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Multi-Laboratory Evaluation of an Automated Microbial Detection/Identification System

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An automated and computerized system (Automicrobic System [AMS]) for the detection of frequently encountered bacteria in clinical urine specimens was tested in a collaborative study among six laboratories. The sensitivity, specificity, reliability, and reproducibility of the AMS were determined, and the system was compared with conventional detection and identification systems. In this study, pure cultures and mixtures of pure cultures were used to simulate clinical urine specimens. With pure cultures, the sensitivity of the AMS in identifying the nine groups of organisms most commonly found in urine averaged 92.8%. The specificity averaged 99.4%, and the reliability of a positive result averaged 92.1%. The latter value was strongly influenced by a relatively high occurrence of false positive *Escherichia coli* results. The AMS was capable of detecting growth of most organisms, including those which it was not designed to identify. However, it identified some of these incorrectly as common urinary tract flora. Reproducibility of results, both within laboratories and among different laboratories, was high. Fast-growing organisms, such as *E. coli* and *Klebsiella/Enterobacter* species, were detected often at cell populations well below the AMS enumeration threshold of 70,000/ml. In mixed culture studies, high levels of sensitivity and specificity were maintained, but when *Serratia* species were present in mixtures with other organisms, there was often a false positive report of *E. coli*. The overall performance of the AMS was considered satisfactory under the test conditions used.

A novel system for the automated and computerized detection, enumeration, and identification of organisms in urine, the Automicrobic System (AMS), has recently been described by Aldridge et al. (1). This system represents a drastic departure from conventional methodology in microbiology, in that no prior isolation and purification of organisms is required; instead, the AMS is designed to detect and identify specific groups and/or species of organisms in urine specimens representing either single or mixed infections.

During its developmental stages, the AMS was evaluated with both seeded (simulated) and clinical specimens in a study reported by Sonnenwirth (2). He found about 90% agreement between AMS results and conventional results with *Escherichia coli, Klebsiella/Enterobacter* species (the AMS does not differentiate them), *Serratia* species, *Proteus* species, *Citrobacter freundii*, group D enterococci, and yeasts (primarily *Candida* and *Torulopsis* species), but only about 75% agreement with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Sonnenwirth's study also indicated the need for improvement of (i) reagents, (ii) sensitivity and specificity for some organisms, and (iii) manual methods used for comparison and validation of results.

The present collaborative study was undertaken after the manufacturer had instituted some of the recommended improvements and had modified the instruments. A two-stage, multi-laboratory evaluation of this system was undertaken to (i) enlarge upon the data generated by the two preliminary reports (1, 2), (ii) test urinary tract isolates from widely dispersed geographic areas, (iii) evaluate the intra-laboratory and inter-laboratory reproducibility of the system, and (iv) challenge the sensitivity and specificity of the AMS. Seeded specimens were used in this evaluation. In another evaluation

study, to be reported, clinical specimens from various hospitals will be used.

MATERIALS AND METHODS

Cultures. In the first phase of this study, in which only pure cultures were used, bacterial and yeast cultures recently isolated in the various collaborating laboratories were lyophilized, coded, and distributed as unknowns to each laboratory by the Center for Disease Control. The selection of cultures used (see Table 1) was not based on the distribution normally encountered in clinical urine specimens, but rather on the idea of extensively testing each identification capability of the AMS system. Additional cultures, which were not among those that the AMS was designed to identify, were taken from the Center for Disease Control stock culture collections, coded, and distributed as viable subcultures on agar slants. In the second phase of the study, in which mixed cultures were used, one culture of each of the various groups or species identifiable by the AMS was selected, coded, and distributed as a viable agar slant to each participating laboratory. In each laboratory, these cultures were mixed according to specific instructions. In all instances, the codes for the unknowns were not broken until results had been obtained.

Equipment. The instrumentation and disposable components of the AMS, including the Identi-Pak, have been thoroughly described by Aldridge et al. (1). The system is based on the principle of utilizing an array of selective media to permit significant growth of only one organism, or a group of closely related organisms, in specific microchambers. The system permits desired organisms to outgrow competing ones, if present, and automatically compares initial readings with subsequent ones. A separate enumeration system, based on most-probable-number theory and utilizing non-inhibitory media, gives approximate total counts of all organisms present. Both the identification and enumeration systems have adjustable thresholds which must be exceeded before a positive result is recorded. In this study, the enumeration threshold was set at 7×10^4 colony-forming units (CFU) per ml, but lesser populations could be detected because of the separate enumeration and identification systems and because of the highly sensitive selective media employed.

The Identi-Paks used in this study were essentially the same as those described by Aldridge et al. (1), except that some selective media formulations had been changed slightly, following Sonnenwirth's recommendations.

The manufacturer provided each laboratory with the complete system, all necessary materials, and specific instructions for use. These instructions have also been described (1). The AMS model employed had a capacity of 120 specimens, contained a tape deck for recording time-history profiles, and automatically printed all results after 13 h of incubation. Preliminary results could be obtained at any time, if desired.

Media and reagents. All bacteriological media and reagents required in the manual method (see below) were supplied to the collaborating laboratories from Regional Media Laboratories, Inc., Lenexa, Kans. Insofar as possible, all laboratories used media and reagents from the same production batches. Media and reagents used in the routine method (see below) were those normally used in the participating laboratories and came from different sources.

Methods. (i) Pure culture study. Each laboratory used three distinct identification schemes on each unknown specimen: (i) the AMS procedure, as directed by the manufacturer, (ii) a manual method upon which all collaborators agreed, and (iii) whatever routine method each of the six laboratories normally used. The procedures employed in the AMS and manual methods are described further. In addition, the Center for Disease Control laboratory also provided a separate "reference" identification for each culture employed.

For the AMS procedure, each coded lyophilized bacterial culture was rehydrated with sterile Trypticase soy broth, streaked onto a 5% sheep blood agar plate, and incubated for 18 to 24 h at 35 to 37°C. Identical isolated colonies were picked into sterile, pooled normal urine (supplied lyophilized to all collaborators) to form a suspension of about 10^7 CFU/ml (by comparison with a turbidity standard), and two 10-fold dilutions were made of this suspension, again with sterile urine used as diluent. This final suspension was called the "seeded specimen" and served as inoculum for the AMS and for other procedures. Every fifth specimen received two additional 10-fold dilutions which were used to determine some of the lower bacterial populations which the AMS could detect. Cultures of yeasts were handled similarly to those of bacteria, except that the original cell suspensions from isolated colonies were adjusted to a turbidity equal to about 10^8 CFU/ml. Colony counts were made of all cultures from the seeded specimen by using either a model 480 Artek-Fisher Automatic Bacterial Colony Counter or conventional colony counting methods. All Identi-Paks which (i) detected the presence of an organism but failed to identify it or (ii) failed to detect an organism when one was known to be present by the manual method were entered aseptically through the plastic membrane of the Identi-Pak by means of a sterile needle and syringe. Contents of the positive control chamber were withdrawn and examined by the manual method.

The manual method was recognized to have certain deficiencies, but was considered to be a reasonably adequate system for the identification of organisms commonly encountered in urine and to have a performance level comparable to that of the AMS. The colony characteristics of each bacterial culture were observed on the blood agar plate inoculated in the AMS procedure described above. Selected well-isolated colonies were then identified by the procedure shown in Fig. 1. It should be noted that identification of streptococci was presumptive, and that serological confirmation was not done. Yeasts were identified from colonies on blood agar plates by means of the following tests: colonial and microscopic morphology; germ tube formation; pellicle formation; capsule production (India ink stain); urease; dextrose, fermentation; and assimilation of sucrose, maltose, raffinose, galactose, trehalose, and melibiose. Colony counts were made on each culture from the seeded specimen

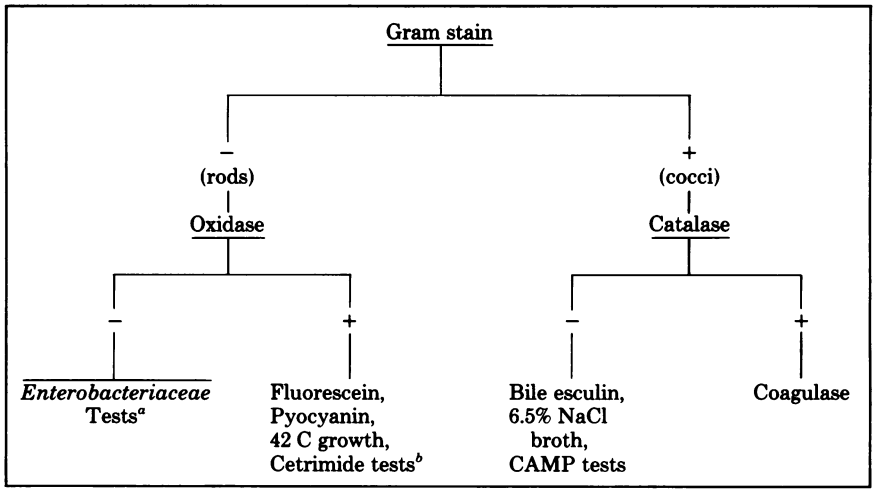

FIG. 1. *Manual method identification procedure. (a) Enterobacteriaceae tests: Triple sugar iron, Christensen urea agar; lysine and ornithine decarboxylase; arginine dihydrolase; indole; Simmon citrate; motility agar; deoxyribonuclease; phenylalanine deaminase; arabinose, inositol, and adonitol fermentation. (b) If Acinetobacter, Flavobacterium, etc. are suspected, use procedure of individual choice.*

prepared in the AMS procedure. Both blood agar plates and MacConkey agar plates were used for counts of bacteria; only blood agar plates were used for the yeasts.

(ii) Mixed culture study. One culture was selected from each of the groups or species of organisms listed in Table 1. All possible combinations of two cultures were used (e.g., cultures A and B, A and C, A and D, B and C, B and D, etc.), and each combination was tested in four different ratios of the two strains involved. For example, *P. aeruginosa* and *E. coli* were tested in ratios of 10^7:10^5, 10^6:10^4, 10^5:10^7, and 10^4:10^6, respectively. The identification procedures via the AMS and manual methods were the same as previously described. Yeasts were not employed in this phase of the study.

Data handling. All data, including tapes and handwritten reports from the AMS, manual, and routine methods, were forwarded to the AMS computer facility of the McDonnell-Douglas Astronautics, East Corp. Both individual laboratory and summary results were complied and returned to the collaborating laboratories. The various authors also compiled some data. In this study, sensitivity was defined as the ratio of true positive reports to the sum of the true positive and false negative reports, i.e., the ability of the AMS to detect a particular organism when it was shown to be present by conventional methods. Specificity was defined as the ratio of the true negative reports to the sum of the true negative and the false positive reports, i.e., the ability of the AMS not to detect an organism when it was not detected by conventional methods. Reliability was defined as the ratio of the true positive reports to the sum of the true positive and false positive reports, i.e., the confidence that could be placed in an AMS report that an organism was present.

RESULTS

Pure culture study. The sensitivity, specificity, and reliability of the AMS in detecting and identifying nine groups of organisms commonly found in urine are shown in Table 1. The basis for this tabulation was the results obtained by the manual method. The sensitivity of the AMS ranged from 83.3% for *Serratia* to 98.9% for *E. coli*, with a mean of 92.8% and a median of 95.1%. Both the positive control and enumeration chambers of the Identi-Pak were affected by tests in which very small populations of organisms (ca. 10^3/ml) at levels well below the thresholds set for detection and identification were used.

Specificity values ranged from 98.1% for *E. coli* to 100% for group D streptococci, with a mean of 99.4% and a median of 99.7%. The positive control specificity is not a significant figure because of the very small number of "true" negative results. The enumeration specificity value (83.4%) indicates a tendency of the AMS to report higher counts on about 17% of the specimens with bacterial populations evaluated manually at levels below 70,000/ml.

The percent reliability calculations reflect the degree of confidence to be placed in a positive AMS result. Reliability percentages ranged from a low of 71% for *E. coli* to 100% for group D streptococci, with a mean of 92.1% and a median of 95.4%. The low value for *E. coli* resulted from an appreciable number of Identi-Pak chambers permitting other organisms tested, not *E. coli*, to attain detectable cell densities. This same tendency was noted later in the second phase of the study, in which mixed cultures were used.

In addition to testing the AMS with pure cultures of the nine groups or species listed in

Table 1, which the system was designed to identify, 224 challenges were made with other organisms which may occur in urine (although infrequently), but which the system was not designed to identify. These were used to test the limits of differential capability of the AMS, and the results of these challenges are shown in Table 2. With the exception of certain species of *Pseudomonas*, particularly *P. stutzeri, P. maltophilia, P. diminuta*, and *P. fluorescens*, most of the strains tested grew in the Identi-Pak positive control chamber and were not identified. However, 45 of the 167 strains which grew were named, although incorrectly. Most of the named ıres consisted of group B streptococci which ..re incorrectly identified as group D streptococci. Somewhat disturbing, although not unexpected, was the misidentification of 10 strains of *Salmonella* as *E. coli*.

The agreement of identification by the AMS was measured on both an inter-laboratory and intra-laboratory basis. Inter-laboratory agreement is indicated in Table 3, which shows agreement of results from all six collaborating laboratories, and of five of the six laboratories, with 152 cultures. The manual method results agreed with the reference results in all six laboratories in 89.5% of the tests and in five of the six laboratories in 97.5% of the tests. Interestingly, AMS results agreed slightly better with the reference results than with the manual method results, indicating some faults in the latter. The AMS

TABLE 1. *Summary of pure culture study results: AMS sensitivity, specificity and reliability, based on manual method results*[a]

Organism	No. of challenges	AMS results[b]				Sensitivity (%)	Specificity (%)	Reliability (%)
		T+	F−	F+	T−			
P. aeruginosa	125	119	6	22	1,819	95.2	98.8	84.4
Proteus species	116	104	12	3	1,886	89.7	99.8	97.2
C. freundii	95	82	13	3	1,904	86.3	99.8	96.5
Serratia	90	75	15	7	1,902	83.3	99.6	91.5
E. coli	90	88	1	36	1,811	98.9	98.1	71.0
Klebsiella/Enterobacter	110	103	7	5	1,880	93.6	99.7	95.4
Yeasts	348	331	17	4	1,597	95.1	99.8	98.8
Group D *Streptococcus*	140	137	3	0	162	97.9	100.0	100.0
S. aureus	144	137	7	9	728	95.1	98.8	93.8
Positive control	1,540	1,406	134	0	13	91.3	100.0	100.0
Enumeration	1,540	1,121	419	81	407	72.8	83.4	93.3

[a] Sensitivity = [(T+) × 100]/[(T+) + (F−)]; specificity = [(T−) × 100]/[(T−) + (F+)]; reliability = [(T+) × 100]/[(T+) + (F+)].

[b] T+ = AMS +, manual method +; F− = AMS −, manual method +; F+ = AMS +, manual method −; T− = AMS −, manual method −.

TABLE 2. *AMS response to challenge with miscellaneous organisms*

Organism	No. tested	Correct AMS response		Incorrect AMS response		
		Growth in positive control	Not identified	Incorrect genus	Correct genus, wrong species	No growth
Pseudomonads, other than *P. aeruginosa*	79	36	30	4	2[a]	43
Aeromonas	12	12	11	1	0	0
Group B streptococci	29	26	4	0	22[b]	3
α-Streptococci, not group D	30	25	24	1	0	5
S. epidermidis	17	14	12	0	2[c]	3
Salmonella	12	12	2	10[d]	0	0
Flavobacterium	12	12	10	2	0	0
Acinetobacter	22	19	18	1	0	3
Providencia	11	11	11	0	0	0
Total	224	167	122	19	26	57

[a] Both identified as *P. aeruginosa*.

[b] All identified as enterococci.

[c] Both identified as *S. aureus*.

[d] All identified as *E. coli*.

and manual methods produced the same results, however, in 83% of the participating laboratories (five of six) in 93.4% of the challenges made and in all six laboratories in 81.6% of these challenges. Intralaboratory agreement of results, with 136 challenges in each laboratory, is shown in Table 4. Obviously, the data were in excellent agreement in the various laboratories, and there is no significant variation in these results among the laboratories.

Table 5 presents the sensitivity, specificity, and reliability data for each laboratory and for each group of organisms tested in the pure culture study. The greatest variation in sensitivity occurred with *C. freundii* and *Serratia* cultures, and it appears that relatively poor performance in two laboratories reduced the overall values obtained (see Table 1). Specificity was extremely high and uniform in all laboratories. Reliability was the most variable of these parameters, particularly with *P. aeruginosa* and *E. coli*. Two laboratories obtained low values for *P. aeruginosa*, thus reducing the overall reliability from over 94% to 84.4% (Table 1). Similarly, two very low values were obtained for *E. coli*, with a resultant low overall reliability (Table 1). However, reliability of a positive *E. coli* result was a common problem among all six laboratories, with none of them achieving higher than 83.3%. There was no indication from the sensitivity or reliability data that any one laboratory consistently performed less well than did other laboratories.

The three Identi-Paks which were inoculated with every fifth specimen were used to define, to some extent, the lower limits of detection and identification capabilities of the AMS. Some indication of this is shown in Table 6, where the AMS results are tabulated in relation to cell population and organism. Certain organisms, such as *P. aeruginosa*, *S. aureus*, and yeast, were often not detected at low cell concentrations (10^3/ml), although others, such as *E. coli* and group D streptococci, were not only detected but were also identified at such populations. At cell populations generally considered to be indicative of probable urinary tract infection, i.e., between 10^4 and 10^5/ml, the first three organisms mentioned above were still not accurately identified in most tests. The variation observed with yeasts was partly due to the variety of genera and species tested, some of which grew faster than others. In 13 tests with yeast in populations of 10^4 to 9×10^4/ml, no growth occurred in the positive control chamber of the Identi-Pak. In contrast, normally fast-growing organisms, such as *E. coli*, were occasionally detected and identified at populations of less than 10^3/ml (data not shown).

TABLE 3. *Interlaboratory agreement of identification (152 challenges)*

Comparison	Agreement by all 6 laboratories		Agreement by 5 of 6 laboratories	
	No.	%	No.	%
Manual vs reference	136	89.5	148	97.4
AMS vs reference	127	83.6	145	95.4
AMS vs manual	124	81.6	142	93.4

TABLE 4. *Intralaboratory reproducibility of AMS results (136 challenges)*

Laboratory no.	AMS vs reference	AMS vs manual	Positive growth	No. false positive	No. false negative
1	124	124	132	5	4
2	127	127	131	2	3
3	124	123	131	6	5
4	128	127	128	5	2
5	128	129	134	0	6
6	122	121	125	1	3

Mixed culture study. The unknowns distributed for this phase of the study were mixed in each collaborating laboratory according to a protocol which resulted in all cultures being tested in pairs and each pair of cultures being tested in four different ratios of organisms. The detection and identification capabilities of the AMS for these mixtures are shown in Table 7, which is comparable to Table 1. The lowest values for both sensitivity and specificity were observed for *E. coli*; the highest values were observed with *C. freundii*. The mean sensitivity for these organisms, even in the presence of other organisms, was 96.3%, with a median of 98.3%. The specificity ranged from 96.6% for *E. coli* to 100% for *C. freundii* and the *Klebsiella/Enterobacter* species, with a mean of 99.3% and a median of 99.8%.

Another point of investigation in this mixed culture study was the effect, if any, of the presence of one particular organism on the ability of the AMS to detect another organism when present simultaneously; Tables 8 to 10 contain these data. In Table 8, the false positive and false negative ASM results are tabulated with respect to both laboratory and organism. It shows that *E. coli* was detected in 22 instances when it was not present (false positive), and in 12 instances it was not detected when it was present (false negative), as determined by the manual method. Thirteen of the 22 false positive *E. coli* results were from one laboratory—a variation of about

TABLE 5. *Pure culture study: sensitivity, specificity, and reliability results by organism and laboratory*[a]

Determination	*P. aeruginosa*	*Proteus*	*C. freundii*	*Serratia*	*E. coli*	*Klebsiella/Enterobacter*	Yeasts	Group D *Streptococcus*	*S. aureus*
Sensitivity									
Laboratory 1	82.6	94.7	83.3	87.5	100	100	96.4	100	96.9
2	100	89.4	93.7	87.5	100	100	93.3	100	88.8
3	95.2	90.0	84.2	88.2	100	94.7	93.3	94.1	84.8
4	100	88.2	100	84.6	94.1	89.4	96.6	100	100
5	100	80.9	83.3	66.6	94.1	100	96.5	96.2	100
6	94.7	95.0	75.0	90.0	100	83.3	94.3	100	100
Specificity									
Laboratory 1	99.3	100	99.7	99.7	97.0	100	99.6	100	100
2	99.6	100	100	99.3	99.0	100	100	100	96.9
3	97.2	100	100	99.7	98.1	100	100		98.4
4	97.0	99.6	100	99.6	98.0	100	99.5	100	99.2
5	99.3	99.3	100	99.6	98.7	98.7	99.6		99.1
6	100	100	99.3	99.6	97.3	99.6	99.6	100	100
Reliability									
Laboratory 1	90.4	100	90.9	93.3	54.5	100	98.2	100	94.1
2	95.0	100	100	87.5	83.3	100	100	100	72.7
3	68.9	100	100	93.7	71.4	100	100	100	93.3
4	70.3	93.7	100	91.6	76.1	100	98.3	100	96.1
5	92.3	89.4	100	92.3	80.0	80.0	98.2	100	96.7
6	100	100	85.7	90.0	63.6	95.2	98.0	100	100

[a] Definitions for sensitivity, specificity, and reliability are given in the text and the footnotes for Table 1.

TABLE 6. *AMS response to different bacterial populations*

Organism	Range of cell populations					
	10^3/ml			10^4/ml		
	No growth	Positive control	Positive identification	No growth	Positive control	Positive identification
P. aeruginosa	11	13	2	1	15	15
Proteus species	0	8	5	0	5	8
C. freundii	0	8	6	0	7	9
E. coli	0	0	11	0	0	7
Klebsiella/Enterobacter	0	8	6	0	2	14
Serratia	0	14	1	0	8	9
Yeast	15	8	2	13	15	21
Group D *Streptococcus*	1	5	18	0	0	24
S. aureus	11	15	0	3	11	12

TABLE 7. *Summary of mixed culture study results: AMS sensitivity, specificity, and reliability, based on manual method results*

Organism	Challenges	AMS result[a]				Sensitivity (%)	Specificity (%)	Reliability (%)
		T+	F−	F+	T−			
P. aeruginosa	119	111	8	1	639	93.2	99.8	99.1
Proteus species	129	127	2	2	648	98.4	99.6	98.4
C. freundii	122	122	0	0	655	100.0	100.0	100.0
Serratia	132	125	7	9	646	94.6	98.6	93.2
E. coli	117	105	12	22	629	89.7	96.6	82.6
Klebsiella/Enterobacter	221	215	6	0	500	97.2	100.0	100.0
Group D *Streptococcus*	115	114	1	1	649	99.1	99.8	99.1
S. aureus	119	117	2	2	643	98.3	99.6	98.3
Positive control	841	840	1	0	0	99.8		100.0
Enumeration	841	841	0	1	1	100.0	50.0	99.8

[a] See footnote *b*, Table 1.

seven times the average occurrence of such reports in the other laboratories. There was no unusual distribution of false negative reports of *E. coli* among the six participating laboratories.

Table 9 presents in more detail the conditions under which the false positive AMS results were obtained. It is apparent that every false report of *E. coli* occurred when *Serratia* were actually present. Similarly, six of the nine false reports of *Serratia* occurred when a group D streptococcus was present. The precise reasons for these associations are not known, but one reason might be that certain combinations of organisms act synergistically to overcome the specific inhibitors present in the *E. coli* or *Serratia* growth chambers of the Identi-Pak.

The false negative reports observed in the mixed culture study are listed in Table 10. *E. coli* was missed most often (12 times), and most of these misses occurred when there was growth in the *Klebsiella/Enterobacter* chamber of the Identi-Pak. *C. freundii* was most frequently present when the AMS failed to detect another organism, and most of these associations were with false negative *Serratia* and *E. coli* results. This could perhaps be attributed to an antagonistic relationship between certain combinations of organisms, but there is no additional evidence to support this. Even if this is true, a reciprocal relationship between *C. freundii* and *Serratia* or *E. coli* was not observed, because *C. freundii* was not missed during this phase of the study.

DISCUSSION

The concept of an automated and computerized system for enumeration, detection, and identification of organisms in urine, in the presence of other organisms, is a dramatic departure from the concepts involved in ingrained, conventional pure culture techniques. The AMS relies on the principle of utilizing specific compounds that selectively favor the growth and metabo-

TABLE 8. *Mixed culture study: AMS false positive and false negative results*

Organism	False positive in laboratory no.:							False negative in laboratory no.:						
	1	2	3	4	5	6	Total	1	2	3	4	5	6	Total
P. aeruginosa	0	0	0	1	0	0	1	1	5	0	0	0	2	8
Proteus species	0	0	0	1	1	0	2	0	0	0	0	0	2	2
C. freundii	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Serratia	0	4	1	1	2	1	9	1	1	1	1	2	1	7
E. coli	2	0	13	4	1	2	22	3	3	0	0	3	3	12
Klebsiella/Enterobacter	0	0	0	0	0	0	0	3	2	0	0	1	0	6
Group D *Streptococcus*	0	0	0	1	0	0	1	0	0	0	0	0	1	1
S. aureus	0	0	0	1	0	1	2	2	0	0	0	0	0	2

TABLE 9. *Mixed culture study: AMS false positive results*

Organisms present	*Pseudomonas*	*Proteus*	*C. freundii*	*Serratia*	*E. coli*	*Klebsiella*	*Enterobacter*	Group D streptococci	*S. aureus*
C. freundii/Serratia					1[a]				
S. aureus/Serratia					8				
Klebsiella/Serratia					1				
Enterobacter/Serratia					3				
Proteus/Serratia					2				
P. aeruginosa/Serratia					3				
Group D streptococci/*Serratia*					4				
P. aeruginosa/Group D streptococci				4					
Klebsiella/group D streptococci				2					
C. freundii/group D streptococci		2							
P. aeruginosa/Klebsiella				1					1
Proteus/Enterobacter				1					1
E. coli/S. aureus				1				1	
Group D streptococci/*S. aureus*	1								

[a] Figures indicate number of occurrences of a false positive report when the indicated pairs of organisms were actually present.

TABLE 10. *Mixed culture study: AMS false negative results*

Organism also present	Organism missed								
	Pseudomonas	*Proteus*	*C. freundii*	*Serratia*	*E. coli*	*Klebsiella*	*Enterobacter*	Group D streptococci	*S. aureus*
Pseudomonas		1					1		
Proteus	1						2		
C. freundii	1			5	4		2		
Serratia	2						1		
E. coli									1
Klebsiella	2	1			4			1	
Enterobacter	1			1	4				
Group D streptococci				1					1
S. aureus	1								

lism of specific groups or species of bacteria, and combines this with automated and computerized instrumentation.

The system is designed to be used for screening urine specimens of the types most commonly encountered, in which infection is usually associated with bacterial counts of 10^5 or more organisms per ml. Specimens collected by catheterization, suprapubic aspiration, or from patients undergoing cystoscopy, in which low counts may be clinically significant, should not be tested in the AMS, but should receive special handling.

Published developmental studies on this system (2) were encouraging in that more than 92% correlation was obtained with conventional manual results on about 3,400 simulated urine specimens, and, with the exception of *P. aeruginosa*, more than 93% correlation was obtained with 1,500 clinical urine specimens. Problems were encountered, however, with both false positive and false negative results and with various technological difficulties.

A later study of the AMS, in which a preprototype instrument and both simulated and clinical specimens were used (1), showed high levels of correlation (>90%) for most organisms, but also revealed weaknesses in the identification capabilities of the AMS for *P. aeruginosa*, group D streptococci, and *S. aureus* in clinical specimens. Reproducibility experiments in this same study, with two machines, suggested that more variation was encountered in conventional manual methods than in the AMS procedure.

The multi-laboratory collaborative study reported here has evaluated the sensitivity, specificity, and reproducibility of the AMS. The techniques employed, i.e., use of lyophilized pure cultures, conventional media from a single supplier, and a manual method, were designed to minimize variation in experimental conditions. Nevertheless, some variations did occur. Urine Identi-Paks from the same batch could not be provided to all participants for the duration of the study; thus, several production batches of Identi-Paks were employed. Furthermore, during the study some of the specific chamber media used in the Identi-Paks were found to be deficient. They were later modified, thus invalidating results previously obtained. Additionally, it was recognized that "human error" could not be totally eliminated from the manual manipulations involved in setting up the various tests, as partially shown in Table 8. Finally, as mentioned previously, the manual method used in all six laboratories was neither an exhaustive nor an extremely abbreviated identification system; it was a compromise between these extremes. The method was reasonably accurate and reflected the level of recognition possessed by the instrument. It still permitted some subjective conclusions, and thus some variations occurred. These variations undoubtedly had some adverse influence on the experimental data presented. Conversely, they more closely approximated conditions which might be encountered if the AMS were used in a number of widely scattered laboratories.

We consider the results of this study to be satisfactory despite the many variables inherent in it. With pure cultures, five of the nine group/species tested showed sensitivity of over 95%, compared with the manual method; one was 93.6%, two were between 85 and 90%, and only one (*Serratia*) was between 80 and 85%. The specificity of the AMS was extremely high for all of the organisms tested, being at least 98% or better. The reliability of the AMS showed a wider range than did either the sensitivity or specificity. The data (Table 1) show that an AMS result of *E. coli* was recorded 124 times, yet *E. coli* were actually present only 88 times. This was one of the most disturbing problems encountered, because of both the frequency and the significance of *E. coli* in urinary tract infections. The reliability value observed for *E. coli*

was, however, not representative of values obtained for other organisms. Only one other value, that for *P. aeruginosa* (84.4%), was below 90%.

The limits of the AMS for detection and identification were severely tested by including various organisms which are seen only rarely, but still may occur, in urine specimens (Table 2). The slow-growing pseudomonads were not detected for the most part, whereas most of the other organisms tested were detected but were not identified. The misidentifications of group B streptococci as group D streptococci and of *Salmonella* as *E. coli* illustrate limitations in the capabilities of the AMS Identi-Pak to differentiate selectively among related organisms. These limitations (misidentifications) could have some serious therapeutic consequences, but they should not be overemphasized. Probably the most commonly encountered problem here would be misidentification of group B streptococci, because they are not uncommon in urine from females. The reason for this error is not clear, because hydrolysis of esculin and tolerance to high salt concentrations are required for AMS identification of group D streptococci. Most group B streptococci will tolerate 6.5% salt, but they do not hydrolyze esculin. Possibly there was sufficient change in turbidity, because of growth of group B streptococci to register a positive result, but this does not account for the hydrolysis of esculin. Certainly conditions existent in a microchamber are different from those in conventional test tubes, and this may also be a factor. The significance of these data lies in the recognition that such discrepancies may occur, and so microbiologists and clinicians must view them in the proper perspective.

The problems mentioned in the two preceding paragraphs regarding specificity, sensitivity, and reliability are influenced not only by the selective compounds employed in the specific identification chambers, but also by the threshold levels set for each chamber. These thresholds are adjustable, and vary from chamber to chamber, so that only the desired organisms be detected. It is appropriate to mention that this investigation was designed to aid in fine-tuning the system before its use in the clinical setting and that future adjustment of both selective compounds and identification chamber threshold levels may modify, if not alleviate, some of the problems encountered.

Agreement of identification of AMS results was considered to be very good, both among the six collaborating laboratories and within each of these laboratories. Table 3 shows that the manual and reference results agreed among all six laboratories in 136 of 152 challenges and in five of six laboratories in 148 of 152 challenges. The figures used for the "AMS versus manual" comparison were 124/152 (for all six laboratories) and 142/152 (for five of six laboratories). However, a more realistic measure might be the use of the "manual versus reference" figures as the denominators in these calculations, thus changing the percents to 91.2 and 95.9, respectively. These more accurately reflect the agreement of the AMS and the manual method results. Some of the variation in sensitivity noted among individual laboratories (Table 5) was probably the result of batch-to-batch variation in urine Identi-Paks.

This study confirmed, in six laboratories, the report by Sonnenwirth (1) that the AMS was capable of detecting and of sometimes identifying specific organisms at levels of 10^3 to 9×10^3 CFU/ml. This was particularly noted with relatively fast-growing organisms. Only with slower growing organisms, such as *Staphylococcus aureus*, yeasts, and pseudomonads, did significant numbers of tests result in no growth in the Identi-Pak at such low cell concentrations.

At concentrations of 10^4 to 9×10^4 CFU/ml, the AMS nearly always detected the presence of organisms and usually correctly identified them. Only yeast cultures still were not detected in a significant number of tests when present at this concentration. Thus, the ability of the AMS to recognize the presence of microorganisms in simulated urine specimens at levels below those considered indicative of urinary tract infection was established.

It should be reemphasized that in this study the enumeration threshold was set at 70,000 CFU/ml. It might be argued that the detection and identification of small cell populations ($<10^4$ CFU/ml) by the AMS is problematical from a clinical standpoint, because such populations are usually regarded as insignificant. Thus, detection of low populations by the AMS might be falsely interpreted as representing significant bacteriuria. This possibility is not considered very likely, however, because the AMS will report whether counts are above or below 70,000 CFU/ml. Another point of consideration is that patients with urinary tract infection often have extremely high-count bacteriuria (10^7 to 10^9 CFU/ml). In the present study, populations of cells in seeded urines rarely exceeded 10^7 CFU/ml, so the question might arise as to whether the specificity of the AMS would remain as high with significantly higher count urines. This cannot be answered on the basis of results of this study; the answer will depend on results of clinical studies with the AMS.

We wish to emphasize that the AMS enumer-

ation and identification reports on mixed specimens do not reflect the proportions in which various organisms are present, and thus give equal weight to all of them, whereas only one may actually be present in significant numbers. Some information may be gained from comparing the time periods required for identifying two organisms, but this is not totally reliable because of the varying growth rates of different organisms in their respective identification chambers.

The mixed-culture phase of this study was intended to explore the effect of one organism on the AMS's ability to detect another organism, when both were present simultaneously. Only a low level of false reports were encountered: 4.4% false positive and 4.5% false negative. These values compare to 4.4% and 4.0%, respectively, in the pure culture study. The only associations that could be made relating to the occurrence of false results were as follows: (i) a correlation between false positive reports of *E. coli* when *Serratia* were also present (Table 9); (ii) a tendency for *Serratia* to be reported when group D enterococci were present (Table 9); and (iii) a possible, but not clear-cut failure to identify *Serratia* when *C. freundii* was also present (Table 10). The first of these associations seems the clearest, but, because of the small amount of data available, confirmation by testing of fresh urine specimens is highly desirable.

The present study was designed to test various capabilities of the AMS, by using pure cultures and simulated mixed cultures of organisms found in urinary tract infections. No attempt was made to simulate the distribution of organisms which occur most frequently in urine specimens of patients with urinary tract infections. Thus, this study is weighted towards organisms less commonly encountered in the usual clinical practice. Nevertheless, such a study is necessary to properly define some of the limits of performance of any device intended for clinical application. Stock cultures of some *Enterobacteriaceae* may not be as metabolically active as fresh clinical isolates, but even the largest clinical laboratories do not encounter sufficient numbers of some organisms to permit a complete study of this taxonomic family. Thus, this study has attempted to provide a rigid test of the AMS, under controlled conditions, and has shown that the AMS appears to have potential for use in clinical microbiology. Clinical studies now in progress should focus on this potential and further define the AMS's capabilities and limitations in relation to specimens from patients suspected of having urinary tract infections. In addition, our early experiences with defective lots of Identi-Paks show that the manufacturer is obligated to maintain a high level of quality control in the large-volume production of these products, and the user is obligated to perform his own quality control tests with known cultures at appropriate intervals.

LITERATURE CITED

1. **Aldridge, C., P. W. Jones, S. Gibson, J. Lanham, M. Meyer, R. Vannest, and R. Charles.** 1977. Automated microbiological detection/identification system. J. Clin. Microbiol. **6**:406–413.
2. **Sonnenwirth, A. C.** 1977. Preprototype of an automated microbial detection and identification system: a developmental investigation. J. Clin. Microbiol. **6**:400–405.