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Analysis of Cost and Accuracy of Alternative Strategies for *Enterobacteriaceae* Identification

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Analysis of the cost of time and material required for the diagnosis of *Enterobacteriaceae* isolates indicated that a conventional 17-tube (20-test) setup costs $7.98 per isolate identified. Using the API 20E, a similar identification cost $3.02. A conventional 7-tube (10-test) setup cost $3.60, whereas the comparable API 10S cost $2.33. Compared with the API 10S, using the API 20E increased costs by 30% while increasing the number of isolates identified correctly by 3%. Other strategies using the API 10S in combination with the API 20E or a deoxyribonuclease test were also evaluated for cost and accuracy.

A number of kits are commercially available for the diagnosis of medically important bacteria, particularly in the family *Enterobacteriaceae*. Evaluation of these methods has generally demonstrated satisfactory performance when compared with more traditional methods. In addition, some of these kits have utilized the power of computer diagnosis to increase their accuracy by analyzing data bases derived from their own results or extrapolated from existing data. The laboratory director is confronted with the problem of whether switching to one of these newer methods is advantageous and also financially practical. With these considerations in mind, we have performed a detailed time-and-cost analysis that compares a more traditional diagnostic approach with one of these new systems. A standard 7-tube (10-test) and expanded 17-tube (20-test) setup is compared with the API 10S and API 20E kits (Analytab Products Inc., Plainview, N.Y.) from the standpoints of time expended, cost of material, and diagnostic accuracy.

MATERIALS AND METHODS

The API system provides a scheme for identifying *Enterobacteriaceae* on the basis of 21 biochemical tests performed with the API 20E kit. The biochemical reactions beta-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate, hydrogen sulfide, urease, tryptophan deaminase, indole, Voges-Proskauer, gelatin, glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, arabinose, and oxidase are read as positive or negative at 18 to 24 h. A smaller version of the kit (API 10S) includes the 11 tests beta-galactosidase, glucose, arabinose, lysine decarboxylase, ornithine decarboxylase, citrate, hydrogen sulfide, urease, tryptophan deaminase, indole, and oxidase.

By using the plastic API Coder (Analytab Products Inc.), test results are reduced to a unique profile number. With the API 20E, the user simply looks up the profile number in a directory to find the genus and usually the species corresponding to the observed pattern of test results. An interpretive pattern directory has also been developed for the API 10S kit (5).

For comparison purposes, a set of 17 tubes was chosen corresponding to the tests included in the API 20E kit: Kligler's iron agar (lactose instead of beta-galactosidase, glucose, and hydrogen sulfide), lysine iron agar (lysine deaminase instead of tryprophan deaminase; lysine decarboxylase), arginine dihydrolase, ornithine decarboxylase, citrate, urease, indole, Voges-Proskauer, gelatin, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, and arabinose.

The 7 tubes corresponding to the API 10S kit include Kligler's iron agar (lactose instead of beta-galactosidase; glucose; and hydrogen sulfide), lysine iron agar (lysine deaminase instead of tryptophan deaminase; lysine decarboxylase), arabinose, ornithine decarboxylase, citrate, urease, and indole.

To evaluate the cost of alternative methods of identification, the following diagnostic strategies were considered: API 20E alone; API 10S alone; API 10S on all isolates followed by an API 20E on those isolates having an ambiguous pattern at the genus level or at the species level; API 10S and a deoxyribonuclease (DNase) plate on all isolates; API 10S on all isolates followed by DNase on selected isolates; 7-tube (10-test) setup alone; and 17-tube (20-test) setup alone.

All identification strategies include streaking the colony on one-half of a blood agar plate and one-half of a MacConkey agar plate to check purity. When DNase testing is performed, a single plate suffices for eight isolates. The cost of oxidase testing has not

been shown; all strategies considered in this study presuppose that the isolate has been demonstrated to be oxidase negative.

The costs of media and kits shown in Table 1 reflect market prices for modest-volume users in late 1975. The $6.00 hourly rate for technologist time corresponds to a GS-7 step 3 laboratory worker employed under the U.S. Civil Service system. No allowance has been made for overhead costs, such as the cost of ancillary personnel, fringe benefits for personnel, equipment costs, or the cost of obtaining an isolated colony ready for identification.

In estimating the worker time required for completing the various procedures described in this study, standard industrial engineering techniques (4) were used by a management analyst experienced in using these techniques in a clinical laboratory setting.

The total process of performing the laboratory determination under each method was divided into its individual steps (Tables 2 and 3). This assured consistency among the times for the alternative methods by making certain that the times being compared were for like elements of work.

Where steps were identifiable as part of a standard laboratory process, use was made of data from a work measurement study of procedures in hospital clinical laboratories, published by the Chicago Hospital Council (1). Use of such predetermined time standards offers the objectivity of applying standard motion/time elements to the basic activities needed to accomplish a given task. Their use also takes advantage of the multitude of validation observations that have been made by the publishers of the standards. These factors minimize observer bias in the time estimates. In all instances where the predetermined time standards were used, the analyst verified the validity of these standards in the observed situation.

Where the predetermined time standards were not applicable, either because of judgmental steps within the operation or because of unique laboratory layouts or other local characteristics, the analyst reverted to basic stopwatch time studies. Such elements as marking cards and plates, examining plates, reading and encoding results, and writing reports were among those studied by actual observation. The times thus determined were reviewed with the technologists for reasonableness.

The published predetermined time standards are generally expressed in "standard hours" per operation. This means that the published figure for hours includes a 15% allowance for operator factors. Although this adjustment is a generally accepted method of translating stopwatch time studies into staffing projections, the time estimates of this study were simply for the individual operations. To make the predetermined time standards comparable to the stopwatch time studies, the 15% allowance was deducted from the published standards.

In estimating the accuracy of various strategies, the API 20E has been used as the standard for comparison. The percentage of isolates that would receive a different identification using the API 10S kit has been calculated from the API 10S pattern directory. When two or more different species produce the same pattern of test results, all isolates showing that pattern are assigned the identification most frequently associated with that pattern (5). In estimating the accuracy when DNase testing is included, the following rates of DNase positivity have been used: *Serratia marcescens*, 96.7% (2); *S. liquefaciens*, 88.3%; *S. rubidaea*, 100.0% (3); all others, 0%.

A direct comparison of the accuracy of the kits and the conventional methods was not possible, since the 37,000 isolates used to construct the API directories were not available to us for retesting by the 7- and 17-tube conventional setups. Because the conventional biochemical tests were chosen to parallel those included in the kits, we presume that the accuracy achieved would parallel that of the kits using the same number of tests.

For strategies requiring additional testing of some isolates, the total cost is obtained by adding the cost of the basic testing to the cost of the additional testing multiplied by the fraction of isolates requiring additional testing.

RESULTS

Table 1 shows the cost figures used for material and technologist time. Table 2 details the time analyses in terms of fractions of an hour expended for each step of the methods using tubed media. The summation of these components provides an estimate of the time expended by an average laboratory worker to identify an isolate. Table 3 analyzes the steps used with the API 10S and 20E kits. In addition, the time analysis to perform an additional DNAse test is also included. Table 4 summarizes the costs and accuracy of each method. Various operational approaches using combinations of the API kits with or without DNase testing are included.

Performing 20 tests with the API 20E kit cost $3.02, whereas the comparable 17-tube method cost $7.98 per isolate. The diagnostic approach with the lowest cost was use of the API 10S kit alone with a total cost of $2.33 per diagnosis. The comparable standard 7-tube setup cost $3.60 per diagnosis. The diagnoses were theoretically accurate 96.9% of the time at the genus level and 95.9% at the species level using

TABLE 1. *Cost of time and material*

Item	Unit cost ($)
API 20E kit	2.05
API 10S kit	1.50
Tube for biochemical test	0.30
Blood agar plate	0.29
MacConkey plate	0.33
DNase plate	0.45
Technologist time, per hour	6.00

the API 10S. Using a DNase test on all isolates in conjunction with the API 10S increased the cost to $2.49, but increased the theoretical accuracy to 97.8% at the genus level and 96.8% at the species level.

DISCUSSION

Table 4 indicates that the kits are less costly than the traditional methods in terms of both cost and materials. In fact, performing 20 tests with the API 20E kit costs less than performing only 10 tests with tubed media. The table indicates several possible trade-offs between cost and accuracy. Switching from the API 10S kit to the API 20E kit increases the number of isolates identified correctly at the genus level by 3% while increasing the cost by 30% ($0.69 per isolate). Setting up a DNase plate with the API 10S kit increases the accuracy by 1% and the cost by 7% ($0.16 per isolate).

To reduce cost, it might be suggested that the API 10S be used for screening and the API 20E be used on either all ambiguous patterns or on patterns ambiguous at the genus level. It can be seen that these two approaches are not only more time consuming but also more costly. A possible approach might be to add a DNase test in those circumstances where it would be useful. Although this adds little cost, it increases the time to final diagnosis.

Although the DNase test frequently helps separate *Serratia* from *Enterobacter*, for certain patterns of API 10S results it is misleading. For example, profile 3700 (positive results for beta-galactosidase, glucose, lysine decarboxylase, ornithine decarboxylase, and citrate) was observed in 391 *S. marcescens* and 1 *Enterobacter aerogenes* (5). Without the DNase test all the *Serratia* would be correctly identi-

TABLE 2. *Time analyses for 7- and 17-tube methods*

Operation	Hours required	
	7 tubes	17 tubes
Assemble tubes	0.00257	0.00623
Label 1 tube	0.00316	0.00316
Label 2 purity plates	0.00722	0.00722
Stamp test names on worksheet	0.00083	0.00083
Inoculate tubes	0.10606	0.25758
Cover decarboxylases with oil	0.00197	0.00394
Streak 2 purity plates	0.00812	0.00812
Examine 2 purity plates	0.01344	0.01344
Read tubes	0.04709	0.11424
Record results	0.00195	0.00473
Identify organism	0.00162	0.00394
Record identification	0.00513	0.00513
Total	0.19916 h 11.949 min	0.42856 h 25.713 min

TABLE 3. *Time analyses for API procedures*

Operation	Hours required	
	10S	20E
Pick colony and make suspension	0.00879	0.00879
Label kit	0.00316	0.00316
Label purity plates	0.00722	0.00722
Load pipette	0.00111	0.00111
Pipette suspension to kit and purity plates	0.01740	0.03190
Cover appropriate tests with oil	0.00581	0.00775
Put water in base	0.00194	0.00194
Streak 2 purity plates	0.00812	0.00812
Examine 2 purity plates	0.01344	0.01344
Read and encode results	0.00625	0.01250
Identify organism	0.00833	0.00833
Record identification	0.00513	0.00513
Total	0.08670 h 5.2020 min	0.10939 h 6.5634 min
Mark DNase plate	0.00361	
Pipette 1 drop	0.00145	
Streak plate	0.00406	
Examine plate	0.00672	
Total	0.01584 h 0.9504 min	

TABLE 4. *Costs and accuracy of alternative strategies*

Strategy	Time (min)	Cost of time ($)	Cost of material ($)	Total cost ($)	Incubation cycles (days)	Differences from API 20E (%)	
						Genus	Species
API 20E	6.56	0.66	2.36	3.02	1.00	0.0	0.0
API 10S best judgment	5.20	0.52	1.81	2.33	1.00	3.1	4.1
API 10S on all; API 20E on all ambiguities	8.01	0.80	2.82	3.62	1.43	0.0	0.0
API 10S on all; API 20E on ambiguous genera	7.74	0.77	2.72	3.49	1.39	0.0	1.0
API 10S and DNase on all	6.15	0.62	1.87	2.49	1.00	2.2	3.2
API 10S and selective DNase	5.32	0.53	1.82	2.35	1.13	2.2	3.2
Routine 7-tube setup	11.94	1.19	2.41	3.60	1.00		
Routine 17-tube setup	25.71	2.57	5.41	7.98	1.00		

fied and the single *Enterobacter* would be misidentified. Considering the DNase test, positive isolates would be called *Serratia* and negative isolates called *Enterobacter*. Thus the single *Enterobacter* isolate would be correctly identified, but 11 DNase-negative *Serratia* would be misidentified as *Enterobacter* (only 96.7% of *Serratia marcescens* are DNase positive).

Compared with traditional tube methods, the kits offer savings in both time and material costs. In addition to the savings detailed in Table 4, kits require less storage and incubator space and simplify quality control. They provide standardization of methods, permitting use of interpretive pattern directories based on the study of a large number of isolates with the same test systems. Thus, test pattern interpretation is standardized and can be done by a less sophisticated operator. This may result in further cost reduction and/or improvement in accuracy.

From a diagnostic standpoint more data is better than less, but it is harder to manipulate and assimilate. Therefore, when dealing with 10 tests or fewer, manual interpretation is possible, but without the computer analysis it is difficult to have precise information on what patterns are inherently more ambiguous than others. As the number of tests performed increases, the time required for their interpretation increases disproportionately. Thus the time in Table 2 for interpretation of the 17 tubes probably represents a considerable underestimate.

In calculating the cost of technologist time, no allowance for operator factors has been included. Typically the times would be increased by an arbitrary 15%. Had we included such an allowance, the difference between the kits and traditional methods would be even greater.

Although accuracies and costs have been compared with those of the API 20E, in practice, problem isolates might receive tests going beyond those included in the API 20E; this would increase the total cost. Although the accuracy of the 7- and 17-tube setups may correspond to that of the two kits, we do not have data directly demonstrating this. If in fact the traditional tube method is less accurate because of poorer quality control or less sophisticated interpretive aids, this would be a further advantage for the kits.

It is anticipated that other kits would show similar or greater savings in time; however, the overall cost and accuracy would have to be evaluated on an individual basis. As new kits and other groups of organisms are studied, similar evaluations must be performed. These analyses will of necessity be limited until the data bases for each methodology encompass a sufficiently large number of isolates.

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