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Automated Microbiological Detection/Identification System

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An automated, computerized system, the AutoMicrobic System, has been developed for the detection, enumeration, and identification of bacteria and yeasts in clinical specimens. The biological basis for the system resides in lyophilized, highly selective and specific media enclosed in wells of a disposable plastic cuvette; introduction of a suitable specimen rehydrates and inoculates the media in the wells. An automated optical system monitors, and the computer interprets, changes in the media, with enumeration and identification results automatically obtained in 13 h. Sixteen different selective media were developed and tested with a variety of seeded (simulated) and clinical specimens. The AutoMicrobic System has been extensively tested with urine specimens, using a urine test kit (Identi-Pak) that contains selective media for *Escherichia coli*, *Proteus* species, *Pseudomonas aeruginosa*, *Klebsiella-Enterobacter* species, *Serratia* species, *Citrobacter freundii*, group D enterococci, *Staphylococcus aureus*, and yeasts (*Candida* species and *Torulopsis glabrata*). The system has been tested with 3,370 seeded urine specimens and 1,486 clinical urines. Agreement with simultaneous conventional (manual) cultures, at levels of 70,000 colony-forming units per ml (or more), was 92% or better for seeded specimens; clinical specimens yielded results of 93% or better for all organisms except *P. aeruginosa*, where agreement was 86%. System expansion in progress includes antibiotic susceptibility testing and compatibility with most types of clinical specimens.

In 1966 McDonnell Douglas Astronautics Co. (MDAC-E) Bioscience Laboratory proposed to the National Aeronautics and Space Administration (NASA) the development of a system for detecting specific microorganisms in a spacecraft environment. NASA responded by sponsoring a program for the development of a system known as the microbial load monitor. This effort with NASA was performed in several phases (1, 2, 4). In 1973 McDonnell Douglas Corp. initiated a separate program for the purpose of transferring the basic microbial load monitor technology to clinical applications. The resulting system, the AutoMicrobic System (AMS), represents a sharp departure from traditional methods and techniques used in microbiological detection and identification. The system reflects its aerospace beginnings in the use of a disposable miniaturized plastic specimen-handling system, solid-state optics for microbial detection, and a minicomputer for control and processing. However, the greatest break with tradition is conceptual, for the system is designed to identify specific organisms or groups of organisms directly from clinical specimens in the presence of competing organisms.

This novel approach became possible because of the development of selective growth media specifically tailored to a sealed microenvironment (microcuvette), using very small volumes of selective broths (liquid volume of 32 μl of broth). After inoculation with a clinical specimen, the microcuvettes are incubated in an automated instrument equipped with a solid-state optical system that monitors changes in light transmission through the microcuvettes. When all predetermined conditions have been met, the minicomputer displays results and provides printed copy after 13 h of incubation.

Several engineering models of the instrument have been tested in the MDAC-E Bioscience Laboratory with urine, feces, sputum, and throat specimens. In addition, the capability to perform simultaneous microbial detection, enumeration, and antibiograms has been demonstrated. However, since urine specimens represent a major portion of the clinical laboratory's workload, the first application of the AMS has been the development of a capability for handling large numbers of urine specimens by automated techniques with results obtained considerably faster than with conventional manual procedures. This report presents a description of the AMS instru-

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ment, the urine test kit, and of developmental data obtained with urine specimens.

MATERIALS AND METHODS

AMS instrument. The AMS instrument is modularized and accepts from 1 to 240 individual clinical specimens per 13-h incubation and reading period. The instrumentation, which includes the diluent dispenser, filling module, reader-incubator, computer module, and data terminal, is shown in Fig. 1. The complete test kit for urine screening is illustrated in Fig. 2. The left-hand unit, with the two reservoirs, is the sample injector, which holds the specimen and a diluent mixture during the inoculation process. The plastic rectangular card on the right is the Identi-Pak, which contains the lyophilized media in the growth chambers. Figure 3 depicts schematically all elements of the AMS system and the successive steps involved in the work flow.

In preparation for inoculation of the test kits, a defined quantity of the specimen is placed into each reservoir (chamber) of the sample injector, where it is diluted and mixed. The disposable test kits are then automatically inoculated in the filling module with the diluted specimen and are then placed in the reader-incubator, which is electronically monitored to maintain a temperature of 35 ± 0.5°C. Each test kit identity number is sensed by solid-state optics, and the information is stored in the system's computer. By preprogrammed schedule, the computer calls for a reading of each test kit. The readings are made with arrays of light-emitting diodes whose peak emission is 665 nm; there are no optical condensers or lenses used. The system is self-calibrating; i.e., the current is automatically altered as necessary to maintain a constant light source over a period of months.

The system's high-volume capacity is a result of the reader-incubator arrangement. In each reader-incubator module, a carrousel with tray mountings accepts four trays of test kits with a tray capacity of 30 tests each. During the reading operation, the computer commands carrousel rotation to the proper tray. In sequence, (i) the reading head is positioned to perform an optical calibration check, align the tray-carrousel combination, and begin the reading sequence; (ii) the test kit is pulled from the tray, placed into and then removed from the reading head, and placed back into the tray, with (iii) the head continuing down the stack. Multiple detectors are used to scan the growth wells and the identity segments once each hour, allowing the computer to discriminate against bubbles and poor-quality specimen identification marks.

The instrument takes initial readings on each growth chamber, and the computer stores these values as base lines, thus compensating for different colors and densities of media and specimens. The computer uses these data to calculate subsequent percent changes in light attenuation. The calculated values are compared to the stored present thresholds for determination of a positive result. At the end of a 13-h test cycle, the results are automatically reported at the command of the computer.

Test kit. The test kit consists of two parts (Fig. 2); both are plastic and disposable: (i) the card (Identi-Pak), containing wells with lyophilized (growth) culture media, and (ii) the sample injector, which provides reservoirs (chambers) for diluent and specimen mixing used for inoculation of the growth wells. The sample injector consists of two chambers. Chamber A (right), containing 1.8 ml of diluent (distilled H_2O with 0.5% NaCl), receives 0.2 ml of undiluted urine specimen (1:10 dilution). After mixing, 0.05 ml is transferred from chamber A to chamber B (left), containing 5 ml of diluent (resulting in 1:1,000 dilution). Chamber A feeds through a needle inoculating the specific growth wells, and chamber B feeds through a separate needle to inoculate the enumeration wells.

A detailed view of the Identi-Pak before rehydration is shown in Fig. 4. The various media have been placed into the wells and lyophilized. These wells after rehydration with the diluted urine form microcuvettes in the optical system. The card includes provisions for specimen identification numbers and carries a unique code to signify the type of test kit. During manufacture of the cards, the selective media are always placed in the same corresponding growth and detection wells; therefore, the computer identifies the well being monitored and applies predetermined information to "detect" a positive result for each of the selective media.

The two isolated fluid circuits, 1 and 2 (Fig. 4), correspond to the two chambers in the sample injector. Circuit 1 interconnects the wells in columns A_1, A_2, and A_3, in the right-hand portion of the Identi-Pak. These wells serve for identification of specific organisms or groups of organisms; 10 of the 15 wells contain separate and unique media; the other 5 are currently empty, but provide for expansion to include additional selective broths. Circuit 2 (the 5 wells in the left-hand portion of the card) is used for enumerating the total

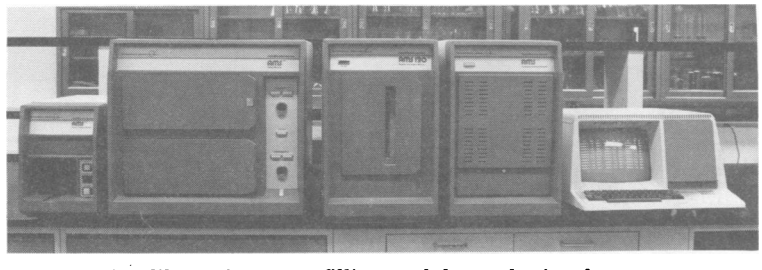

FIG. 1. *AMS (left to right: diluent dispenser, filling module, reader-incubator, computer module, and data terminal).*

number of organisms in the original specimen. The enumeration technique is based on theoretical analysis, using the most-probable-number technique and empirical data, which indicate a high probability of the presence of 7×10^4 (or more) colony-forming units (CFU) per ml when growth is detected in 3 or more of the 5 wells.

Test kit media. Presently, the urine test kit card contains separate, selective media for the detection of *Pseudomonas aeruginosa, Proteus* species, *Citrobacter freundii, Serratia* species, *Escherichia coli, Klebsiella-Enterobacter* species, yeasts (*Candida* species and *Torulopsis glabrata*), *Staphylococcus aureus*, and group D enterococci. In addition, a special growth medium (all-purpose positive control) is provided to detect any organism(s) that may occur in the urine specimen regardless of its identity. The positive control broth also functions as the enumeration medium; it contains no inhibitors. A positive result in the selective broth is evidenced by a visible reaction in the growth well of the test kit. Description of the reconstituted broths and their visible (positive) reactions are as follows:

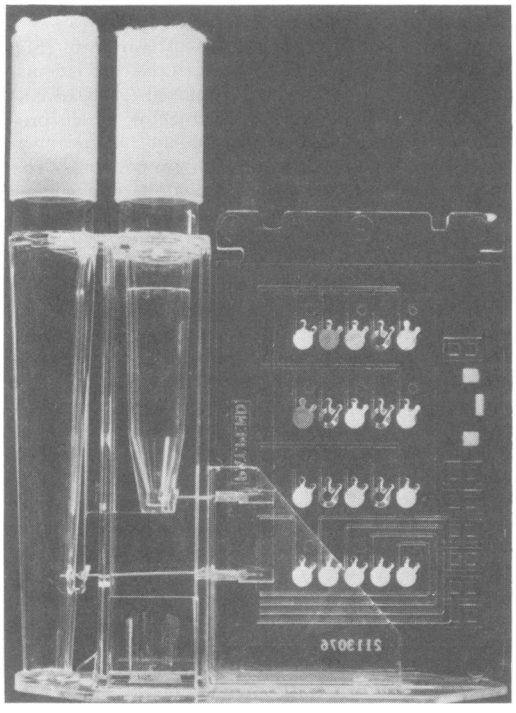

FIG. 2. *Urine test kit.*

(i) *P. aeruginosa*: Cream color. A positive reaction is evidenced by turbidity with or without pigment; it indicates resistance to cetyltrimethylammonium bromide (cetrimide).

(ii) *Proteus*: Yellowish green or blue green. A deep-blue color with or without turbidity indicates positivity, i.e., urease activity in the presence of inhibitors for organsims other than *Proteus*.

(iii) *C. freundii*: Blue green. A positive reaction is indicated by a milky turbidity resulting from utilization of rhamnose and palatinose. Acid production precipitates the bile salts in the broth.

(iv) *Serratia*: Light-straw color. When positive, a smoky-black color develops due to the alteration of plant glycosides in the medium.

(v) *E. coli*. This broth is light cream to light blue, depending upon the urine inoculum. When positive, the broth changes to a milky blue due to the conversion of arabinose and lactose to acids, changing the color of the indicator and precipitating the bile salts. The inhibitors and pH indicator are sensitive to oxidation by O_2 and therefore are protected by the addition of sodium thioglycolate.

(vi) *Klebsiella-Enterobacter*: Clear lime green. When positive, the broth appears milky green, after utilization of cellobiose and inositol in the presence of inhibitors. Resistance by *Klebsiella* and *Enterobacter* to the inhibitors is enhanced by the addition of *d*-biotin.

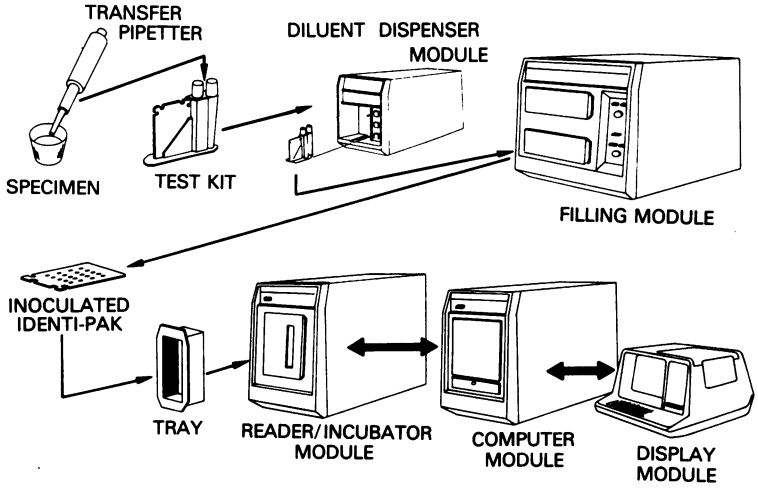

FIG. 3. *Work flow.*

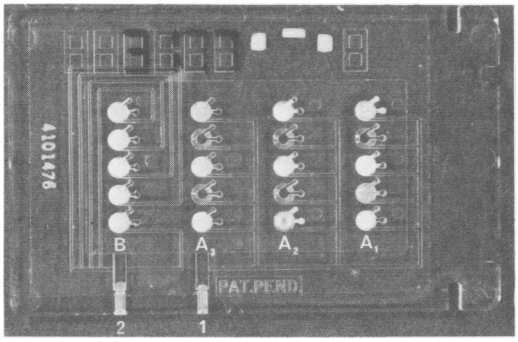

FIG. 4. *Detailed view of an Identi-Pak kit before rehydration.*

(vii) Group D enterococcus: Cream color. A positive reaction is indicated by the development of a black precipitate, formed when esculin is hydrolyzed in the presence of iron compounds. A positive result also indicates high salt tolerance.

(viii) *Staphylococcus*: Light pink to reddish purple. When positive it changes to deep bluish purple and a coagulum is formed. This indicates tolerance to 6.5% NaCl, deoxyribonuclease activity, and plasma coagulation.

(ix) Yeast: Light blue to cream. When positive it changes to an intense blue, occurring when dextrose is fermented, resulting in acid production and pH change. Inhibitors prevent the positive reaction from occurring with bacteria and more yeasts with the exception of *Candida* species, *T. glabrata*, and a few strains of *Saccharomyces cerevisiae*.

(x) Positive control and enumeration. The two broths have identical formulation and, when reconstituted, are cream to very light blue. When positive, the color changes to bright blue. These are general-purpose broths (similar to brain heart broth) that contain multiple peptones, vitamins, and growth factors X and V. A color change results when acid is produced from dextrose, and the pH indicator changes from colorless at pH 7.4, to blue at 6.8.

The kits are manufactured by placing 3.5-mil (88.9 μm) FEP Teflon tape on the bottom of a polystyrene blank from a master mold that has had two silicone rubber septa installed and cured. The liquid selective media are automatically dispensed into the taped polystyrene, and the materials are then flash-frozen at −36°C or colder and placed in the freeze-dryer. After lyophilization, the kits are completed by placing 3.5-mil (88.9 μm) FEP Teflon on the top of the freeze-dried kit, thereby completely sealing the lyophilized material. All handling after lyophilization takes place in a dry nitrogen environment to prevent moisture damage to the dried media. Since FEP Teflon does permit water vapor to penetrate, the kits are further protected by sealing in polyethylene-aluminum foil laminated pouches. The kits are stored at approximately 4°C.

Specific formulations are tailored to the plastic sealed environment and patents are pending.

Instrument detection/identification cycle. Figure 5 illustrates the manner in which the computer monitors data during the microbial detection cycle. Each detection well in the system contains a specific, dedicated, selective broth; thus, the computer always recognizes the type of broth monitored. In this example, the selective broth is for *E. coli*. The two curves depict time history profiles generated by the presence of *E. coli* and *Klebsiella*. These are not ordinary growth curves, for the system monitors only the amount of detectable light penetrating the growth well; therefore, light penetration is affected by turbidity due to bacteria and chemical precipitation. In addition, dyes and color indicators incorporated in the media react to the presence of certain bacteria. Thus, the light is absorbed by biomass, by precipitates, and by color changes in the selective broth. When *E. coli* is present, all three of these reactions occur simultaneously. At the start of the testing period (time 0), the amount of light detected is automatically set as the initial value, and each subsequent reading is computed as the percent change in light intensity. As the incubation and monitoring progress, subsequent readings are compared with the initial reading. The time required to detect a positive reaction varies with each medium and the number of microorganisms present in the original specimen. Typically, when *E. coli* is present in the original specimen at 10^6 CFU/ml, detection may occur between 3 and 5 h. When the original specimen contains only a few thousand CFU per milliliter, the detection time may be as long as 13 h.

The presence of *E. coli* in this broth results in a 50% change in light detected. Experience has shown that when only competing organisms are present in this broth, the percent change in detected light rarely exceeds 30. Therefore, by programming the computer to ignore light changes of less than 40%, organisms other than *E. coli* are ignored. The example of *Klebsiella* illustrates the fact, and, whereas there may be an initial detection and monitoring by the computer, the subsequent lysis and destruction of *Klebsiella* is ignored because the threshold of 40% light absorbed was not exceeded. The characteristics of each selective formulation vary, and, therefore, the computerized thresholds also vary. Each broth threshold was determined empirically after the analysis of thousands of tests.

Conventional test methods. The identification of gram-negative, oxidase-negative, fermentative, fa-

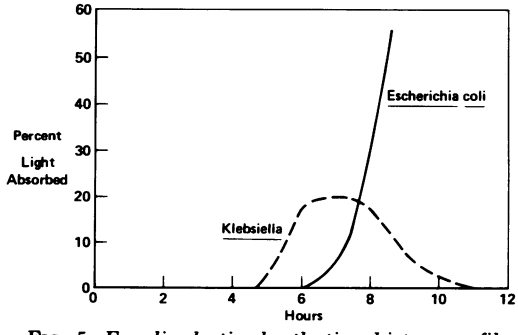

FIG. 5. *E. coli selective broth: time history profiles monitored by the AMS computer.*

cultative rods was routinely performed with API 20E test kits. When necessary, biochemical tests according to Ewing were performed for final identification (3); nonfermenters were identified by standard procedures (5). Staphylococci were tested for deoxyribonuclease activity, tolerance to 6.5% NaCl, and coagulase activity with reconstituted lyophilized rabbit plasma. Streptococci were tested for ability to grow in the presence of 6.5% NaCl and with the bile-esculin test. Yeasts were identified by germ tube formation and carbohydrate fermentation and assimilation tests. Conventional (manual) cultures were performed by spreading 0.1-ml amounts of serial 10-fold dilutions (in 0.85% saline) of urine samples on the surfaces of Trypticase soy-blood agar and MacConkey agar plates, incubation at 35°C overnight, and performance of colony counts with the aid of a Quebec colony counter.

Specimens. (i) Seeded (simulated) urine specimens were prepared by seeding clinical isolates and, in some instances, laboratory stock strains in both sterile 0.5% NaCl solutions and pooled filter-sterilized urines obtained from healthy human males. (ii) Clinical (clean voided) urine specimens, refrigerated within 1 h of collection, were obtained from local hospitals and transported on ice to the laboratory. Specimens were diluted 1:10 in the smaller kit chamber and 1:1,000 in the larger chamber with 0.5% NaCl. The test units were then moved to the filling module, where an evacuation-repressurization cycle of 5-min duration transferred the diluted urine into the kits' growth chambers. After filling, each kit was separated from the sample injector, fitted into a tray, and loaded into the reader-incubator. This completed the manual portion of the test. The AMS assumed control and incubated and monitored the status of the test specimens automatically. At the end of a 13-h test cycle, the results were automatically reported.

The selective broths that have been formulated and tested successively in an engineering model, a preprototype, and a prototype AMS instrument over a period of 10 years are: (most extensively challenged) *E. coli, Proteus* species, *P. aeruginosa, Klebsiella-Enterobacter, S. aureus, C. freundii, Serratia* species, group D enterococci, yeast (*Candida* species, *T. glabrata*), and positive control and enumeration (nonselective) and (less extensively challenged) *Candida albicans, Aspergillus niger*, group A β-hemolytic streptococci, *Haemophilus, Acinetobacter, Salmonella* species, and *Shigella* species. The selective formulations that have been successfully applied in this system for urine culture were *E. coli, Proteus* species, *P. aeruginosa, Klebsiella-Enterobacter* species, *S. aureus, C. freundii, Serratia* species, group D enterococcus, yeast, and positive control and enumeration broths.

RESULTS

Summary results obtained with 3,370 seeded urine specimens cultured in the AMS system are shown in Table 1. In all instances, the percent positive correlations were calculated by dividing true positives by the sum of true positives plus the false negatives and multiplying by 100. The percent negative correlation was calculated by dividing true negatives by the sum of true negatives and false positives and multiplying by 100. In all cases, positive correlation was 92% or greater. The lowest percent positive correlation was attained with *Serratia* species (92%), and the next higher one with group D enterococci (94%). The three commonest organisms isolated from urinary tract infections (*E. coli, Proteus*, and *Klebsiella-Enterobacter*) were detected and identified with positive correlations of 97, 96, and 98%, respectively.

Although not shown in detail, analysis of the results in Table 1 revealed certain typical patterns in the selective media designed for grouping several species together. In the *Proteus* species broth, all but one of the false negative findings were due to *Proteus morganii* strains. In the *Serratia* broth, the majority of false neg-

TABLE 1. *Seeded urines—3,370 samples*[a]

Selective broth	Challenges[b]					Correlation (%)	
	Total +	T+	F−	F+	T−	+	−
P. aeruginosa	301	286	15	11	3,058	95	99
Proteus species	458	438	20	5	2,907	96	99
C. freundii	179	170	9	3	3,188	95	99
Serratia	198	182	16	29	3,143	92	99
E. coli	532	515	17	36	2,802	97	99
Klebsiella-Enterobacter	768	756	12	22	2,580	98	99
Yeast	177	173	4	9	3,184	98	99
Group D enterococcus	682	644	38	51	2,637	94	98
S. aureus[c]	346	27	0	4	314	100	99
Positive control	3,340	3,273	67	1	29	98	97
Enumeration	3,276	3,014	262	9	85	92	91

[a] Concentration of organisms: 7×10^4 to 6×10^6 CFU/ml.
[b] T, True; F, false; +, positive; −, negative.
[c] 346 challenges after reformulation.

atives were due to *S. liquefaciens*; however, some strains of *S. marcescens* that failed to meet the computer threshold requirement did grow in the *Serratia* medium as evidenced by its color change. In the *Klebsiella-Enterobacter*-group broth, false negatives were confined entirely to *Enterobacter* strains, i.e., *E. hafniae* and *E. agglomerans*. Both species grew in the medium, but at a rather slow rate, when the inoculum was 10^5 CFU/ml or less. No distinctive pattern was seen with the false negative yeast findings; these likely reflect low or borderline counts.

A general pattern was noted with all AMS selective media in regard to false positive readings. Many of the false positive readings occurred when very large (10^8 CFU/ml or greater) inocula overloaded the limits of the selective media in the system. However, confined to an upper test limit of 6×10^6 CFU/ml, some false positives were still observed (Table 1). *P. aeruginosa* was noted to occasionally grow in *Proteus* broth and also a few times in *Serratia* broth. A *Proteus rettgeri* strain was encountered (isolated from a clinical specimen) that grew equally well in the *Proteus* or *Serratia* broth, and one strain of *Serratia rubidaea* grew faster in the *E. coli* broth than in its *Serratia* broth. The frequency of strains that could not be sequestered in their respective selective media during testing of seeded specimens was, however, less than 1%.

Results of AMS tests with 1,486 consecutive clinical urine specimens obtained from various local hospital laboratories are shown in Table 2. Both positive and negative correlations were 93% or higher, except for specimens containing *P. aeruginosa*, where the positive correlation was 86%. Organisms failing to grow in the selective broths were detected in the positive control broth (positive correlation 99%).

With clinical specimens, no general pattern could be discerned in the occurrence of false positives and false negative results. There seemed to be a somewhat greater tendency of these to occur in polymicrobic high-count specimens. In the false negative readings with *P. aeruginosa*, it was noted that most of the failures to detect the presence of *P. aeruginosa* were associated with only two lots of test kits (out of a total of seven used).

DISCUSSION

The AMS represents a novel, fully automated, total system approach to detection, enumeration, and identification of various bacteria and yeasts in clinical specimens. The studies of the AMS instrument and its urine Identi-Pak presented here show an overall agreement of better than 90% between the AMS system and conventional (manual) culture methods. In the 3,370 seeded (simulated) specimens, all eight bacterial species/groups and yeast species/groups were detected and identified at levels of 92% or higher positive correlation, when present at levels of 7×10^4 to 6×10^6 CFU/ml. Similarly, with 1,496 clinical urine specimens, seven bacterial species/groups and two yeast species/groups yielded 93% or better positive correlations, with the sole exception of *P. aeruginosa* (86%).

These results were obtained in a total elapsed time of 13 h from placement of the inoculated test kit into the AMS instrument to the appearance of the final report, thus providing a timely report to the clinician and allowing for earlier decision making, or change of therapy, by the physician.

Problems encountered involved small num-

TABLE 2. *AMS system results with 1,496 clinical urine specimens*[a]

Selective broth	Challenges[b]						
	Total +	T+	F−	F+	T−	Correlation (%)	
						+	−
P. aeruginosa	21	18	3	4	1,471	86	99
Proteus species	80	78	2	13	1,403	98	99
C. freundii	2	2	0	3	1,491	100	99
Serratia species	0	0	0	0	1,496		
E. coli	172	165	7	4	1,320	96	99
Klebsiella-Enterobacter	43	40	3	2	1,451	93	99
Yeast	8	8	0	2	1,486	100	99
Group D enterococcus	47	45	2	4	1,445	96	99
S. aureus	7	7	0	6	1,483	100	99
Positive control	347	345	2	1	1,148	99	99
Enumeration	316	303	13	27	1,153	96	97

[a] Concentration of organisms: $\geq 7 \times 10^4$ CFU/ml.
[b] T, True; F, false; +, positive; −, negative.

bers of both false negative and false positive results. In the case of false negative results (no growth, i.e., no detection/identification in selective broth) with seeded specimens, the majority was detectable by growth in the all-purpose growth control broth and most (but not all) were the results of borderline counts (7×10^4 to 1×10^5 CFU/ml). In a few instances, specific organisms (*P. morganii, S. liquefaciens, E. hafniae,* and *E. agglomerans*) were noted to be responsible for false negative results, likely requiring some adjustments of the selective broths; with clinical specimens, this phenomenon was not particularly noted (probably because of the relatively rare occurrence of these organisms in clinical urine specimens).

It should be noted (Table 1) that the number of strains in seeded urines tested for each species/group bears no relationship to the frequency of occurrence of these organisms in clinical urine specimens (Table 2). Testing with seeded specimens was designed to present the highest possible challenge to the system. Of the 3,370 seeded samples, 198 (or 5.9%) contained *Serratia* species, whereas the occurrence of *Serratia* species in the 1,496 clinical urines examined was 0%; Sonnenwirth (6) similarly failed to detect any *Serratia* species in a group of 648 specimens and noted 0.3% *Serratia* species in another group of 1,040 clinical specimens. Similarly, 9% of seeded specimens contained *P. aeruginosa*, whereas the species was detected in only 1.4% of clinical specimens. Thus, the high-frequency testing of such clinically rarer organisms in seeded samples by the AMS should be a reasonably accurate predictor of the efficiency of the system with clinical specimens containing such organisms.

Some of the problems peculiar to the system, requiring continuing close monitoring on the manufacturing level, are best illustrated by the developmental difficulties encountered. Acquisition of data for evaluation of the AMS took several years; this was due to the necessity for developing and evaluating new, untested, empirical manufacturing skills and methods before tests could be designed for comparison on the AMS with conventional bacteriological methods. For example, the optimum amount of each selective broth to be placed in the kit's card wells before lyophilization was a particularly difficult problem because of the miniaturization involved. Technology for automatic, accurate pipetting of 18 to 20 μl at speeds up to 17 kits per min did not exist, yet slower speeds risked deterioration of the media awaiting dispensing and freeze-drying. Large-scale testing had to await development of manufacturing technology capable of producing sufficient numbers of test kits. The data presented here were obtained with several consecutive lots of test kits (instead of the ideal single uniform lot); the results include early lot-to-lot variables and therefore represent a realistic evaluation of the system's performance using various production lots.

Quality control procedures for the test kits originally involved a total of 54 separate bacterial strains; by judicious selection the number of test strains was finally reduced to 30. An example of this process is the *E. coli* broth. Originally tested separately with "typical," H_2S+, mucoid, and lactose-negative *E. coli* strains, it is now tested only with typical *E. coli* strains, since it was noted that when the broth failed with any one type of *E. coli*, it failed with all *E. coli* strains. Conversely, the broth performs well with all types of *E. coli* when properly compounded and lyophilized.

Since nine different selective broths are placed in the kit simultaneously, the chance for error in compounding the broths or properly dispensing them was considerable; in fact, the first 24 production runs were rejected due to failure of one or more broths in the test kits. Detailed analysis revealed that many of the failures were associated with freeze-drying profiles and assorted physical phenomena such as cracked pH electrodes in contact with the broths or absorption and removal of cetrimide from the *Pseudomonas* broth by plastic walls of containers before dispensing into the kits. The FEP Teflon film (used for sealing tops and bottoms of kits) was also a possible source of failure with the *Pseudomonas* broth; the amount of O_2 passing through different lots of the tape varied by at least 40%.

Expanded testing, especially with polymicrobic specimens, revealed that, for the most part, the media formulations gave acceptable results (approximately 92% or better correlation with conventional methods); however, some microbial combinations necessitated changes in media formulations or in computer instructions to improve instrument-manual technical correlations. Further large-scale tests will determine the need for such additional adjustments and/or refinements.

The promising results reported here, the encouraging findings of an independent evaluation of a preprototype AMS system (6), and feasibility studies of antimicrobial susceptibility testing with the AMS system prompted the initiation of a large-scale, multilaboratory collaborative project for the assessment of the AMS as a complete system for microbiological diagnosis of urine, which is now under way. Studies for using the AMS system with a variety of specimens other than urine are in progress.

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