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The Automicrobic System for Urines

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An evaluation of the Automicrobic System (AMS) for Urines (Vitek Systems, Inc.) was carried out under the auspices of the Product Evaluation Committee of the College of American Pathologists from the period June 1977 through October 1978. Data generated during this evaluation indicated that, when comparing the AMS methodology to our clinical microbiology laboratory methodology, a 37% time saving could be realized by utilizing the AMS. Quantitation with the AMS showed a 99% correlation with the clinical microbiology laboratory method except for yeast which correlated only 50% of the time. The average overall identification accuracy was 95%. Negative response accuracy was 99%. Other members of the *Enterobacteriaceae* which the instrument is not designed to identify may produce erroneous results if they occur in urine specimens. Specimens containing two organisms were identified with a 94% correlation when compared to our conventional methodology. The time when a well becomes positive may be used as a fairly reliable indicator of significance (count greater than 70,000 colony-forming units per ml) for *Escherichia coli, Klebsiella-Enterobacter* group, and group D *Enterococcus,* but not for *Proteus* sp., *Pseudomonas aeruginosa,* and yeast. Specimen collection must be performed properly since specimens considered as contaminated by conventional plating-out techniques may be reported out by the AMS as only one or two organisms and thus lead to an erroneous assumption as to significance. Cost per specimen was $1.83 more by utilizing the AMS method as compared to our conventional method. This is offset by a saving of 1.74 h daily of personnel time and a final report in 13 h. At least 30 urine specimens would be needed daily to pay for the instrument and specimen costs in 1 year. The AMS can provide significant aid to a clinical microbiology laboratory when all factors are considered.

The Automicrobic System (AMS) for Urines by Vitek Systems, Inc. was evaluated from June 1977 through October 1978 under the auspices of the Produce Evaluation Committee of the College of American Pathologists. Several investigators have published the results of their evaluations (1-6), and numerous papers have been presented at various professional society meetings (P. W. Jones, C. Aldridge, R. A. Charles, and J. B. Frankel, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C73, p. 38; C. Aldridge, S. Gibson, J. Lanham, M. Meyer, R. Vannest, P. Jones, and R. Charles, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C74, p. 38; A. C. Sonnenwirth, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, C75, p. 38; E. L. Marso, J. Cue, C. Oshima, V. Wilkinson, and W. J. Martin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C148, p. 301; H. D. Isenberg, M. Telenson, J. Washington, A. C. Sonnenwirth, and W. Taylor, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C149, p. 302). The primary purpose of our evaluation was to determine the validity of the promotional claims made by the manufacturer. Secondarily, we desired to determine the feasibility of incorporating the AMS into our clinical microbiology laboratory (CML). During the course of the evaluation, several items of interest to clinical microbiologists became apparent, and we feel they are worthy of reporting.

MATERIALS AND METHODS

Specimens. Initially all urine specimens received in the CML for routine culture were inoculated into AMS identification cards. After 500 true negative results were obtained, all urine specimens were visually screened grossly against a lighted background and all clear specimens were discarded to decrease the number of negative responses. Since *Serratia* sp. and *Staphylococcus* sp. (particularly *Staphylococcus aureus*) occur at a low frequency in urine specimens in our laboratory, all isolates of these two organisms from other types of specimens were saved and used for seeded urine specimens. Some *Serratia* sp. cultures were obtained from outside laboratories. Fresh isolates of other organisms, which the AMS is not designed to identify, were also obtained from the CML and utilized for seeded specimens.

Seeded specimens were prepared by making a suspension of the organism in 5 ml of 0.5% sodium chloride, adjusting to an 0.5 McFarland turbidimetric standard, transferring 0.2 ml from the suspension to 1.8 ml of 0.5% saline, and then transferring 0.2 ml of this suspension to 1.8 ml of sterile pooled urine (see *AMS Operators Manual,* Vitek Systems, Inc.).

Equipment. The AMS consists of five modules: the diluent dispenser, filling module, reader-incubator module, computer/control module, and cathode ray tube data terminal with printer. These five units occupy 0.73 m^2 of space and exhibit a total weight of 248 kg. A dedicated electrical circuit of 110 to 120 V, 60 Hz, and 25 A is needed. The reader/incubator module can accommodate 120 specimens and controls but can be expanded to accommodate 240 specimens with controls.

Detection, identification, and quantitation is accomplished by the AMS urine identification card, which is a sealed, disposable, 20-well plate which is inoculated with the diluted specimen by means of the filling module. Nine of the wells contain selective media formulated to allow growth of only one organism (or closely related group of organisms) in a specific well. One other well contains nonspecific media and serves as the control well, allowing most organisms to grow in it. Five additional wells are responsible for quantitation.

The inoculated identification cards are incubated at 35 ± 1°C in the reader-incubator. Identification cards may be added at all times, except for a maximum of 6.5 min during each 15-min interval when the AMS is reading incubating cards. They are electro-optically scanned over a 13-h period. Preliminary results are available via the cathode ray tube terminal after the first 3 h of incubating except for a 5-min delay if final results are being printed. Final results are printed out at the end of 13 h.

Culture interpretation. During the course of this evaluation, if the CML or the AMS result, or both, indicated more than two organisms, that specimen was considered unsatisfactory due to contamination and the results were discarded.

A true positive result was indicated when both the AMS and our method indicated the same organism with a count of at least 70,000 colony-forming units (CFU) per ml.

A true negative result occurred when both the AMS and our method indicated "no growth" or revealed a count of less than 70,000 CFU/ml.

Discrepancy investigation. A discrepancy investigation was instituted if the identification or quantitation, or both, differed between the CML and the AMS methods. This consisted of streaking out the positive well of the AMS urine identification card to a blood agar and eosin methylene blue plate, incubating overnight, and submitting the plates to the CML for identification. Additionally, the original urine culture plates and urines were saved under refrigeration and reexamined in an attempt to settle differences.

Methods. (i) Conventional methodology. Conventional methodology in our CML consists of quantitative loop (0.001 ml) plating-out of urine specimens onto blood and eosin methylene blue agar plates, incubating at 35°C for 18 to 24 h, and then examination. Gram-negative rod isolates were identified to the species level by use of Russell double sugar, Simmons citrate, and SIM media initially, with the addition of lysine iron agar, urease medium, the usual fermentation sugars, oxidation-fermentation medium, and deoxyribonuclease medium as needed. Gram-positive isolates were identified by use of the following tests as determined by the technologist: catalase, coagulase, bile esculin medium, 6.5% sodium chloride medium, sorbitol, mannitol, arabinose, litmus milk, and oxidation-fermentation sugars. Yeasts were identified by sugar fermentations, germ tube test, chlamydospore agar, and urease. Problem yeasts were further screened by use of the Corning Yeast Uni-tek (Corning Laboratories).

(ii) Time savings. Time/motion studies were done for both the AMS and our CML method utilizing the College of American Pathologists timing study format. These studies were carried out on single and batched specimens on five different occasions for each group. Comparisons of each method were made to the same level of identification.

(iii) Quantitation. The AMS quantitation is accomplished by five wells in the identification card, which contain noninhibitory media, and is based on the most-probable-number theory. The approximate total counts of all organisms present in the specimen that will grow in the media are reported as less than 70,000 CFU/ml or greater than 70,000 CFU/ml as determined by the number of enumeration wells exhibiting growth. Comparison of the AMS result with the quantitative count reported by the CML was made for each specimen included in the evaluation.

(iv) Accuracy of identification. The results obtained by the AMS method were compared with the CML results. Seeded urine specimens were set up using the protocol outlined in the *AMS Operators Manual.*

(v) Other organism response. Organisms belonging to the same genus or a group closely related to those identifiable by the AMS were obtained from the CML and used for seeded urine specimens. This approach enabled us to assess the differentiating capabilities of the AMS.

(vi) Polymicrobic specimen response. Polymicrobic is defined in this study as a urine specimen which contains two different organisms. Urine specimens with more than two different organisms were considered as contaminated. This phase of the evaluation was carried out to determine if the presence of a second organism would affect the accuracy of identification. A comparison of the results obtained by the CML and by the AMS on all urine specimens containing two organisms was carried out.

(vii) Identification time. Utilization of the AMS can result in a positive response in 3 to 13 h. A final result with quantitation is printed out at 13 h. The time needed for a positive response to become available both above and below 70,000 CFU/ml was recorded for all specimens containing organisms.

(viii) Response comparison. The different combinations of results obtainable by comparing the CML results with the AMS results were recorded. These data were then utilized to determine if misleading results are generated by the AMS.

(ix) Cost effectiveness. Media costs were determined using current commercial prices. The average hourly wage was determined for the various levels of employees involved. Equipment costs were current quoted prices. Overhead costs, such as electricity, heat, and water, were not included. Comparison was made with the identification methodology in use in the CML at the time of the evaluation. The urine specimen workload and charges were obtained from current data.

RESULTS

Time savings. The routine methodology used in our CML required 4.25 min per specimen, as compared to 2.66 min per specimen by utilization of the AMS methodology. Use of the AMS represents a 1.59 min-per-specimen or a 37% (1.59/4.25) time saving.

Quantitation. Table 1 contains the data correlating the level of agreement of the quantitation results obtained by the CML method and the AMS method. Each organism or organism group identifiable by the AMS was compared separately. The majority of the specimens contained organisms in quantities of 10^6 CFU/ml or more. However, each organism or organism group identifiable by the AMS contained some specimens with counts ranging from 900,000 down to 10,000 CFU/ml. Specimens with counts less than 70,000 CFU/ml were considered as negative results and are not included in Table 1. The overall correlation value for all organisms except yeast was 98.8%. The correlation value for yeast (*Candida* sp. and *Torulopsis glabrata*) was 50% due to the varying growth rates of different strains. Only one specimen was encountered in which the AMS result indicated a count greater than 70,000 CFU/ml, whereas the CML result indicated less than 70,000 CFU/ml.

The seeded specimens in Table 1 are not paired with a result from the CML.

TABLE 1. *Quantitation correlation summary*[a]

Organism or organism group	A	B	% Correlation
E. coli	234[b]	1	99.6
KE group	85	0	100
P. aeruginosa	84	1	99
Proteus sp.	82	0	100
Group D *Enterococcus*	59	0	100
Staphylococcus	30	2	94[c]
sp.	103[d]	3[d]	97[e]
Yeast	46	47	50
Serratia sp.	87[d]	0	100

[a] A, Number of urine specimens shown by both AMS and CML to have counts >70,000 CFU/ml. B, Number of urine specimens shown by CML to have counts >70,000 CFU/ml but by AMS to have counts <70,000 CFU/ml. Percent correlation = A/A + B × 100.

[b] Number of urine specimens compared with this response.

[c] Percent determined only on actual urine specimens.

[d] Seeded specimens adjusted to a MacFarland 0.5 standard.

[e] Percent determined only on seeded urine specimens.

Identification accuracy. Table 2 contains the data showing the accuracy of the AMS in identifying the following organisms when in concentrations of 7×10^4 CFU/ml or greater: *Escherichia coli, Klebsiella-Enterobacter* group (KE group), *Pseudomonas aeruginosa, Proteus* sp., group D *Enterococcus, Staphylococcus* sp., *Candida* sp. and *T. glabrata* (yeast), and *Serratia* sp. Identification accuracy was 94% for *E. coli,* 95% for KE group, 98% for *P. aeruginosa,* 99% for *Proteus* sp., 92% for group D *Enterococcus,* 88% for *Staphylococcus* sp., 99% for yeast, and 92% for *Serratia* sp. The 88% accuracy of *Staphylococcus* sp. identification reflects the tendency for some strains (particularly *Staphylococcus epidermidis*) to be inhibited and slow to grow. This is seen usually when counts are below 10^6 CFU/ml. This also necessitates additional incubation time beyond 13 h to see if the *Staphylococcus* sp. well will change to positive. The growth of some strains of *Serratia* sp. was not inhibited in the *E. coli* well, thus resulting in a false-positive report of *E. coli* being present.

The "other" column (Table 2) showing an accuracy of 55% should be considered cautiously since it represents many specimens seeded with various organisms that the AMS is not capable of identifying. Some of these organisms would be rare isolates from urine specimens. Others would be considered as nonpathogenic or contaminants when isolated from urine specimens. Further discussion of organisms involved in this column is found in a later section.

The accuracy of true negative responses was determined to be 99%.

The average overall accuracy of organism identification was 95%.

Other organism response. The differentiating capabilities of the AMS was tested by challenging with 188 strains of other organisms which the system was not designed to identify, but which may occur in urines, however infrequently (Table 3). Smith et al. (5) commented on the erroneous identification of group B streptococci as group D enterococci. The 13 strains of group B streptococci which we tested gave an "unidentified organism" response. *Citrobacter diversus* was misidentified in 9 out of 10 strains tested. Three of four strains of *Salmonella* sp.

TABLE 2. *Identification accuracy*

	Organism or organism group	CML results									
		E. coli	KE group	*P. aeruginosa*	*Proteus* sp.	Group D *Enterococcus*	*Staphylococcus* sp.	Yeast	*Serratia* sp.	Other[a,b]	No growth[c]
AMS results	*E. coli*	316							8_s	15	3
	KE group	5	143							3	2
	P. aeruginosa	1	1	117						2	
	Proteus sp.	3	1		123	1	1			1	1
	Group D *Enterococcus*	2				90	1				
	Staphylococcus sp.	1					42 95_s[d]			1	
	Yeast							114			
	Serratia sp.				1				88_s	2	
	Other	8	6	2		7	4 12_s	1		100	
	No growth	1								59	1,248

[a] Others = Unidentified organisms, organisms which grew but could not be identified by the AMS, or organisms known to be present but which did not grow in their homologous wells.
[b] See later section for organism breakdown.
[c] No growth = Either no organisms cultured or else the count was less than 70,000 CFU/ml with two or less organisms present.
[d] Subscript s indicates seeded specimens used.

were erroneously identified. *Providencia stuartii, Enterobacter hafniae, Enterobacter agglomerans, Alcaligenes odorans,* and *Alcaligenes faecalis,* although few strains were tested, appeared to give variable responses. It is also interesting to note that a significant number of strains of organisms commonly found in urines, i.e., alpha-hemolytic streptococci, nonhemolytic streptococci, *Lactobacillus* sp., *Corynebacterium vaginalis,* and diphtheroids, did not grow in the control media.

Polymicrobic specimen response. The results on 96 urine specimens containing two different organisms identifiable by the AMS were compared to our conventional methodology in Table 4. Complete agreement was obtained in 90 specimens for a 94% correlation.

One discrepancy represented the failure of a *P. aeruginosa* to grow in the identification card. Another discrepancy represented a difference in count, with the CML reporting *E. coli* and group D *Enterococcus* greater than 70,000 CFU/ml and the AMS reporting the same two organisms at a count of less than 70,000 CFU/ml. Three of the remaining discrepancies were due to one of the organisms present growing in and triggering one of the other wells. The remaining difference occurred in a specimen containing group D *Enterococcus* and KE group, with the AMS giving an unidentified organism response for unknown reasons.

Identification time. The AMS is so programmed that a status report for each culture may be requested and obtained at the end of 3 h of incubation. It would be of considerable aid if the time to positivity could be used as a presumptive indication as to whether the count is over 70,000 CFU/ml. The critical factor here is how many cultures containing organisms in quantities less than 70,000 CFU/ml are also positive at the same time. Table 5 contains the percentage of each organism present in quantities greater than 70,000 CFU/ml identified by a certain hour of incubation. It can be seen that 81% of the specimens containing *E. coli* in quantities greater than 70,000 CFU/ml were positive by 6 h. At this same time interval, none of the 108 strains of *E. coli* present in quantities less than 70,000 CFU/ml had triggered a positive response. At 7 h, however, 3% of these strains were positive. KE group gave a 53% positive response at 7 h. None of the 18 strains of the KE group present in quantities less than 70,000 CFU/ml were positive at this time, but 6% became positive at 8 h. *Proteus* sp. identification is interesting in that 71% of the strains gave a positive response by 3 h of incubation. Twenty-five percent of the 32 strains of *Proteus* sp. present in quantities less than 70,000 CFU/ml were also positive after 3 h of incubation. *P. aeruginosa* results are similar to *Proteus* sp. except that it takes 7 h of incubation to reach a 61% positivity level. During this same time period, 34% of the 32 strains of *P. aeruginosa* present in quantities less than 70,000 CFU/ml had become positive. Seventy-one percent of the group D *Enterococcus* strains were positive by 7 h of incubation. It necessitated 8 h of incuba-

TABLE 3. *Organisms not identified by the AMS*

CML organism identification	AMS result	No. of strains giving listed response
1. *Citrobacter diversus*	UIO[a]	1
	E. coli	3
	E. coli and KE group	5
	KE group	1
2. *Providencia stuartii*	*P. aeruginosa*	1
	UIO	7
	Proteus sp.	1
3. *Providencia alcalifaciens*	UIO	5
4. *Salmonella* sp. (group B, C)	UIO	1
	E. coli	2
	E. coli and KE group	1
5. *Enterobacter hafniae*	*E. coli*	3
	UIO	10
6. *Enterobacter agglomerans*	UIO	6
	C. freundii and *E. coli*	2
	KE group	1
	E. coli	1
7. *Alcaligenes odorans*	No growth	1
	UIO	2
	Serratia sp. and *P. aeruginosa*	2
8. *Alcaligenes faecalis*	UIO	3
	Serratia sp.	1
9. *Acinetobacter* sp.	UIO	16
10. *Pseudomonas maltophilia*	UIO	9
11. Beta-hemolytic streptococci	UIO	16
	Group B = 13	
	Group A = 3	
12. *Streptococcus pneumoniae*	UIO	3
13. Alpha-hemolytic streptococci	No growth	11
	UIO	7
14. Nonhemolytic streptococci	No growth	4
	UIO	2
15. *Micrococcus* sp.	No growth	3
	Staphylococcus sp.	1
16. *Lactobacillus* sp.	UIO	1
	No growth	14
17. *Corynebacterium vaginalis*	UIO	2
	No growth	18
18. Diphtheroids	No growth	6
	UIO	8
19. *Neisseria* sp.	UIO	3
	No growth	2

[a] UIO, Unidentified organism.

TABLE 4. *Two organisms per specimen comparison*

CML results	AMS results	No. of specimens	Discrepancy explanation
1. *C. albicans*, KE group	Yeast, KE group	4	
2. KE group, *Proteus* sp.	KE group, *Proteus* sp.	9	
3. *Proteus* sp., group D *Enterococcus*	*Proteus* sp., group D *Enterococcus*	3	
4. KE group, *C. freundii*	KE group, *C. freundii*	2	
5. *C. freundii, Proteus* sp.	*C. freundii, Proteus* sp.	1	
6. *P. aeruginosa*, group D *Enterococcus*	*P. aeruginosa*, group D *Enterococcus*	6	
7. *P. aeruginosa*, KE group	*P. aeruginosa*, KE group	4	
8. *P. aeruginosa, Serratia* sp.	*P. aeruginosa, Serratia* sp.	1	
9. *P. aeruginosa, Proteus* sp. *P. aeruginosa, Proteus* sp.	*P. aeruginosa, Proteus* sp. *Proteus* only	5	
10. *P. aeruginosa*, yeast	*P. aeruginosa*, yeast	8	
11. Yeast, *Staphylococcus* sp.	Yeast, *Staphylococcus* sp.	1	
12. *E. coli*, yeast	*E. coli*, yeast	4	
13. *E. coli, Staphylococcus* sp.	*E. coli, Staphylococcus* sp.	2	
14. *E. coli*, group D *Enterococcus*	*E. coli*, group D *Enterococcus*	22	
E. coli, group D *Enterococcus*: >70,000 CFU/ml	*E. coli*, group D *Enterococcus*: <70,000 CFU/ml	1	
15. *E. coli, P. aeruginosa*	*E. coli, P. aeruginosa*	2	
16. *E. coli*, KE group	*E. coli*, KE group	10	
E. coli, KE group	Yeast, KE group	1	KE group triggering yeast well
17. *E. coli, Proteus* sp.	*E. coli, Proteus* sp.	5	
E. coli, Proteus sp.	*E. coli, Proteus* sp., KE group	1	*E. coli* triggering KE well
18. Group D *Enterococcus*, yeast	Group D *Enterococcus*, yeast	3	
19. Group D *Enterococcus*, KE group	Group D *Enterococcus*, KE group	2	
a) Group D *Enterococcus*, KE group	Group D *Enterococcus*, KE group, *E. coli*	1	KE triggering *E. coli* well
b) Group D *Enterococcus*, KE group	Unidentified organism	1	Both organisms present in control well
20. *Staphylococcus* sp., *Proteus* sp.	*Staphylococcus* sp., *Proteus* sp.	2	

tion before any of the 51 strains of group D *Enterococcus* present in amounts less than 70,000 CFU/ml elicited a positive response. Yeast (*Candida* sp. and *T. glabrata*) was more difficult to analyze due to the fact that quantitation is only 50% accurate. Of the 62 strains of yeast in which the AMS and CML agreed that the count was greater than 70,000 CFU/ml, 66% produced a positive response by 7 h. There were 31 strains of yeast in which the AMS reported less than 70,000 CFU/ml, whereas the CML reported greater than 70,000 CFU/ml. Thirty-one percent of these produced a positive response during the 7-h interval. The 14 strains of yeast on which the AMS and CML agreed that the count was less than 70,000 CFU/ml did not produce a positive response until after 10 h of incubation. The results listed for *Staphylococcus* sp. and *Serratia* sp. are based entirely on seeded specimens containing greater than 70,000 CFU/ml. As would be expected with a standardized inoculum, the time of identification of the majority is fairly well grouped from 8 through 12 h.

Response comparison. As would be expected, the AMS and the CML did not always agree. Table 6 illustrates the discrepant results obtained. If one accepts the thesis that bacterial counts below 70,000 CFU/ml are not significant, then many of these differences would not present problems in interpretation. Additionally, since the presence of more than two organisms indicated a contaminated specimen, further differences are readily resolved. The above then leads one to the conclusion that only items 3 and 5c2 in Table 6 represent significant discrepancies in identification. A total of 15 specimens out of 3,595 specimens set up, where either the AMS

TABLE 5. *Time for positive identification*

Organism	Percentage of organisms identified by indicated time elapsed (hours):													
	0[a]	1[a]	2[a]	3	4	5	6	7	8	9	10	11	12	13
E. coli (303)[b]	0	0	3	.19	52	70	81	88	92	96	97	97	99	100
KE group (134)	0	0	1	2	8	16	30	53	70	81	90	96	99	100
Proteus sp. (113)	14	44	60	71	76	84	89	92	97	98	99	99	99	100
P. aeruginosa (113)	4	4	4	7	12	21	38	61	76	83	89	96	99	100
Group D *Enterococcus* (87)	0	0	1	9	23	48	67	71	86	91	94	98	99	100
Yeast (62)	2	3	5	7	13	31	48	66	79	90	95	95	100	100
Staphylococcus sp. (98)[c]	0	0	3	3	4	4	4	5	9	33	74	93	100	100
Serratia sp. (143)[c]	0	0	0	0	1	3	10	25	40	57	64	78	92	100

[a] The report obtainable at 3 h may list some specimens as positive at 0, 1, or 2 h.
[b] Number of organisms involved (>70,000 CFU/ml) are indicated in parentheses.
[c] Seeded specimens over 70,000 CFU/ml.

or CML reported an organism and the other method reported no growth, would seem to be an insignificant difference.

The false-positive responses shown in items 9 and 10 in Table 6 are usually due to bubbles in the case of *P. aeruginosa* and *Staphylococcus* sp. and apparently are due to a pH change for *Proteus* sp. The majority of these are readily apparent upon examination of the identification card. Items 13, 14a, and 15a (Table 6) are interesting in that, with just the AMS result, a misleading report could be generated. These three groups represent approximately 3% of the total specimens run. Item 19 (Table 6) is involved with count differences other than those seen with specimens containing yeast.

Cost effectiveness. Table 7 compares the cost per urine specimen by both methods. The personnel costs are less for a urine culture utilizing the AMS method due to the need for less technologist time. This is offset by a considerably higher cost for expendables. Consequently, it costs $1.83 more per urine specimen for the AMS method. Technologist time savings of 630 h yearly with personnel cost savings of $4,200 per year are realized by using the AMS method (Table 8).

The number of daily, monthly, and yearly specimens needed to cover the cost of personnel time and expendable items, and the above two plus the instrument cost, are shown in Table 9. To pay for the instrument-plus-specimen costs in 1 year would necessitate 30 specimens daily at our charge rate of $12.00 per urine specimen. Table 9 also shows that, with our workload of 21,000 urine specimens per year, we could pay for the instrument and the yearly specimen cost in 5.7 months.

DISCUSSION

The AMS represents a significant step in providing automation to clinical microbiology through the ability of this system to automatically analyze a patient urine specimen and provide quantitation and identification of the more common urinary tract pathogens. During the course of our evaluation we found the instrument to be very reliable and to require only around 5 min of routine maintenance daily. When problems did arise, the company responded rapidly.

Our studies in general agree with published studies on the AMS, confirming tbe fact that this system can contribute significantly in the examination of urine specimens in a CML (1-6).

A total time savings of 1.74 h per day would presumably be realized in our laboratory by using the AMS. This would have more significance if it involved one technologist or station. In our laboratory it would be divided among five to six technologists, thus somewhat reducing the value of its addition to the laboratory.

Quantitation obtainable by the AMS is quite accurate when the count is over 70,000 CFU/ml, except for yeast. The quantitative streaking-out of a blood agar plate with each specimen would alleviate the problem with yeast quantitation. We had 284 specimens with counts below 70,000 CFU/ml by the AMS method which correlated well with the CML method. Only seven specimens in this study were reported as having greater than 70,000 CFU/ml by the CML, whereas the AMS reported less than 70,000 CFU/ml. The reverse was true in only one specimen. The trend would seem to indicate fairly good correlation down to 10,000 CFU/ml. However, definite conclusions cannot be made due to the small number of specimens in this range. Until additional data are available, specimens in which counts less than 70,000 CFU/ml may be significant (catheterized, suprapubic aspirate, or cystoscopy specimens) should not be tested only by the AMS. The presence of more than one organism identifiable by the AMS obviously pro-

TABLE 6. *AMS responses versus CML results*

CML	AMS	No. of occurrences
1. No growth	UIO[a], <70,000 CFU/ml	116
2. No growth	One of eight identifiable organisms, <70,000 CFU/ml	131
3. No growth	One to two identifiable organisms, >70,000 CFU/ml	6
4. No growth	Three or more organisms, >70,000 CFU per ml/<70,000 CFU per ml	2/3
5. Organisms	No growth	74
a. 34 organisms not identifiable by AMS (alpha-hemolytic, nonhemolytic streptococci; *Lactobacillus* sp.; diphtheroids; etc.)		
b. 14 organisms identifiable by AMS but very low counts (<16,000 CFU/ml)		
c. 25 organisms identifiable by AMS		
1. 16 organisms counts <70,000 CFU/ml (20,000–60,000 CFU/ml)		
2. 9 organisms counts >70,000 CFU/ml (80,000–3×10^6 CFU/ml)		
6. Three or more organisms	No growth	2
7. Multiple[a]	No growth	56
8. Variable[b]	Card fill error[c]	45
9 Variable	False positive,[d] >70,000 CFU/ml (usually *P. aeruginosa, Proteus* sp., or *Staphylococcus* sp.)	20
10. Variable	False positive, <70,000 CFU/ml (60 of the false positives occurred before October 1977)	81
11. Multiples	One to two organisms, <70,000 CFU/ml	77
12. Multiples	Three or more organisms, >70,000 CFU per ml/<70,000 CFU per ml	31/4
13. Multiples	One to two organisms, >70,000 CFU/ml	54
14. Multiples and/or organism(s)[e]	UIO	
	a. >70,000 CFU/ml	10
	b. <70,000 CFU/ml	66
15. Multiples plus organism(s)	One to two organisms	
	a. >70,000 CFU/ml	47
	b. <70,000 CFU/ml	10
16. Multiples plus organism(s)	Three or more organisms	20/2
	a. >70,000 CFU per ml/<70,000 CFU/ml	
17. More than two organisms (AMS and/or CML)		
a. Same number	Same number	8
b. Fewer organisms than AMS	More organisms than CML	
	1. Set up same day	43
	2. Overnight delay	18
c. More organisms than AMS	Fewer organisms than CML	11
18. Two organisms	One organism >70,000 CFU per ml/<70,000 CFU per ml	14/2
19. Count differences		
a. <70,000 CFU/ml	>70,000 CFU/ml	4
b. >70,000 CFU/ml	<70,000 CFU/ml	5

[a] UIO, Unidentified organism.

[b] Multiple, Multiple organisms present suggesting an improperly collected specimen.

[c] Variable, Responses cover many different possibilities from no growth through multiples.

[d] Card fill error, This response does occur occasionally and examination of the AMS identification card may reveal bubbles in several wells, a positive organism well with negative control and enumeration wells, positive enumeration wells with all other wells negative, or no apparent change in any well.

[e] False positive, Usually due to bubbles in *P. aeruginosa* and *Staphylococcus* sp. wells. *Proteus* sp. response apparently due to pH change and is usually present immediately.

[f] Multiples and organism(s), If an organism(s) is present in quantities greater than two times any of the other organisms making up the multiples response and it is a common urinary tract pathogen, it will be identified and listed separately.

duces a problem in determining the significance of each organism relative to quantities.

Identification accuracy for all organisms identifiable by the AMS is good except for *Citrobacter freundii.* The negative or no growth correlation is also excellent.

The AMS system as it is marketed contains the capability of identifying *C. freundii.* Since this is an uncommon isolate from urine specimens in our laboratory, isolates from other types of specimens were utilized for seeding. We obtained a 60% identification accuracy and felt the media was at fault. However, results from other investigators indicate a high accuracy of identification (5). Consequently, we are not reporting any *C. freundii* results but are attempting to determine the reason for this discrepancy.

As it now stands, the AMS does not define the species of all the organisms it is capable of identifying in urine specimens. This may seem to present a problem from an epidemiological or recurrent infection standpoint. We feel these are minor drawbacks in that, if the hospital epidemiologist is closely following all hospital-acquired infections, a change in incidence should become apparent whether the organism is identified just to the genus level or all the way to the species level. Any change in incidence would then necessitate identifying to species. The physician caring for a patient with recurrent urinary tract infection should request the laboratory to identify to species if any organism from the same genus is isolated. Additionally, sensitivity patterns would be of considerable help in both cases.

Previous reports have indicated that organisms closely related to the ones identifiable by the AMS may give rise to false-positive reports (3, 5). We found this to be true also. One report (5) which indicated that group B streptococci were often reported out as group D enterococci was not substantiated by our data. The investigators have no ready explanation for this discrepancy at this time. Our data also show that many strains of alpha-hemolytic streptococci, nonhemolytic streptococci, *Micrococcus* sp., *Lactobacillus* sp., *C. vaginalis,* diphtheroids, and *Neisseria* sp. will not grow in the control medium. These organisms would be of questionable significance in most urine cultures. Organisms other than those identifiable by the AMS are capable of causing urinary tract infections. The occurrence of these organisms is uncommon in our experience, although this would undoubtedly vary from one laboratory to another depending on the patient population. Many of these organisms give an unidentified organism response from the AMS. Additional identification procedures can then be carried out. A solution to some of these problems could be the streaking-out of a blood and eosin methylene blue agar plate with each urine specimen. A quick comparison could be made after overnight incubation in an attempt to catch any obvious errors. Thus, a printout of *E. coli* and KE group

TABLE 7. *Urine specimen direct cost analysis*

Item	CML cost	AMS cost
Personnel	$0.39	$0.19
Media and supplies	$1.16	$3.19
Cost per specimen	$1.55	$3.38

TABLE 8. *Time and savings comparison*

Personnel	Time needed per specimen (h)[a]		Cost per specimen ($)[b]	
	CML[1]	AMS	CML	AMS
Chief technologist	0.001	0.001	0.01	0.01
Clinical lab technician I	0.031	0.001	0.21	0.01
Lab assistant II	0.023	0.030	0.10	0.13
Clerk II	0.016	0.010	0.07	0.07
Total	0.071	0.042	0.39	0.19

[a] With an 0.03 h time savings per specimen using the AMS times 21,000 specimens tested yearly, the total time savings per year would be 630 h.

[b] With a $0.20 cost savings per specimen using the AMS times 21,000 specimens tested yearly, the total cost savings per year would be $4,200.

TABLE 9. *Amortization by direct cost analysis*[a]

System used	Cost/ specimen ($)	No. of specimens/yr	Yearly cost ($)	Charge/ specimen ($)	No. of specimens needed to cover cost		
					Yearly	Monthly	Daily
CML	1.55	21,000	32,550	12.00	2,713	226	8
AMS							
Expendables only	3.38	21,000	70,980	12.00	5,915	493	16
Including instruments			120,480	12.00	10,040	837	30

[a] Total yearly charges = (21,000 specimens/year) × ($12.00/specimen) = $252,000. Supply and instrument cost: (1,750 specimens/mo.) × ($12.00/specimen) = $21,000/mo. Total yearly cost of $120,480 ÷ $21,000 = 5.7 mo. needed to amortize supply and instrument cost.

in a single specimen while the plates show a pure culture would necessitate additional work-up. How much of a problem this could represent will undoubtedly vary depending upon the incidence of specific organisms in the patient populations being cultured in different laboratories around the country.

The presence of two AMS-identifiable organisms in a patient urine specimen produced a 94% correlation between the two methods. This would indicate that the AMS can correctly process urine specimens with up to two organisms present. In an additional experiment, 120 specimens containing various combinations of at least two organisms utilizing our control strains were set up. A high degree of correlation was obtained except for those combinations containing the *E. coli* strain and KE group strain, in which the latter was antagonistic to the former and inhibited the growth of the *E. coli*. Utilization of additional strains of *E. coli* and KE group failed to show other strains with this propensity. Again one has to remember that the presence of more than one organism produces problems in interpreting the significance of each isolate, since quantitation is based on all organisms present and not each one separately. Although the time when a well became positive may aid in making an interpretation of significance, it is not an absolute criterion, since there is variation in rate of growth among different strains. Additionally, any antagonistic or synergistic in vitro responses would affect the time to positivity.

Comparisons were made between the time when an organism triggered its homologous well and whether the final count was greater than or less than 70,000 CFU/ml in an attempt to determine the probable clinical significance of an isolate as reflected by the count. If the homologous well becomes positive within 6 to 7 h, in most cases the count will be over 70,000 CFU/ml for *E. coli*, KE group, and group D *Enterococcus*. At 7 h, 92 and 61% of *Proteus* sp. and *P. aeruginosa* isolates, respectively, with counts greater than 70,000 CFU/ml will have exhibited a positive response whereas 47 and 34% of the same organisms, respectively, with counts less than 70,000 CFU/ml will also have triggered a positive response. Of more interest is the fact that, at 3 h of incubation, 71% of the *Proteus* sp. present in amounts greater than 70,000 CFU/ml will have given a positive response whereas 25% of the *Proteus* sp. present in amounts less than 70,000 CFU/ml will also have triggered a positive response. This obviously inserts a large degree of uncertainty in assigning significance to these organisms. Since yeast enumeration is only 50% accurate when the count is greater than 70,000 CFU/ml, this parameter cannot be used as a predictor of significance. Due to the few patient urine specimens that contain *Staphylococcus* sp. and *Serratia* sp. in our hospital, comment concerning time to positivity significance based on clinical specimens is not warranted.

The different responses which occur when comparing the AMS and CML method could be cause for concern (Table 6). A determination of whether a urine specimen containing less than 70,000 CFU/ml is significant would depend on the method of collection, time of collection (early morning specimen versus midmorning specimen), possible presence of antimicrobial agents, and whether a previous specimen contained the same organism (follow-up culture after treatment). Table 6 shows that 407 specimens fall in this category when using the AMS response only. The fact that 111 cultures with counts over 70,000 CFU/ml containing multiple organisms were reported out by the AMS as one to two AMS-identifiable organisms or as unidentified organisms greater than 70,000 CFU/ml would seem to represent a risk of leading to unnecessary patient antimicrobial therapy. If the problem of presumably improperly collected specimens, which lead to the multiple organism response, cannot be resolved, then the best solution would appear to be the streaking-out of a blood and eosin methylene blue agar plate, incubation, and then comparison of the plates with the AMS response. It would be hoped that, through an educational campaign, improvement could be obtained in specimen collection. The accuracy of the AMS methodology for identification of more than two organisms in urine specimens will have to await additional data.

Although direct cost analysis indicates that the AMS method is more expensive than our conventional method (Table 7), several other factors have to be considered in attempting to determine cost effectiveness. It is apparent (Table 8) that a significant amount of personnel time is saved on a daily basis by utilizing the AMS. Hopefully this time would be utilized to accomplish other tasks. It would also be hoped that the availability of a culture report in 13 h or less would lead to more rapid and correct treatment of patients with urinary tract infections. This would depend on the availability of a physician to institute treatment. It would also necessitate an educational campaign on the part of the laboratory so that the physician would be aware of the availability of urine culture reports in a much shorter period of time than was previously possible. This presumably would lead to a more rapid discharge from the hospital and consequently a more marked saving in patient

charges. Setting up an antibiotic susceptibility on the positive cultures before or at the end of the 13-h incubation period would give a complete report within 24 h. This not only should benefit the patient from the treatment and cost standpoints but should enhance the microbiology laboratory status due to the markedly shortened turnaround time.

Utilizing direct costs only and not considering indirect costs, such as overhead, it is readily apparent from Table 9 that the cost of the AMS methodology can readily be amortized in our hospital. The ability to amortize within 5.7 months would seem to indicate that the charge per urine specimen should be significantly reduced. The cost effectiveness is going to vary from one laboratory to another depending upon methodology, personnel used, and number of specimens.

It would appear that the AMS could make a significant contribution to clinical microbiology by providing a urine culture result in 13 h or less. The upcoming availability of other AMS specimen procedures represents a giant step toward automation of microbiology and shortening of specimen turnaround time.

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