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Preprototype of an Automated Microbial Detection and Identification System: a Developmental Investigation

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The AutoMicrobic System is an automated, computerized instrument that uses highly selective media and an optical system for detection, enumeration, and identification of bacteria and some yeasts in 13 h. A preprototype instrument (AutoMicrobic System-1) and its urine culture kit (Identi-Pak), developed for the detection, enumeration, and identification of eight species or groups of bacteria and of *Candida* species and *Torulopsis glabrata* in urine specimens, was evaluated during its development. An overall agreement of approximately 90% between the preprototype instrument and conventional (manual) culture methods has been obtained both with 1,473 seeded (simulated) and 1,688 clinical (mono- or polymicrobial) specimens containing 70,000 (or more) colony-forming units per ml of *Escherichia coli, Klebsiella-Enterobacter* species, *Proteus* species, *Citrobacter freundii, Serratia* species, group D enterococci, or yeasts (*Candida* species and *T. glabrata*). Lower agreements in identification were obtained with *Pseudomonas aeruginosa*-containing (average of 75% in clinical specimens) and *Staphylococcus aureus*-containing (76%) specimens. Comparison of specimens tested simultaneously in two preprototype systems resulted in ≤4% disagreement; true negativity agreements in all specimen groups tested were at least 94%. Among problems remaining are adaptation of system for specimens other than urine, improvement of sensitivity for *P. aeruginosa* and *S. aureus*, and standardization of manual methods used for comparison and validation.

A variety of mechanized or automated systems for detection, characterization, and drug-susceptibility testing of microorganisms have been recently described or are in the process of development (2). Practically all of these systems were originally designed to perform a single function only, e.g., (i) detection, by such methods as differential light scattering, radiometric measurement of $^{14}CO_2$ from labeled substrates, bioluminescence assay for adenosine triphosphate, measurement of electrical impedance, microcalorimetry, and gas chromatography; (ii) identification, by combinations of pyrolysis and either gas-liquid chromatography or mass spectrometry, gas chromatography, optical scanning of growth stimulation or inhibition, microcalorimetry, and forward light scattering (electrical impedance now also being investigated for possible use in identification); or (iii) antimicrobial sensitivity testing, e.g., by forward light scattering for qualitative or quantitative testing in 4 to 5 h.

A recent development, the AutoMicrobic System (AMS; McDonnell Douglas Corp., St. Louis, Mo.) (1), was designed for the detection, enumeration, identification, and drug-susceptibility testing of microorganisms in clinical specimens, with all of its functions to be performed simultaneously or consecutively by the same instrument. It uses a novel array of highly selective media in which a mixture of substrates and inhibitors is used to permit the growth of one or a group of closely related microorganisms, to concurrently inhibit growth of any other organisms present, and to enumerate the total number of organisms in the sample. The lyophilized media are incorporated in wells of a small, sealed, disposable plastic cuvette (card), inoculated, and incubated in an automated instrument equipped with an optical system. The optical system monitors light transmission changes in the card wells and transmits optical measurements to a minicomputer, which compares them with preset thresholds and then displays results in 4 to 13 h. A preprototype AMS instrument and its urine culture kit (Identi-Pak), for the detection, enumeration, and identification of eight species or groups of bacteria and of yeasts (*Candida* species and *Torulopsis glabrata*) in urine specimens was investigated during its development.

This report presents the results of a study

designed to (i) compare the AMS preprototype system with conventional culture methods, (ii) ascertain its reproducibility by testing identical split specimens in two identical instruments, and (iii) define the performance limits of the AMS both with seeded (simulated) urines and with clinical urine specimens.

MATERIALS AND METHODS

Organisms. The organisms used for preparation of 90% of simulated specimens were current isolates from clinical specimens obtained from three St. Louis area hospitals that were reidentified by conventional methods (3) and maintained on Trypticase soy agar slants; for the remaining 10% of simulated specimens, laboratory stock strains were used. Single strains were employed for seeding 1,326 stimulated specimens, and 2 organisms were used for seeding each of 147 simulated specimens.

Specimens. (i) Seeded. Simulated urine specimens were prepared by seeding pooled, filter-sterilized urine from healthy human males to a final concentration of 10^6 to 10^7 colony-forming units (CFU) per ml. All seeded specimens were coded, and the identity of the organism(s) was not known to the instrument's operator until the code was broken after completion of both the instrument run and conventional culture. Seeded specimens were processed within 2 h of preparation. A total of 1,473 simulated specimens were tested.

(ii) Clinical. These were unselected, clean, voided urine specimens from patients hospitalized at The Jewish Hospital of St. Louis. Specimens were refrigerated for a maximum of 12 h, with the great majority tested within 4 h of refrigeration. A total of 1,688 clinical specimens were used.

Conventional culture protocol. Serial 10-fold dilutions of urine samples (seeded or clinical) were prepared with saline, and 0.1-ml amounts of dilutions were spread on the surface of MacConkey agar and Trypticase soy blood agar plates with a glass "hockey-stick." After overnight incubation at 35°C, counts were performed (i) manually for a period of 3 months with a Quebec colony counter and (ii) for the next 9 months with a Model 480 Artek-Fisher Automatic Bacterial colony counter. The colony counter was regularly checked with manual counts. Fermentative gram-negative rods were identified with the API 20E kit, and oxidizers and nonfermenters were identified with the use of conventional techniques (3), including oxidation-fermentation media. Coagulase tests with staphylococci were done by the tube method; bile-esculin agar (40% bile) and 6.5% NaCl tests (for growth) were used for presumed enterococci, and germ-tube, fermentation, and assimilation tests were performed on yeastlike organisms.

Preprototype instrument. The preprototype instrument was similar to the production model described by Aldridge, et al. (1) with the following exceptions: (i) it has only a 30-specimen capacity; (ii) results were displayed by teletypewriter instead of electrostatic printer; (iii) the computer (Digital Equipment Corp. model PDP 11-05) was modified with tapes for collection of time history profiles; and (iv) only one light-emitting diode pair was available for reading each growth well, resulting in a relative inability of the instrument to discriminate between growth or bubbles in the wells. The detection threshold of the instrument was set at 7×10^4 CFU/ml.

Urine test kit. The urine Identi-Pak card contained separate, specific, selective media (1) for *Escherichia coli, Pseudomonas aeruginosa, Proteus* species, *Citrobacter freundii, Serratia* species, *Klebsiella-Enterobacter* groups, yeasts (*Candida* species, *T. glabrata*), *Staphylococcus aureus,* and group D enterococci and an enriched broth for positive growth control and enumeration (total count). The maximum (liquid) volume of each broth in the card wells was 32 μl. A two-reservoir sample injector for diluent and specimen mixing and inoculation of the card wells was included in the kit; one reservoir (chamber) feeds through a needle to inoculate the specific growth wells, and the other feeds through a separate needle to inoculate the enumeration wells.

Instrument test protocol. The diluent used was unbuffered distilled water with 0.5% NaCl. To chamber A of the sample injector, containing 1.8 ml of diluent, 0.2 ml of undiluted urine was added. After mixing, 0.05 ml was transferred to chamber B, which contained 5 ml of diluent. The final specimen dilution in chamber A was 1:10, and in chamber B, 1:1,000. Before filling, each card was appropriately marked for instrument recognition with Arabic numerals. The joined test units (injector and card) were placed in the filling module, where the diluted urine was transferred from the injector through the two needles into the card wells (the needles piercing hermetic seals in the card) by a pneumatically controlled evacuation-repressurization process (5 min). After filling, the cards were separated from the injector and loaded into the reader-incubator. At this point, the instrument assumed control; every 30 min it recorded readings (for a total of 12 h), printed interim status reports every h, and at the end of 13 h elapsed time, furnished final reports.

Comparative study. The number, nature, and designation of simulated and clinical specimens tested is shown in Table 1. Group I, a total of 929 seeded urines, was tested with one instrument (AMS-1) and with conventional manual culture methods; group III, 544 seeded urines, was tested with AMS-1 and another identical preprototype instrument (AMS-2) in the manufacturer's laboratory, employing split specimens and conventional culture methods. The split specimens were transported on ice to the second laboratory and were tested within 3 h after dividing the specimen. Group II (648 clinical urines) and group IV (1,040 clinical urines) were tested exactly as groups I and III (Table 1). Whenever discrepancies were noted between instrument and manual method, the card wells were entered with a syringe and needle and cultured by conventional methods; presence of bubbles was noted and recorded.

Comparison of manual and instrument cultures were made by determining correlations based on two formulas:

(i) percent positive correlation:

$$\frac{T+}{(T+) + (F-)} \times 100$$

(ii) percent negative correlation:

$$\frac{T-}{(T-) + (F+)} \times 100$$

Percent positive correlation indicates the success of the AMS instrument in detecting a particular organism (T+, true positive) when it is present in concentrations of 7×10^4 CFU/ml or more (as shown by the manual culture method); i.e., it denotes the sensitivity of the instrument. Percent negative correlation, where T− represents the number of all negative challenged media, shows the accuracy of AMS in not reporting an organism when, in fact, the organism was not present; i.e., it signifies the specificity of AMS. Results considered as false negative (F−) AMS readings (no detection or identification of a particular organism) were calculated by deducting AMS positive readings from positive conventional culture method results (considered as 100%), while false positive AMS readings (F+) represented those not obtained by conventional culture.

RESULTS

Detailed results obtained with 929 seeded urines (group I) are shown in Table 2. Positive correlation (sensitivity) of the AMS system compared with manual culture was 91 to 100% with *Serratia, Klebsiella-Enterobacter,* the yeasts, *E. coli, C. freundii,* and *Proteus,* with over 90% agreement for the majority of the organisms. Negative correlation (specificity) was 98 to 100% for all organisms tested except *S. aureus* (91%). False negative readings by the AMS as compared with conventional culture results occurred with ≲1.4% of *Klebsiella-Enterobacter* group and the yeasts, ≲9% of group D enterococci, *P. aeruginosa,* and *E. coli* (6.6%), and ≲20% of *S. aureus, C. freundii,* and *Proteus* species (13%), for an overall (weighted) false negativity rate of 4.9%. False positive readings ranged from 0 to 4% with the exception of group D enterococci (9%), *P. aeruginosa* (15%), and *S. aureus* (20%), for an overall (weighted) false positivity rate of 3.5%. A second group of seeded urines (Table 3) yielded positive correlations of over 90% for all organisms tested, except for *S. aureus* (84%); negative correlations were 95 to 100%.

Clinical urine specimens test results are shown in Tables 4 and 5. In group II (648 clinical urines), positive correlation (sensitivity) of ≲91% at levels of 70,000 CFU/ml or greater was achieved by the AMS in detecting and identifying *Klebsiella-Enterobacter,* group D enterococci, *Proteus* species and the yeasts; positive correlation was 88% with *E. coli,* 77% with *P. aeruginosa,* and 66% with a small number of *S. aureus* (Table 4), with no *Serratia* or *C. freundii* isolated. In group IV (1,040 split clinical urines), percent positive correlations were considerably higher for *E. coli* (98%) and *S. aureus* (86%) and lower for group D enterococci (80%) and *P. aeruginosa* (73%), with an overall agreement range of 87 to 100% for the other organisms (Table 5).

TABLE 1. *Specimens tested with AMS preprototype*

Designation	No.	Specimens	Compared with:
Group I	929	Seeded (simulated) urine	CM[a]
Group III[b]	544	Seeded (simulated) urine	CM, AMS-2[c]
Total	1,473		
Group II	648	Clinical urine (CVS)[d]	CM
Group IV[b]	1,040	Clinical urine (CVS)[d]	CM, AMS-2
Total	1,688		

[a] CM, Conventional (manual) method.
[b] Split specimens.
[c] Second identical preprototype instrument.
[d] Clean, voided specimen.

TABLE 2. *Group I—seeded urines*[a]

Organisms	Challenges[b]					
	T+	F−	F+	T−	Correlation	
					% Negative	% Positive
P. aeruginosa	77	8	13	831	91	98
Proteus	140	21	1	767	87	99
C. freundii	9	2	0	918	82	100
Serratia	10	0	0	61	100	100
E. coli	150	10	7	762	94	99
Klebsiella-Enterobacter	211	1	8	709	99	99
Yeast	68	1	2	858	98	99
S. aureus	4	1	1	196	80	91
Group D enterococci	10	1	1	103	91	99
Enumeration	728	173	1	27	81	96
Positive control	860	52	0	17	94	100

[a] 929 specimens.
[b] Number of true positive (T+), false negative (F−), false positive (F+), and true negative (T−) challenges.

An overall summary of results obtained in comparison of two instruments (reproducibility) with seeded and clinical specimens in terms of percent positive correlation is shown in Table 6. The two instruments (AMS-1 and AMS-2) were used by different workers in different laboratories employing split clinical specimens. With seeded specimens, there was 6% or less disagreement between the two instruments with *Pseudomonas, Serratia, E. coli,* and *S. aureus* and complete agreement with *Proteus, C. freundii,* and *Klebsiella-Enterobacter.* With clinical specimens, there was complete agreement with *C. freundii, Serratia* species, and yeasts, 2% disagreement with *Proteus* and *E. coli,* 13 and 14% disagreement with *Klebsiella-Enterobacter* and *S. aureus,* respectively, and 21% disagreement with *P. aeruginosa.* Since the positive correlations for each instrument were based on comparisons with manual cultures (also performed on each split sample in both laboratories), analysis of results of the manual culture in the two laboratories was undertaken. The results indicated that the great majority of disagreements between the two instruments, as expressed by each percent positive correlation, was due to discrepant results of the manual culture methods, primarily of colony counts, obtained by the two laboratories. When the manual culture results were excluded from consideration, disagreements between the two instruments were reduced to 4% or less overall.

The distribution and frequency of organisms in the clinical specimens tested, as listed in Tables 4 and 5, generally reflect the prevailing pattern of organisms seen in urinary tract infections of the patient population in this institution, except for *E. coli.* Of 648 specimens shown in Table 4, a total of 150 yielded *E. coli* in concentrations of 7×10^4 CFU/ml or higher, but instrument results for only 81 are listed, since formulation of the *E. coli* selective broth was changed during the study, and, therefore, results of the first 69 *E. coli* challenges could not be included. Likewise, the second group of 1,040 clinical urines (Table 5) yielded 270 specimens containing *E. coli* with results of 212 challenges not included because of the change in formulation. Among the positive clinical specimens, 12.2% contained more than one organism.

Correct enumeration (percent positive correlation of total counts) was achieved by the instrument in an average of 83.5% of the two groups of seeded urines (range, 81 to 86%), and in 86.5% of the two groups of clinical urines (range, 85 to 88%). Overall accuracy of enumeration in negative challenges (percent negative

TABLE 3. *Group III—seeded urines*[a]

Organisms	No. of challenges	Correlation	
		% Positive	% Negative
P. aeruginosa	17	94	96
Proteus	19	100	99
C. freundii	64	97	95
Serratia	163	96	99
E. coli	24	92	100
Klebsiella-Enterobacter	11	100	97
Yeast	28	100	99
S. aureus	90	84	95
Group D enterococci	120	98	100
Enumeration	463	86	100
Positive control	468	86	**[b]

[a] 544 specimens; split samples.
[b] **, No negative challenges.

TABLE 4. *Group II—clinical urines*[a]

Organisms	No. of challenges	Correlation	
		% Positive	% Negative
P. aeruginosa	13	73	98
Proteus	41	100	99
C. freundii	0		99
Serratia	0		100
E. coli	81[b]	88	98
Klebsiella-Enterobacter	32	91	99
Yeast	5	100	99
S. aureus	3	66	99
Group D enterococci	16	94	96
Enumeration	140	88	97
Positive control	170	97	91

[a] 648 specimens.
[b] Total of 150 *E. coli* challenges—change in media formulation.

TABLE 5. *Group IV—clinical urines*[a]

Organisms	No. of challenges	Correlation	
		% Positive	% Negative
P. aeruginosa	15	73	98
Proteus	73	93	98
C. freundii	4	100	99
Serratia	3	100	99
E. coli	58[b]	98	98
Klebsiella-Enterobacter	64	87	99
Yeast	14	100	99
S. aureus	7	86	99
Group D enterococci	44	80	98
Enumeration	281	85	98
Positive control	342	99	91[c]

[a] 1040 specimens; split samples.
[b] Total of 270 *E. coli* challenges—change in media formulation.
[c] Includes bubbles.

TABLE 6. *Comparison of percentage of positive correlation between two preprototype instruments (AMS-1 versus AMS-2, employing split urine specimens) based on conventional colony counts*

Group III—544 seeded specimens		No. of challenges	Organisms	No. of challenges	Group IV—1040 clinical specimens	
AMS-1 (%)	AMS-2 (%)				AMS-1 (%)	AMS-2 (%)
94	100	17	*P. aeruginosa*	15	73	94
100	100	19	*Proteus*	73	93	91
97	97	64	*C. freundii*	4	100	100
96	94	163	*Serratia*	3	100	100
92	96	24[a]	*E. coli*	58[b]	98	100
100	100	11	*Klebsiella-Enterobacter*	64	87	100
100	100	28	Yeast	14	100	100
84	89	90	*S. aureus*	7	86	100
98	99	120	Group D enterococci	44	80	86

[a] Total of 150 *E. coli* challenges; see text.
[b] Total of 270 *E. coli* challenges; see text.

correlation) was 98% with seeded urines (range, 96 to 100%) and 97.5% with clinical urines (range, 97 to 98%). Overall detection rates in the positive control broth were 90% for seeded urines and 99% for clinical urines.

DISCUSSION

The AMS preprototype instrument and urine kit employed in this investigation is the first, and to date only, system capable of performing detection, enumeration, and identification of various bacteria and yeasts commonly found in urine in a fully automated mode. It requires only minimal initial manual handling, with all steps beyond urine sample dilution and loading performed automatically by the instrument. In these studies, involving 1,473 seeded urines and 1,688 clinical urines, the preprototype AMS system was found to have an overall average sensitivity and specificity of approximately 90% in detecting, enumerating, and correctly identifying (at the level of $\geq 7 \times 10^4$ CFU/ml) eight different bacterial species/groups and two yeast species/groups in urine specimens.

In comparison with conventional (manual) culture methods, the AMS was highly successful in detecting and identifying yeasts (*Candida* species and *T. glabrata*) in both seeded urines (average agreement, 99%), and mono- and polymicrobial clinical urines (100% agreement). A similar pattern was noted with *E. coli* (where agreement was 91% in seeded and 93% in clinical urines), *Klebsiella-Enterobacter* species (99.5 and 89%), *Proteus* species (93.5 and 96.5%), *C. freundii* (89.5 and 100%), and *Serratia* species (98 and 100%). Agreements below 90% were found in clinical urines only with *P. aeruginosa* (75%), group D enterococci (87%), and with a small number (10 specimens) of *S. aureus* (76%).

In the early portion of the study, a number of discrepancies between the AMS and manual culture results, expecially false positive AMS findings, were traced to the formation of visible bubbles in the media-containing growth wells, particularly in those serving for selective growth of *P. aeruginosa*. Adjustments in the card manufacturing process were made, and the number of discrepancies due to bubble formation did diminish; however, no allowance for this occurrence was made in the calculation of percent positive correlations, a fact that partially explains the low agreement rate (75%) with *P. aeruginosa* in clinical urines. Another source of difficulty with this organism was the rate of O_2 diffusion through the Teflon tape used for sealing the top and bottom of the card; batches of Teflon varied in their permeability. Some false positives with *S. aureus* were due to problems with rehydration of the *S. aureus* medium. It is noteworthy that the test results reported here were obtained with the use of more than a dozen production lots of test kits, thus inevitably introducing a major variable. This made the testing procedure both more realistic and more severe than it would have been with the use of a single production batch of test kits.

It was also found that the preprototype system was capable of detecting, and in some cases identifying, certain organisms at levels far below 7×10^4 CFU/ml, although this was not explored systematically. In fact, some *Proteus* species were detected and identified at levels as low as 3×10^3 CFU/ml. However, it needs to be emphasized that the threshold for detection and identification in this study was set at 7×10^4 CFU/ml and that the preprototype instrument and kit was not designed to perform at levels lower than this.

The use of a computer equipped with time

history collection tapes afforded some insight into the detection and identification times versus population size relationship. In general, high populations were detected and identified in less time than low populations, but this was not true in all instances. The system occasionally detected and identified organisms in the 1×10^3 to 3×10^3-CFU/ml range; in some instances, false positive AMS results were recorded because the manual culture method missed such low counts, with the presence of the organisms in the proper well (selective medium) proven by conventional culture of the well. However, the AMS did correctly report enumerations of less than 70,000/ml in such cases.

Comparative testing of split urine specimens in two separate, identical AMS instruments revealed yet another variable. Despite the use of a standardized manual culture method, discrepancies were noted in some colony counts obtained in the two laboratories ranging from 0.4 to 1.4 logs. These discrepancies accounted for a major portion of disagreements between the two instruments, since success of the instrumental results was predicated on the manual colony counts. When the latter were excluded from consideration and results of the two instruments were compared directly, disagreements between the two instruments were less than 4%.

In summary, this study has shown that detection, enumeration, and identification of eight bacterial and two yeast species/groups in urines by the AMS system is a promising approach, an overall agreement between the preprototype AMS instrument and conventional manual procedures of approximately 90% having been obtained. Further work is required to raise the system's sensitivity for *P. aeruginosa* and *S. aureus*, to adapt the system for use with specimens other than urine and to improve standardization of manual methods (counts) used for comparison and validation of instrument results. The system also needs to be challenged with (i) organisms for which no selective broths are presently available to further delineate the specificity and limits of sensitivity of the existing formulation and (ii) a larger number of highly polymicrobic specimens (containing four to five different species; these are relatively rare among clinical specimens, except in those from patients with long-term indwelling catheters, e.g., quadruplegics and paraplegics, etc.).

A large-scale multilaboratory collaborative project for the assessment of the AMS system in urine bacteriology (1) should result in elucidating some of the problems delineated and furnishing broad-based data on the inter- and intralaboratory reproducibility of the system.

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