JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1975, p. 322-326
Vol. 2, No. 4

Printed in U.S.A.

Reproducibility of the Analytab (API 20E) System

DIANE A. BUTLER,[1] CORAZON M. LOBREGAT, AND THOMAS L. GAVAN*
Department of Microbiology, The Cleveland Clinic Foundation, Cleveland, Ohio 44106

Received for publication 17 June 1975

The reproducibility of the Analytab (API 20E) system for identification of *Enterobacteriaceae* was evaluated with 110 clinical isolates. Each isolate was identified by two technologists at different times. Genus-species identification was 97.3% reproducible; however, only 55.5% of the strains gave identical reactions in all 20 of the API 20E biochemical tests on repeat testing. Of those strains which varied, 56% possessed only one variable biochemical test. The reproducibility for each biochemical test was calculated and ranged from 89 to 100%. A subset of 20 of the most variable strains was tested further under conditions of varying incubation time (15 and 22 h) and inoculum concentration (10^7, 10^5, and 10^3 colony-forming units per ml), and by having four technologists interpret the test results. The reproducibility for each biochemical test for these 20 variable strains ranged from 86 to 99%. Less variation in interpretation by technologists was seen at an incubation time of 22 h and an inoculum concentration of 10^7 colony-forming units per ml. Consideration of the reproducibility for each biochemical test can aid in determining the probability that two isolates suspected of being the same strain, but with API profiles which differ by one or more biochemical test results, are in fact the same strain. Variables such as inoculum size, incubation time, technologist interpretation, and strip variability affect the API test results and should be standardized to minimize their effects.

The Analytab (API 20E) system has proven to be a valuable aid in identifying bacterial strains in the clinical microbiology laboratory (3, 6). Recently, several reports have further described the system as helpful in biotyping bacterial strains as an aid in epidemiologic studies (T. B. Neblett, E. J. Bottone, and J. F. Eisses, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C116, p.46; N. Trowers, T. Camino, B. Beatty, E. Dorvall, F. Jackson, E. Torres, and M. Brimmage, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C117, p.46). For this purpose, each of the 20 biochemical reactions must exhibit a known and high degree of reproducibility.

The present study, conducted in three phases, was undertaken to determine the reproducibility of each of the 20 biochemical reactions in the API 20E system under routine clinical laboratory conditions, and to evaluate the effects of standardizing certain test conditions.

MATERIALS AND METHODS

Phase I. One hundred and ten *Enterobacteriaceae*, isolated from clinical specimens received by the microbiology laboratory of The Cleveland Clinic Foundation, were used as test strains. The distribution of species among the six genera listed in Table 1 was proportional to their frequency of isolation in this laboratory. Therefore, these strains represent a cross section of clinically encountered strains rather than an attempt to include equal numbers of each genera tested. Identification of each isolate using the API 20E system was performed at two separate times by different skilled technologists.

In this phase, the API 20E test was performed by the manufacturer's instructions as follows. Approximately 5 ml of water was dispensed into the API 20E incubation tray to provide a humid atmosphere during incubation. The inoculum was obtained by selecting an isolated colony from the surface of a solid agar medium with the use of a sterilized wire inoculating loop. The inoculum was dispersed into 5 ml of sterile distilled water, mixed with a 4-ml pipette just before inoculation, and then inoculated into the 20 microtubes of the API 20E test strip. Table 2 lists each of the 20 tests and their abbreviations. Both the microtube and cupule for the CIT, VP, and GEL tests were filled with the inoculum suspension. For all other tests, only the microtubes were filled. The ADH, LDC, ODC, URE, and ARA cupules were filled with mineral oil after inoculation.

TABLE 1. *Organisms tested in phase I*

Species	No. tested
Escherichia coli	53
Proteus sp.	20
Klebsiella sp.	19
Enterobacter sp.	12
Citrobacter sp.	4
Serratia sp.	2

[1] Present address: The Ohio State University, School of Medicine, Columbus, Ohio 43210.

The plastic lid was placed over the incubation tray and strips and incubated 18 to 24 h at 35 C. After incubation, appropriate reagents were added to the VP, IND, and TDA tests and the 20 test reactions (plus the oxidase reaction) were recorded as a seven-digit number as described in the API analytical profile index (Analytab Products Inc., 1st ed., 1974). This seven-digit number is the API profile number. Bacterial identification was determined with the API profile index. No other identification schema was employed.

Phase II. In phase II, variables considered to be possible factors in the reproducibility of the API profile number were tested under controlled conditions on a subset of 20 test strains (selected from phase I strains exhibiting variable API profiles) (Table 3). These strains were tested at three inoculum concentrations (10^7, 10^5, and 10^3 colony-forming units [CFU]/ml) at two incubation times (15 and 22 h), and with four technologists giving their interpretations of each test result. The different inoculum concentrations were obtained by serial 1/100 dilutions in sterile distilled water from which colony counts were performed.

The strips were read by technologists who, although familiar with the routine reading of API strips, were unaware of the specific variables pertaining to each strip. These variables were selected to represent the extremes of variation that might possibly occur in our laboratory, although it is recognized that these extremes are not included in the manufacturer's recommendations. Each test was performed in replicate, using two different API strips (of the same lot number) inoculated from the same bacterial suspension.

Phase III. Phase III consisted of an examination of data obtained from 19 routine repetitive inoculations of three quality control organisms into API 20E test strips. The API tests were performed on different lots of API strips and the results were interpreted by different technologists. The three strains of *Enterbacteriaceae* (*Escherichia coli, Proteus mirabilis,* and *Enterobacter aerogenes*) were among five strains (the other two being non-fermentative gram-negative bacilli) selected to provide both positive and negative reactions for each of the 20 biochemical tests on the API 20E test strip. Only the data for the *Enterobacteriaceae* among the quality control strains were considered in this study.

TABLE 2. *API 20E biochemical tests*

Test sequence	Biochemical test	API abbreviation
1	*o*-Nitrophenyl-β-D-galactosidase	ONPG
2	Arginine dihydrolase	ADH
3	Lysine decarboxylase	LDC
4	Ornithine decarboxylase	ODC
5	Citrate utilization	CIT
6	H_2S production	H_2S
7	Urease	URE
8	Tryptophan deaminase	TDA
9	Indole production	IND
10	Acetoin production	VP
11	Gelatinase	GEL
12	Glucose fermentation	GLU
13	Mannitol fermentation	MAN
14	Inositol fermentation	INO
15	Sorbitol fermentation	SOR
16	Rhamnose fermentation	RHA
17	Sucrose fermentation	SAC
18	Melibiose fermentation	MEL
19	Amygdaline fermentation	AMY
20	Arabinose fermentation	ARA

TABLE 3. *Organisms tested in phase II*

Species	No. tested
Escherichia coli	5
Proteus sp.	2
Klebsiella sp.	6
Enterobacter sp.	4
Citrobacter sp.	2
Serratia sp.	1

RESULTS

Of the 110 strains tested in duplicate during phase I, 61 (55.5%) produced identical API profiles. Forty-six additional strains (41.8%) were identified as the same genus and species, but produced different API profile numbers the second time. Only three strains (2.7%) produced API profiles on the second trial that resulted in bacterial identification that differed in genus or species or both from that determined the first time they were tested.

An analysis of the API profiles of the 49 strains exhibiting changes in one or more of the 20 biochemical tests revealed that 51% of the discrepancies were due to a change in only one biochemical reaction. Table 4 lists the percentage of these strains in which one to four reactions varied. Of the three strains that were identified as different genus or species or both, one demonstrated only a single variable biochemical reaction and the other two differed by two reactions. These changes in identification on duplicate testing were: (i) *E. coli* to *Citrobacter diversus*; (ii) *Serratia liquefaciens* to *Enterobacter aerogenes;* and (iii) *Shigella boydii* to *E. coli*.

The reproducibility of each biochemical test among all 110 strains is given in Table 5. Re-

TABLE 4. *Phase I. Discrepant biochemical tests per API 20E profile among 49 organisms*

No. of discrepancies per API profile	%
1	51
2	29
3	16
4	4

sults of the H_2S, TDA, IND, and MAN tests were 100% reproducible. The remaining 16 biochemical reactions varied in their reproducibility from 89 to 99%. There was a total of 85 (3.9%) disagreements among the 2,200 individual tests performed.

In phase II, for each of the two incubation times (15 and 22 h) and each of the three inoculum concentrations (10^7, 10^5, and 10^3 CFU/ml), the ability of at least three of four technologists to obtain the same API profile number was used to calculate the reproducibility shown in Table 6. Longer incubation time and higher inoculum gave the highest reproducibility of API profiles among four technologists.

Percentage of agreement was also calculated when the same technologist obtained identical API profile numbers after interpreting two API test strips inoculated and incubated under identical conditions. Again, longer incubation time and higher inoculum size gave the highest reproducibility (Table 6). Although these differences are not statistically different, as tested by the chi-square test, there is a trend toward greater reproducibility at longer incubation time and higher inoculum concentration. The calculated reproducibility for each of the 20 API test reactions on these strains is shown in Table 7.

The reproducibility of the 19 duplicate API tests on each of three quality control *Enterobacteriaceae* of phase III ranged from 95 to 100% and is listed in Table 8.

DISCUSSION

An analysis of the duplicate API 20E profile numbers of 110 randomly selected bacterial strains demonstrated 97.3% reproducibility of genus-species identification. Reproducibility of the identical API profile number was 55.5%, and of the remaining 44.5% demonstrating different API profile numbers 51% differed by only one biochemical test. However, the number of variable biochemical reactions may not parallel the probability of a change in the identification assigned the strain. All three strains showing changes in four biochemical reactions were identified as the same genus and species, despite these changes. The three strains identi-

TABLE 7. *Phase II. Reproducibility of API 20E biochemical tests among 20 selected organisms*

Biochemical test	Reproducibility (%)
H_2S, TDA, IND	98–99
GEL, MAN, SAC	97
ONPG, ADH, SOR	95–96
URE, INO, RHA	93–94
ODC, VP, GLU, MEL, AMY, ARA	90–92
LDC	88
CIT	86

TABLE 5. *Reproducibility of API 20E biochemical tests among 110 organisms tested*

Biochemical test	Reproducibility (%)
H_2S, IND, MAN, TDA	100
ADH, ODC, INO, SOR	98–99
GLU	97
LDC, ONPG, URE, GEL, RHA, SAC	95–96
VP, MEL, ARA, AMY	90–94
CIT	89

TABLE 8. *Phase III. Reproducibility of API 20E biochemical tests among three quality control strains*

Biochemical test	Reproducibility (%)
ONPG, ADH, LDC, ODC, CIT, H_2S, URE, TDA, IND, VP, GLU, MAN, INO, SOR, SAC, AMY, ARA	100
RHA, GEL	98
MEL	95

TABLE 6. *Effect of incubation time and inoculum size on subjective interpretation of API 20E test of 20 selected organisms*

API test reading	Reproducibility (%)				
	Incubation time		Inoculum size		
	15 h	22 h	10^7 CFU/ml	10^5 CFU/ml	10^3 CFU/ml
Same API test read by four technologists (three to four technologists agree)	67.5	75.8	77.5	72.5	65.0
Two API tests read by same technologist (both tests agree)	55.4	63.7	67.5	54.4	56.9

fied differently, however, exhibited only one or two variable biochemical reactions.

In phase II, a subset of 20 of the most discrepant strains was tested under conditions of three different inoculum concentrations and two different incubation times. The highest inoculum and the longer incubation were found to give the highest percentage of reproducibility of API profile number among four technologists reading the same API strip (Table 6). The same conditions gave greater reproducibility when two identically prepared API strips were interpreted by the same technologist (Table 6). An increase in inoculum concentration has also been reported to increase the correlation of API test results with macro-identification methods (3). The lower reproducibility of each biochemical test in phase II (Table 7) as compared to phase I (Table 5) is expected, since the strains in phase II were selected for their previously demonstrated variability.

The following two examples selected from phase II data substantiate that these variables can cause significant changes in biochemical test results. In strips inoculated identically but incubated either 15 or 22 h, the results of two biochemical reactions (CIT and INO) differed, causing the identification to be *E. coli* and *Citrobacter freundii* at 15 and 22 h, respectively. Likewise, with strips varying only in the inoculum concentration of a strain of *P. mirabilis* (10^7, 10^5, and 10^3 CFU/ml), five biochemical reactions (ODC, CIT, URE, GEL, and GLU) gave positive reactions at 10^7 CFU/ml, but were negative at either 10^5 or 10^3 CFU/ml (giving a profile number no longer identifiable as *P. mirabilis*). Although these represent extreme examples of separately controlled variables, the results emphasize the importance of standardizing these variables to provide greater uniformity of API profile numbers.

The overall lower reproducibility within the second category of Table 6 (i.e., when two API tests were read by the same technologist) could be the result of either intratechnologist interpretive variability or API 20E strip variability. Since the two API 20E test strips were inoculated from the same bacterial suspension with strips of the same lot number, it is possible to attribute this variability to the individual interpretations of the technologists. However, in 48.7% of the replicate readings of two identically prepared API strips by four technologists, at least three technologists agreed on one API profile number for one strip and also agreed on a different API profile number for the other strip.

The phase III data show good reproducibility of all 20 API 20E biochemical reactions with the quality control strains selected for use in this laboratory, although occasional variability is observed. These strains do not produce known "borderline" biochemical test results, although such strains would perhaps better serve the purpose of controlling any system of bacterial identification employing reactions on differential media.

Since each biochemical test can be assigned a degree of reproducibility, the probability of identity of two isolates (suspected of being the same strain) with API profiles which differ by only one biochemical test result, can be evaluated in terms of the probability of variation of the differing biochemical reaction. Differences between those biochemical tests showing very high reproducibility would tend to support the conclusion that there is a difference between the strains. On the other hand, differences between biochemical tests with lower reproducibility would not confirm a difference between strains and should provide reason for reidentification of all isolates involved with all recognized variables controlled.

Although it was not the purpose of this study to compare the results of the individual API biochemical tests with those of "conventional" media, it is important to comment on the reproducibility of these later methods. Several groups of workers have pointed out that the extent of test errors is much greater than one might expect (1, 2, 5). Sneath and Johnson (5), in evaluating methods for identification of pseudomonads, indicate that the mean percentage of disagreement for replicates within laboratories is about 2.9% with values for individual tests ranging from almost 0 to 12%. Sneath (4) further cites the work of Jones, Wilkinson, and Feltham working in his laboratory on a number of gram-positive and gram-negative bacteria which places the average percentage of disagreement between replicates at about 3% for *Listeria*, 2.5% for enterobacteria, and 2.4% for coryneform bacteria. Comparable figures were obtained in the present study. Of the 2,200 biochemical tests evaluated in phase I there were 85 discrepancies in results between the first and second replicate tests, or 3.9% average disagreement.

Sneath and Johnson (5) indicate that serious errors in bacterial identification do not become significant until the average probability of an incorrect test reading rises above 10%.

Factors in addition to inoculum density, incubation time, and interpretation may have influenced the outcome of the present study. These include the possibility of contamination, clonal

variation on subculture, and clerical errors. Although these factors are difficult to evaluate, few if any clinical laboratories are free of these sources of error and therefore they should be considered. One of the distinct advantages of this multitest system is its ability to reproduce the same genus and species identification of organisms in spite of individual test variability due to whatever cause. It can be expected that, as the data base for the API identification scheme becomes enlarged, the system will become even less sensitive to individual test variation. Indeed, with the latest available version of the profile index and enlarged data base (Analytab Products Inc., 2nd ed., 1975), two of the three genus-species changes observed in phase I would not have been observed. In the third case a suggestion to confirm the identity of the organism as *Shigella* species would have very likely led the user to recognize the error.

In conclusion, the API test demonstrated 97.3% reproducibility of genus-species identification with 110 randomly selected clinical strains of *Enterobacteriaceae*. The reproducibility of each of the individual 20 biochemical reactions ranged from 89 to 100%. Variables such as inoculum size, incubation time, technologist interpretation, and strip variability affect the API test results. When identifying strains for purposes of epidemiological biotyping, the effects of these variables can be minimized by having the same person perform the API test on all strains at the same time, under similar conditions of inoculum and incubation time. Quality control strains, not selected for their borderline reactions, are of less help in detecting the variables influencing epidemiological biotyping than strains selected specifically for this purpose.

LITERATURE CITED

1. Lapage, S. P., S. Bascomb, W. R. Willcox, and M. A. Curtis. 1970. Computer identification of bacteria, p. 1–22. *In* A. Baille and R. J. Gilbert (ed.), Automation, mechanization and data handling in microbiology. Academic Press Inc., London.
2. Lapage, S. P., S. Bascomb, and W. R. Willcox, and M. A. Curtis. 1973. Identification of bacteria by computer: general aspects and perspectives. J. Gen. Microbiol. 77:273–290.
3. Smith, P. B., K. M. Tomfohrde, D. C. Rhoden, and A. Balows. 1972. API system: a multitube micromethod for identification of *Enterobacteriaceae*. Appl. Microbiol. 24:449–452.
4. Sneath, P. H. A. 1974. Test reproducibility in relation to identification. Int. J. Syst. Bacteriol. 24:508–523.
5. Sneath, P. H. A., and R. Johnson. 1972. The influence on numerical taxonomic similarities of errors in microbiological tests. J. Gen. Microbiol. 72:377–392.
6. Washington, J. A., II, P. K. W. Yu, and W. J. Martin. 1971. Evaluation of accuracy of multitest micromethod system for identification of *Enterobacteriaceae*. Appl. Microbiol. 22:267–269.