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Preliminary Evaluation of the autoSCAN-3, an Instrument for Automated Reading and Interpretation of Microdilution Trays: Identification of Aerobic Gram-Negative Bacilli

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The autoSCAN-3, an instrument for the automated reading and interpretation of Microscan microdilution trays, was evaluated for its ability to identify gram-negative bacilli. The results obtained by the visual reading of microdilution trays were compared with the results obtained with the machine. A total of 387 clinical isolates of *Enterobacteriaceae* and nonfermenters were compared by both methods. In 369 instances (95%), the identification obtained by visual reading of the microdilution tray agreed with the identification obtained with the autoSCAN-3. In eight instances, the visual identification differed completely from that of the machine, and in nine cases, the machine was unable to provide an identification.

The growing availability of commercially prepared microdilution trays for bacterial identification and the quantitative determination of antibiotic susceptibility has led to the increased adoption of microdilution methods by clinical microbiology laboratories. After inoculation and incubation, microdilution trays are examined by a technologist, usually with the aid of a viewer, and the results are interpreted. However, it has been found that significant error can occur in the reading and interpretation of microdilution trays (2). Recently, an automated instrument which reads and interprets the microdilution trays of one manufacturer has become available. The present study describes the preliminary evaluation of this instrument in a clinical laboratory setting.

MATERIALS AND METHODS

Organisms. All organisms were recent clinical isolates obtained from adult patients at the Columbia-Presbyterian Medical Center. Colonies of gram-negative bacilli to be tested were picked from MacConkey agar plates (Scott Laboratories, Inc., Fiskeville, R.I., or BBL Microbiology Systems, Cockeysville, Md.) and used to inoculate microdilution trays as described below.

Microdilution tray procedure. Commercially prepared microdilution trays with 96 wells (Microscan, Hillsdale, N.J.) were obtained and stored in the frozen state. Of the 96 wells, 28 contained the biochemical substrates glucose, sucrose, sorbitol, raffinose, rhamnose, arabinose, inositol, adonitol, cellobiose, urea, sodium thiosulfate and iron (H_2S), tryptophan (indole), lysine, arginine, ornithine, tryptophan (deaminase), esculin, *o*-nitrophenyl-β-D-galactopyranoside, citrate, malonate, acetamide, tartrate, glucose (oxidative fermentative), maltose (oxidative fermentative), xylose (oxidative fermentative), starch, nitrate, and buffered broth (Voges-Proskauer). An additional 2 wells contained broth for sterility and growth controls, and the remaining 66 wells contained a variety of antibiotics in dilutions suitable for the determination of minimum inhibitory concentrations.

Four to six 18- to 24-h colonies of the organism to be tested were picked from MacConkey agar plates, placed in 0.5 ml of brain heart infusion broth, and incubated for 3 to 4 h at 35°C. The broth culture was used as inoculum by adding 50 μl to 25 ml of sterile 0.02% Tween 80 in a screw-capped tube. Portions of the broth were also used to inoculate plates of deoxyribonuclease medium and sheep blood agar (Scott); the latter was used as a purity check. Growth from the blood agar was subsequently used to perform an oxidase test (Pathotec CO; General Diagnostics, Morris Plains, N.J.).

The inoculated tube of Tween 80 was mixed by inversion, and the contents were poured into a sterile shallow rectangular plastic seed trough provided by the manufacturer. The lid of this trough was fitted with 95 plastic pegs corresponding to the wells of the microdilution tray; these pegs were immersed in the inoculum when the lid was placed on the trough. The lid was then transferred over the microdilution tray and lowered so that each well was simultaneously inoculated. The lid was then discarded, and wells for the detection of glucose fermentation, urease, arginine dihydrolase, H_2S, and lysine and ornithine decarboxylases were overlayed with mineral oil; wells containing citrate, malonate, oxidative-fermentative glucose, oxidative-fermentative maltose, oxidative-fermentative xylose, acetamide, tartrate, and *o*-nitrophenyl-β-D-galactopyranoside substrates were covered with a strip of cellophane tape. The single uninoculated well served as a sterility control. The microdilution trays were incubated in air at 35°C for 15 to 18 h.

Microdilution trays were read visually at least 10 min after the addition of Kovac's and Voges-Proskauer reagents and 10% ferric chloride solution to the appropriate wells. For nonfermenters, iodine solution was added to the starch well. The results were interpreted by referring to a chart supplied by the manufacturer. Alternatively, a 7-digit biotype number could be generated by assigning a weighted numerical value to all positive reactions. A code book listing the biotypes of aerobic gram-negative bacilli based upon 21 reactions was provided by the manufacturer.

autoSCAN-3 procedure. The instrument under evaluation was a prototype unit consisting of two modules: a reader module with a drawer for receiving the microdilution tray and a display module consisting of a keypad for entering data and controlling machine operation, a slot for the insertion of a multipart report form, and a display panel. The machine was permitted to warm up for 30 min and was then calibrated with a microdilution tray containing 100 μl of sterile distilled water in each well, as recommended by the manufacturer.

Microdilution trays were read visually ca. 10 min after the addition of the chemical reagents; the autoSCAN-3 reading was performed within 45 min of the visual interpretation. The operator did not know the results of the visual interpretation before carrying out the machine reading.

For operating the machine, a microdilution tray was placed in the drawer of the reader module. After the drawer was closed, a single number that programmed the machine for gram-negative organisms was pressed on the keypad, and the report form was inserted into the slot on the display module. The most probable identification of the organism was displayed on the panel within a few seconds, together with the percent probability of correct identification. This identification, the biotype number, and the minimum inhibitory concentrations were printed on the report form. Upon command the instrument could also display the second and third choices, together with their biotypes and probability of correct identification.

If the growth was inadequate or too heavy, the unit displayed the message "sterile—growth insufficient." If the results were not in conformity with the data base (as with a mixed culture), the unit displayed the message "very rare biotype, check manually."

In the case of nonfermenters, the machine required the manual entry of the oxidase and nitrate reduction test results, as well as results of growth on MacConkey agar.

RESULTS

A total of 387 trays with gram-negative isolates were read both visually and with the autoSCAN-3. Of these, 349 (90.2%) showed complete agreement between the visual identification and the first choice of the autoSCAN-3. These organisms are shown in Table 1. An additional 19 isolates (4.9%) showed agreement between visual identification and the second choice of the autoSCAN-3 (Table 2). A single isolate visually identified as *Klebsiella oxytoca* agreed with the third choice of the machine; the first choice was *Klebsiella pneumoniae*, and the second choice was *Enterobacter aerogenes*.

TABLE 1. *Organisms showing agreement between autoSCAN-3 first-choice identification and visual identification*

Organism	No. of isolates in agreement
Escherichia coli	173
Klebsiella pneumoniae	52
K. oxytoca	14
K. ozaenae	1
Enterobacter aerogenes	3
E. cloacae	18
E. agglomerans	9
Serratia marcescens	5
Proteus mirabilis	36
P. vulgaris	3
Morganella morganii	2
Providencia stuartii (urea positive)	1
Citrobacter freundii	2
Pseudomonas aeruginosa	29
Aeromonas hydrophila	1

There were eight instances in which the visual identification failed to agree with any of the choices of the machine (Table 3).

In 10 instances, the autoSCAN-3 was unable to identify the organism and displayed the message "very rare biotype—check manually," indicating that the reactions did not correspond to any of those stored in the data base of the instrument. These isolates were visually identified as *Escherichia coli* (two strains), *K. pneumoniae* (two strains), *Serratia marcescens*, *Serratia liquefaciens*, *Proteus mirabilis*, *Morganella morganii*, *Acinetobacter anitratus*, and *Pseudomonas maltophilia*.

DISCUSSION

Analysis of the discrepant results revealed that in at least 11 instances, the disagreement involved the misinterpretation by the machine of the indole reaction, leading to the confusion of *K. pneumoniae* with *K. oxytoca* and of *P. mirabilis* with *M. morganii*. At the time of testing, the Kovac's reagent employed was prepared with isoamyl alcohol and hydrochloric acid. Positive reactions resulted in a red ring, whereas negative tests often produced a brown oxidation product. These conditions gave rise to false-negative and false-positive interpretations by the machine. The authors have subsequently been advised by the manufacturer that the substitution of amyl alcohol for isoamyl alcohol in the reagent eliminates spurious indole reactions.

A second source of discrepancies involved the

TABLE 2. *Organisms showing agreement between autoSCAN-3 second-choice identification and visual identification*

Visual and autoSCAN-3 second choice	autoSCAN-3 first choice (no. of isolates)	Probability of first-choice identification being correct (%)
Klebsiella oxytoca	*Klebsiella pneumoniae* (5)	99.8
K. pneumoniae	*K. oxytoca*	99.8
Morganella morganii	*Proteus mirabilis* (4)	68–72.7
Proteus mirabilis	*Morganella morganii*	95
Klebsiella pneumoniae	*Serratia rubidaea* (2)	81.7–88.2
Enterobacter cloacae	*S. rubidaea*	99.9
E. agglomerans	*S. rubidaea*	46.7
Escherichia coli	*Klebsiella ozaenae*	88.4
Enterobacter aerogenes	*Enterobacter cloacae*	82.0
Pseudomonas aeruginosa	*Pseudomonas fluorescens* (2)	84.8–90.9

TABLE 3. *Isolates showing complete discrepancy between visual identification and autoSCAN-3 identification*

autoSCAN-3 identification (no. of isolates)	Probability of correct identification (%)	Visual identification
Serratia rubidaea (2)	96.3	*Klebsiella pneumoniae*
S. odorifera	84.0	*Escherichia coli*
Enterobacter agglomerans	62.7	*Enterobacter cloacae*
E. cloacae	82.7	*E. gergoviae*
E. aerogenes	96.6	*E. gergoviae*
CDC-Ve 2 (2)	99.7	*Acinetobacter anitratus*

reading of borderline carbohydrate reactions. Production models of the autoSCAN-3 have been equipped with improved optical filters which are said to result in more clear-cut reactions. The manufacturer strongly advises that any organism with a probability of identification <90% should be examined visually, and other parameters, such as antibiotic susceptibility, should be taken into consideration. Several of the isolates that the autoSCAN-3 failed to identify proved to consist of mixed cultures undetected by visual examination.

Microdilution technology has been shown to be useful for identification of *Enterobacteriaceae* (1, 3, 4). The accuracy of the method depends in part upon the interpretation of the reactions in each well. Visual reading of these reactions is a subjective process which can lead to variability and error. The autoSCAN-3 obviates the necessity for visually examining the tray in most cases; in the present study, 90% of the isolates identified by the machine corresponded to the visual identification. The instrument has the potential for significantly improving the work flow and perhaps the accuracy of results in laboratories that use Microscan trays.

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