, saccharose; MEL, melibiose; AMY, amygdalin; ADH, arginine dehydrolase; V-P, Voges-Proskauer.

[b] Additional test reactions of the fourth tube used when indicated by manufacturer's directions.

Statistical analyses. Data from submittal and reidentification were tabulated, correlated, and analyzed by one of us (V.C.M.) with the aid of a Honeywell 6000 System located in Cleveland, Ohio. Adequate data were available for 290 organisms.

RESULTS

Of the 301 isolates sent to the individual hospital laboratories, data for evaluation were available for 290 organisms. The distribution and number of these organisms contributed by each hospital are listed in Table 2. The distribution and relative proportion of organisms corresponded well to that previously reported for a general microbiology laboratory (1). The distribution and number for each hospital's contribution varied in that OSU had more species of different types represented in their component as compared with those of CCF and SEMC.

At genus and species level of identification, the three methods agreed in 264 of the 290 isolates, or 91% at time of reidentification (Table 3). Two of the three methods agreed in a further 8% of isolates. When identification was taken only to genus level, there was complete agreement with 95% of the isolates. In laboratory to laboratory comparison of identification, each laboratory agreed with at least one other laboratory 95% of the time (Table 4).

With the use of analysis of variance techniques, no significant differences were detected at the 95% confidence level in identification at genus and species levels among the three methods.

The laboratory most often in disagreement with the other two laboratories was SEMC (Table 5). Investigation of the disagreements of both SEMC and OSU resulted in the recognition of technologist failure to interpret correctly test reactions for identification, not system failure. In Conv. and R/B methods, a technologist was required to make an identification based on pattern recognition, whereas API-20E used the number generated by test reactions to search out the identification in the Analytical Profile Index. In two instances of Conv. and five instances of R/B disagreements, the systems had test reactions to make the same identification as the other two laboratories. However, technologist failure to make the proper identification resulted in a disagreement. With R/B, three organisms were called *Proteus mirabilis* when the test reactions were those of *Proteus morganii* as identified by the other two laboratories. Two organisms were identified as *Serratia liquefaciens*, although the system's reactions were those of *Enterobacter cloacae*. With the Conv. system, although the test reactions were for *Citrobacter freundii*, one organism was identified as *E. agglomerans* by the technologist. The second organism was identified as *E. aerogenes* by the technologist when the test reactions indicated *Serratia liquefaciens*. In spite of these technologist failures, and as mentioned previously, no significant differences were detected at a 95% confidence level in identification among the three methods.

There were eight biochemical reactions common to all methods, if phenylalanine deaminase and tryptophane deaminase were considered equivalent (Table 1). These reactions were compared for agreement among the three methods at 24 h (Table 6). With the use of analysis of variance techniques, there was a

TABLE 2. *Submittal identification to genus and species and submittal institution*

Organism	No. of organisms submitted by:			
	OSU	CCF	SEMC	Total
E. coli	17	35	58	110
K. pneumoniae	11	24	15	50
P. mirabilis	8	14	15	37
E. cloacae	12	2	8	22
E. aerogenes	13	4	1	18
P. morganii	5	6	0	11
C. freundii	3	2	1	6
P. vulgaris	4	2	0	6
S. marcescens	4	1	0	5
C. diversus	3	1	0	4
S. enteritidis	3	0	1	4
E. agglomerans	1	1	1	3
A. hinshawii	3	0	0	3
P. rettgeri	2	0	0	2
E. hafniae	1	1	0	2
S. liquefaciens	1	1	0	2
P. alcalifaciens	1	0	0	1
P. stuartii	1	0	0	1
E. tarda	1	0	0	1
Y. enterocolitica	1	0	0	1
Shigella sp.	1	0	0	1
Total	96	94	100	290

TABLE 3. *Overall comparison of organism identification*

No. of systems in agreement	No. of organisms in agreement			
	Genus and species level	(%)	Genus level	(%)
3	264	(91.1)	275	(94.9)
2	23	(7.9)	14	(4.8)
0	3	(1.0)	1	(0.3)

TABLE 4. *Interlaboratory comparison of organism identification*[a]

Laboratory	Agreement (%) at:	
	Genus and species level	Genus level
OSU	98.3	99.3
CCF	97.9	98.6
SEMC	95.8	97.2

[a] Agreement with at least one other hospital.

TABLE 5. *Correlation of systems agreement with organism identification to genus and species*

Reidentification	No. of systems in agreement		System in disagreement		
	3[a]	2	Conv.	API	R/B
E. coli	111	1	0	0	1
K. pneumoniae	47	2	0	1	1
P. mirabilis	34	3	2	0	1
E. cloacae	18	2	0	0	2
E. aerogenes	19	0	0	0	0
P. morganii	7	5	0	1	4
C. freundii	4	4	1	2	1
P. vulgaris	6	0	0	0	0
S. marcescens	5	1	0	1	0
C. diversus	3	1	0	1	0
S. enteritidis	4	0	0	0	0
E. agglomerans	1	0	0	0	0
A. hinshawii	3	0	0	0	0
P. rettgeri	0	2	0	0	2
E. hafniae	0	1	1	0	0
S. liquefaciens	0	1	1	0	0
P. alcalifaciens	1	0	0	0	0
Shigella sp.	1	0	0	0	0
Total	264	23	5	6	12

[a] Number of systems in agreement.

significant difference at the 95% confidence level between LDC of Conv. method and CIT of API-20E and the corresponding tests of the other two systems. The LDC of Conv. and R/B systems and citrate utilization (CIT) of API-20E and R/B had doubtful reactions recorded at 24 h (Table 7). Disagreement among all three systems occurred with nine doubtful LDC reactions of R/B and two doubtful CIT reactions of R/B. These ambiguous reactions in all cases, except one, were associated with agreeing identifications among at least two of the laboratories. All 38 LDC disagreements of Conv. and 24 of the 28 CIT reactions of API-20E occurred in organisms with the same identification as at least one other method.

When the biochemical disagreements were matched with organism reidentification (two or three system agreement), the majority of LDC disagreements were associated with organisms identified as *Escherichia coli* (Table 8). The CIT disagreements were more scattered, with association most often with organisms reidentified as *P. mirabilis*, but also with organisms reidentified as *C. freundii* and *Klebsiella pneumoniae* and with other organisms less frequently. It was also evident that multiple different biochemical tests had disagreements when associated with organisms reidentified as *P. mirabilis*.

Test reactions were compared at 18, 21, and 24 h when possible. IND and PD-TDA reactions could not be compared because these tests were read at 24 h only by one or more of the laboratories. Of the remaining six tests, LDC reactions were most often involved in disagreements at each time interval. Generally, reactions tended to agree more often with the passage of time, but, in most instances, a disagreement at 18 h was also present at 21 and 24 h. The most common pattern of disagreement consisted of a negative reaction in Conv. system and positive reactions by both API-20E and R/B 3 tube systems. As was previously noted, the discrepancies were organism related and involved *E. coli*, insofar as the Conv. system was concerned. However, the final identification of these organisms was not affected by the discrepancies.

The second most frequent test involved in disagreements among the three methods at all timed readings was the CIT reaction. The disagreements clustered about the combination of negative with API-20E when the other two methods recorded a positive reaction at all three times. With the passage of time, there tended to be greater agreement among all three methods. Unlike LDC, disagreements in CIT were spread over a number of organisms as previously noted. Identification of the organisms was not affected in the majority (24 of 28) of API-20E disagreements. In the overall evaluation, the reactions of each of the six tests at 18 h did not appreciably change at 21- or 24-h readings.

When the 8 reactions in common were considered as a "set" of reactions, there were 258 organisms with complete data for these reactions. Of these 258 organisms, there were 153 (59.3%) with exactly the same profile for the eight reactions. For the three most frequent organisms in the study, *E. coli*, *K. pneumoniae*, and *P. mirabilis*, the present agreement of these same set reactions among the three laboratories was 78.7% for *K. pneumoniae*, 48.6% for *E. coli*, and 45.2% for *P. mirabilis*.

TABLE 6. *Comparison of biochemical tests common to three systems*[a]

Biochemical test[b]	Percent agreement			
	Conv.	API-20E	R/B	Overall avg
PD-TDA[b]	99.3	98.3	98.6	98.7
GLU	99.7	100.0	100.0	99.9
H_2S	99.7	98.6	99.0	99.1
LDC	86.5	96.8	96.8	93.4
IND	99.0	98.6	100.0	99.2
ODC	98.6	99.0	97.6	98.4
CIT	96.5	90.3	98.6	95.1
RHA	99.0	99.7	98.3	99.0

[a] Percentage of agreement with at least one other system at the 24-h reading.

[b] Abbreviations as in Table 1.

TABLE 7. *Interlaboratory test agreement: tests common to three systems*[a]

Test[b]	No. of organisms tested (total 290)						Data missing[d]
	No. of systems in agreement			System in disagreement (doubtful)[c]			
	3	2	0	Conv.	API	R/B	
PD-TDA	278	12		2	5	4	1
GLU	286	4		1	0	0	3
H_2S	282	8		1	4	3	0
LDC	225	56	9	32 (6)	9	4 (5)	0
IND	280	10		3	4	0	3
ODC	275	15		4	3	4 (3)	1
CIT	245	43	2	10	27 (1)	3 (1)	1
RHA	281	9		3	1	4 (1)	0

[a] Tested at 24 h.
[b] Abbreviations as in Table 1.
[c] Other two hospitals agreed.
[d] Two hospitals agreed. Number of times one hospital did not submit data for this reaction.

TABLE 8. *Correlation of biochemical disagreements to organism identification: genus and species*

Reidentification	No. of isolates	No. of biochemical tests in disagreement								
		PD-TDA	GLU	H_2S	LDC	IND	ODC	CIT	RHA	Total
E. coli	112	0	1	0	50	1	6	0	3	61
K. pneumoniae	49	0	0	0	8	0	0	5	1	14
P. mirabilis	37	1	0	7	2	2	1	19	2	34
E. cloacae	20	0	0	0	2	0	0	1	1	4
E. aerogenes	19	1	0	0	0	0	0	2	0	3
P. morganii	12	6	0	0	1	0	0	0	0	7
C. freundii	8	0	0	0	1	0	2	6	0	9
P. vulgaris	6	0	0	0	0	2	0	0	0	2
S. marcescens	6	0	0	0	0	0	0	0	1	1
C. diversus	4	0	0	0	0	0	0	2	0	2
S. enteritidis	4	0	0	0	0	0	0	3	0	3
E. agglomerans	1	1	0	0	0	0	1	1	0	3
A. hinshawii	3	0	0	1	0	0	0	2	0	3
P. rettgeri	2	1	0	0	0	1	1	1	0	4
E. hafniae	1	1	0	0	0	0	0	0	0	1
S. liquefaciens	1	0	0	0	0	0	0	0	0	0
P. alcalifaciens	1	0	0	0	0	0	0	1	0	1
Shigella sp.	1	0	0	0	0	0	0	0	0	0
Others (no agreement)	3	0	0	0	1	1	3	1	1	7
Total	290	11	1	8	65	7	14	44	9	159

Another aspect of the study was concerned with reproducibility within each laboratory measured by comparison of identification and sets of reactions obtained at the time of original collection and, subsequently, at the time of reidentification. The time interval between these two identifications by each hospital varied from organism to organism and ranged from 1 to 6 months. Intralaboratory agreement of identification at genus and species was 94% for Conv., 92% for API-20E, and 93% for R/B systems. Disagreements in identification within each laboratory were correlated with the more frequently occurring organisms in the sample of each laboratory. The percent disagreement was similar for *E. coli* and *P. mirabilis* in all three methods. API-20E and R/B methods had the same percent disagreement for *K. pneumoniae*, whereas the Conv. method had no disagreements at time of submittal and reidentification (Table 9). The sample size of the remaining organisms for each laboratory was too small for further consideration from this viewpoint.

When considering each method's various tests as a set of reactions, the overall reproducibility for each system was 60% for Conv., 45% for API-20E, and 61% for R/B. Direct comparison of these results is difficult due to the differ-

ences in organisms tested by each method. However, when specific organisms were correlated to the set reactions, some differences between the methods regarding reproducibility were noted. The profiles of disagreement by system were considered for the most frequent organisms, *E. coli*, *K. pneumoniae*, and *P. mirabilis* (Table 10). For *E. coli*, Conv. and R/B methods had similar profiles of disagreements involving gas, LDC, and motility. API-20E had a very different profile of disagreements involving rhamnose, arabinose, and melibiose. *K. pneumoniae* had few disagreements in the Conv. system, but in API-20E, urea and citrate were in disagreement most often. The few discrepancies in the R/B system were lactose reactions. The last organism, *P. mirabilis*, had different profiles of disagreements in each method. The only reactions to be found in disagreements in two methods were H_2S of API-20E and R/B, citrate of API-20E and Conv. methods, and gas of Conv. and R/B methods.

DISCUSSION

The overall agreement in identification of the sample of organisms tested was 91% at genus and species level, and two of the three methods agreed in a further 8%. There was not a significant difference at the 95% confidence level among the three methods regarding agreement

TABLE 9. *Intralaboratory agreement of identification related to organism*

Submittal identification	Reidentification agreement					
	Conv.		API-20E		R/B	
	No. of pairs agreeing	Total pairs	No. of pairs agreeing	Total pairs	No. of pairs agreeing	Total pairs
E. coli	17	18	34	36	57	60
P. mirabilis	7	8	14	16	14	16
K. pneumoniae	11	11	22	26	13	15

TABLE 10. *Profiles of reaction disagreements for specific organisms: submittal and reidentification agreement*

Reaction	No. of tests in disagreement								
	E. coli			*K. pneumoniae*			*P. mirabilis*		
	Conv. (17)[a]	API-20E (34)	R/B (57)	Conv. (11)[a]	API-20E (22)	R/B (13)	Conv. (7)[a]	API-20E (14)	R/B (14)
PD-TDA[b]	0	0	0	0	0	0	0	0	0
LAC	1		2	1		4/11[c]	0		2/5
H_2S	0	0	0	0	0	0	0	2	4
GLU	0	0	0	0	0	0	0	0	0
GAS	2		5/56	1		0/12	3		5/11
LDC	6	1	12	1	1	0	0	1	0
IND	0	0	0	0	0	0	2	0	0
ODC	1	0	1	0	0	0	0	0	0
MOT	3		3	0		0	1		0
CIT	0	0	0/56	0	4	1	4	7	0/4
RHA	0	4	0	0	0	0	1	0	0
SOR		1			0			0	
ARA		4			0			0	
URE		0			11			0	
INO		0			0			0	
ADH		0			0			0	
ONPG		0			0			0	
MAN		0			0			0	
VP		0			1			0	
GEL		0			0			2	
SAC		0			0			0	
MEL		7			0			0	
AMY		1			0			0	

[a] Numbers in parentheses indicate number of organisms.

[b] Abbreviations as in Table 1.

[c] Missing data, total number of pairs indicated by second number.

of identification at genus and species level. The percentage of agreement compared well with other studies (2–7). If operator failure had been eliminated, the percent agreement of identification would have been greater. This observation emphasizes the necessity to have well-trained personnel using the systems, particularly in the pattern recognition of organisms. At the same time, those individuals using a numerical system of identification must not become enamored with numbers at the expense of recognizing patterns of reactions, and other characteristics such as colony morphology, and of the use of other reactions not contained within the system. In the methods used, the number of tests varied from 11 to 20. The systems were incomplete for identification of all organisms tested, and additional tests were recommended for definitive identification of some organisms. This requirement for further testing varied according to the method and organism tested. The findings also suggested that an increased number of tests in a system did not necessarily improve accuracy of identification.

The LDC of the Conv. method had a significant difference in interpretation as compared with the same test of API-20E and R/B. It was noted, however, that the majority of the discrepancies were related to organisms identified as *E. coli* but did not affect the identification of these organisms by OSU. The relative lack of discrepancies in the three methods with other species suggests that the LDC of these methods, but especially of Conv., are less sensitive for the detection of LDC in *E. coli*. This lack of sensitivity did not affect the final identification in any of the methods.

The CIT reaction of API-20E was significantly different from the CIT reactions of the other two methods. Unlike LDC, there was no apparent relationship to a particular organism species. The CIT reactions were in disagreement over a broader spectrum of organisms, suggesting that the CIT of API-20E is less sensitive than are the other two methods for several organisms. In the majority of organisms, however, there was no effect on identification of the organism. This apparent lack of sensitivity has probably been compensated for in the profile index of the system.

The remaining six tests common to the three methods, PD-TDA, IND, ODC, GLU, RHA, and H_2S, had no significant differences among the three systems.

As far as reproducibility is concerned, the results of this study, although limited, were good regarding identification of a species. However, if reproducibility concerned with biochemical biotyping is examined, the highest percentage of duplication was only 61% with R/B, and the lowest was 45% with API-20E. This degree of reproducibility of reactions for API-20E is somewhat lower than those previously reported by the same laboratory (1). The reasons for this difference are not apparent at this time. The use of these methods for biochemical biotyping is limited. There was some correlation of a system's capability to reproduce defined sets of reactions for particular organism species. This was evident in the different profiles of disagreements on repetitive testing by all methods for *P. mirabilis* and *E. coli*. In contrast, the Conv. method had good capability for reproducing sets of reactions for strains of *K. pneumoniae*.

In summary, there was no significant difference among the three methods in the ability to identify the study set of organisms to genus and species level. The LDC of Conv. method and CIT of API-20E were less sensitive than were the same tests of the other two methods. The LDC sensitivity appeared to be organism related.

Some aspects of reproducibility were considered. Results suggested that sets of reactions be used with extreme caution for biochemical biotyping of organisms.

ACKNOWLEDGMENTS

This study was supported in part by Corning Diagnostics, Roslyn, N.Y. Technologists who participated in the study were: M. Hommonay, J. Silverman, L. Whitbeck, M. Gregory, A. Dieter, J. Barnishan, and M. Smith at Ohio State University; G. Puhl, J. Stich, and W. Stinson at Cleveland Clinic Foundation; M. Brandon, J. Kertesz, G. Houston, C. Jeffries, and N. Shafer at St. Elizabeth Medical Center. Secretarial assistance was provided by E. Rosenzweig.

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