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Clinical Laboratory Evaluation of Automated Microbial Detection/Identification System in Analysis of Clinical Urine Specimens

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More than 4,000 clinical urine specimens were evaluated with an automated microbial detection/identification system compared to a standardized manual analysis and the routine modalities used in five peer-group laboratories. The comparison indicates that the automated system recognizes the nine groups of significant microorganisms in urinary tract infections in hospitalized patients with the same efficiency as a standardized manual method. The automated system's ability to enumerate the bacterial populations in the original clinical specimen attained a high degree of accuracy.

Earlier papers (1-4) have described in detail the design and function of the Auto Microbic System (AMS, Vitek Systems, Inc.), its approach to clinical microbiology, and its initial performance in several laboratories with seeded, simulated specimens which challenged the urine Identi-Pak, the first available test procedure which permits an automated system to address a clinical specimen. This report summarizes the experiences of five clinical laboratories in which clinical urine specimens were analyzed by the AMS, the routine procedures of each laboratory, and an agreed-upon standardized manual method.

(Results of work reported here were presented in part at the 78th Annual Meeting of the American Society for Microbiology, Las Vegas, Nevada, 1978.)

MATERIALS AND METHODS

The participating laboratories included the clinical microbiology laboratories of the Mayo Clinic, Rochester, Minnesota; the Cleveland Clinic Foundation, Cleveland, Ohio; the Jewish Hospital of St. Louis, St. Louis, Mo.; the Long Island Jewish-Hillside Medical Center, New Hyde Park, N.Y., and St. Mary of Nazareth Hospital, Chicago, Ill. The introduction of specimens into the instrument and the methods for evaluating numbers and establishing the generic and species groups for the protista most frequently encountered in urine specimens of patients have been described in detail previously (1, 3, 4).

All clinical urine specimens received in each laboratory for routine microbiological analysis were introduced into the AMS in the same manner as previously described (3). The specimens were evaluated by the same manual procedure in all of the laboratories as reported earlier (3).

Briefly, these methods consisted of introducing 200 μl into the first compartment of the specimen injector. A second 5-μl amount of the urine specimen was introduced into the second compartment. The volumes were diluted automatically with 0.5% NaCl and introduced through an evacuation step into the actual specimen cards (Identi-Pak). The latter were then introduced into the instrument for incubation and scanning. The agreed-upon ("standard") evaluation of the urine specimen consisted of seeding 0.1 ml of 1:100 and 1:1,000 dilutions of the urine specimen on two blood agar plates and one MacConkey agar plate. After incubation, enumeration and further identifications were carried out. The two dilutions tested corresponded to 1:1,000 and 1:10,000 dilutions of the original urine. In addition, each laboratory analyzed each specimen in accordance with the procedures used routinely in that respective laboratory. Table 1 summarizes the different methods. No laboratory performed the analysis of urine specimens in exactly the same fashion. This table indicates that laboratory workup followed collection at relatively short intervals, that detrimental delay in processing was prevented by refrigeration which did not exceed 24 h, that the bacterial densities were evaluated by different methods, and that all participants used media to detect the *Enterobacteriaceae*.

Results of the instrument analysis were recorded automatically by a tape cassette in each instrument. These cassettes along with the findings of analyses achieved by standard and routine methods were introduced into the computer based in the McDonnell-Douglas Corp. Comparisons of the findings were accomplished by this means.

TABLE 1. *Routine methods of participant laboratories*

Method	No. of laboratories using
Length of time before workup:	
Less than 30 min	2
Less than 2 h	2
Unknown	1
Refrigerated specimens	3
Dilutions performed, samples cultured	2
Calibrated loop	
−0.001 ml	1
−0.01 ml	2
Media:	
Blood agar	4
Separate medium for detection of *Enterobacteriaceae*	5
Separate medium for detection of enterococci	2

As a result of the filling mode by vacuum and the nature of the original lyophilized material within each well of the urine Identi-Pak cards, a number of air bubbles appeared occasionally within some of the wells. These bubbles, recognized initially by the instrument as microbial growth, were recorded mistakenly by the machine as positive. Inspection by technologists at the conclusion of the run indicated the lack of microbial presence in wells recognized as positive because of the bubbles. These observations were duly recorded, followed by manual analysis of the well contents, and considered during the evaluation. Eventually, a discriminating scanner was installed in some of the instruments and used by several of the peer group members. This scanner differentiates between air bubbles and microorganisms in the enrichment wells of the urine Identi-Pak card.

The sensitivity of the AMS was established by multiplying the positive reactions recorded by the instrument, as confirmed by the standard, manual analysis, by 100 and dividing by the sum of the truly positive specimens plus false-negative specimens, i.e., specimens not identified or recognized correctly by the AMS but determined via the manual method. Specificity, on the other hand, was defined as the organisms designated as negative by the machine (confirmed by standard laboratory tests), multiplied by 100, divided by the sum of the negative identifications confirmed by the machine, plus those organisms identified as belonging to a specific category in the AMS but not confirmed in standard laboratory testing.

To avoid misunderstanding, the original machine without the bubble scanner is referred to as the prototype AMS; the findings obtained with the scanner attached are referred to as evaluation with the scanner-improved AMS.

RESULTS

Table 2 shows the performance of the prototype AMS in the peer group laboratories. Specificity ranged from 94 to 99% for 4,184 clinical urine samples. Sensitivity, on the other hand, did not achieve similar levels, primarily because of the aforementioned problem with the formation of bubbles in the wells. *Pseudomonas* detection was only 75%; staphylococci were recovered at a 82% level, the *Klebsiella-Enterobacter* group and *Serratia marcescens* at 87%, yeast at 88%, *Citrobacter freundii* at 89%, and the remaining bacteria at 94%.

The introduction of the scanner (Table 3) led to improvement in the specificity of the instrument, with the recognition accuracy of important groups being greater than 96.8%. The sensitivity of the urine Identi-Pak, namely its ability to recognize the presence of a specific organism or a defined group of organisms, was enhanced considerably for all categories except *S. marcescens*. Subsequent to this collaborative study, investigations in laboratories of two participants (H.D.I., T.L.G.) have demonstrated that recognition threshold adjustments in the AMS software and fine-tuning of the enrichment medium constituents have brought the sensitivity capability of the AMS in properly identifying *Serratia*, *Pseudomonas*, and staphylococci to levels in excess of 90%.

Table 4 shows the performance of the individual laboratories in the original evaluation with the prototype AMS. The results indicate that not all participants encountered each and every organism that can be detected by the Identi-Pak. Other differences were distributed in a ran-

TABLE 2. *Collaborative analysis of clinical specimens with prototype AMS*[a]

Organism	No. isolated	Sensitivity	Specificity
Pseudomonas aeruginosa	124	75	96
Proteus sp.	233	94	97
C. freundii	19	89	99
S. marcescens	15	87	98
E. coli	608	94	99
Klebsiella-Enterobacter group	232	87	98
Yeast	72	88	98
Enterococci	358	94	95
Staphylococcus aureus	22	82	94
Enumeration		91	95

[a] Standard manual evaluation served as control. Sensitivity was calculated as: {True (+)/[True (+) + False (−)]} × 100. Specificity was calculated as: [True (−) × 100]/[True (−) + False (+)]. Of 4,184 specimens, 1,683 (above) had positive results and 2,501 had negative results; 1,195 specimens (29%) yielded organisms in numbers >70,000 colony-forming units (CFU) per ml by both the standard method and AMS. The standard method detected microorganisms in numbers <70,000 CFU/ml in 274 (6%) additional specimens; the AMS detected numbers <70,000 CFU/ml in 488 (11%) additional specimens.

dom fashion; there was not one laboratory which performed poorly as compared to the others. The ability of each laboratory to analyze monomicrobic and polymicrobic seeded samples satisfactorily with the prototype instrument was predicated, to a degree, on visual inspection of each card at the completion of incubation, the recording of air bubbles, and, in some instances, cultural analyses of those wells which displayed bubbles (3). A similar evaluation of results obtained with protoype instruments in this investigation resolved most differences in the participating laboratories. As mentioned above, the use of the scanner-improved AMS avoids this problem entirely.

The ability of the prototype AMS to enumerate the microbial densities in clinical urine samples exceeded 90% in this trial. The five wells separately inoculated from the injector reservoir receiving 5 μl of the original specimen provided an acceptable account of the colony-forming units (CFU) in the original specimen, i.e., more than or less than 70,000 CFU/ml.

TABLE 3. *Collaborative analysis of clinical specimens with scanner-improved AMS*[a]

Organism	No. isolated	Sensitivity	Specificity
P. aeruginosa	37	85.7[b]	99.4
Proteus sp.	106	96.2	97.7
C. freundii	16	100.0	99.7
S. marcescens	4	33.3[b]	99.4
E. coli	333	92.4	98.3
Klebsiella-Enterobacter group	102	95.7	98.7
Yeast	33	100.0	99.3
Enterococci	181	94.7	98.3
S. aureus	17	87.5	96.8
Enumeration		95.4	94.7

[a] Standard manual evaluation served as control. Sensitivity and specificity are as defined in the footnote of Table 2. Of 1,773 specimens, distinct and different from specimens described in Table 2, 829 had positive results and 304 had negative results. A total of 640 specimens (36%) yielded organisms in numbers ≥70,000 CFU/ml by the standard method and AMS. The standard method detected microorganisms in numbers <70,000 CFU/ml in 569 (32%) additional specimens; the AMS detected numbers <70,000 CFU/ml in 829 (47%) additional specimens.

[b] Now improved by adjustments of enrichment milieu.

DISCUSSION

The studies with clinical specimens indicate that the AMS evaluates actual clinical specimens with the same accuracy manifested with seeded specimens (3). The instrument is capable of recognizing with efficiency the most important and most common microorganisms isolated from urinary tract infections encountered in patients. The instrument is capable of performing these tasks with a minimum of intervention by technical personnel and it reports the findings with an acceptable accuracy. Since the operator has the capability of requesting the machine to report ad lib, the time to detect a significant microbial organism in any one urine specimen is limited only by the initial number and the sensitivity threshold incorporated in the software of the instrumentation. However, the total incubation of 13 h is required to conclude that a culture is negative. Furthermore, the instrument provides the opportunity to confirm the identification of each of the organisms as well as the chance to submit the organisms within the wells of the card to further biochemical or biological testing. This task can be performed easily with a 10-μl pipette. Several studies reported by Isenberg et al. at the Interscience Conference on Antimicrobial Agents and Chemotherapy, 1977,

TABLE 4. *Individual laboratory results of clinical specimen evaluation with prototype AMS laboratory*

Organism	Laboratory									
	A		B		C		D		E	
	SENS[a]	SPEC	SENS	SPEC	SENS	SPEC	SENS	SPEC	SENS	SPEC
P. aeruginosa	76.4	98.6	65.6	98.4	80	99.2	100	97.1	71.4	99.3
Proteus sp.	97	93.7	95	93.8	82.2	97.4	91.6	93.8	90	94.5
C. freundii	33.3	98.8	50	99.1	—	99.7	—	96.9	100	98.3
S. marcescens	50.0	99	—	98.2	66.6	99.6	100	98.6	—	98.9
E. coli	94	90	97.8	98.3	88.3	88.8	92.3	94.2	91.2	91.5
Klebsiella-Enterobacter group	89.1	95	77.4	98.4	96.5	99.3	100	95.9	91.8	97.0
Yeast	100	99.8	—	96.5	100	99	83.3	98.6	93.3	99.4
Enterococci	93.4	92.6	92.3	93.5	81.4	93.7	79.1	97.5	86.9	89.9
S. aureus	100	97.6	100	93.9	—	98.2	100	94.8	85.7	95.3
Enumeration	87.9	96.1	95	69.5	81.4	96.7	93	92.5	92.3	89.3

[a] SENS, Sensitivity; SPEC, specificity. Sensitivity and specificity are as defined in the footnote of Table 2.

utilized this mode of entry to establish antibiotic susceptibility profiles for *Escherichia coli*. They indicated ease of performance and the capability of further analysis by either manual or automated methodologies.

The early experience with the bubbles led to an improved addition to the detecting mechanisms of the device. Whereas the presence of such physical expressions of filling are dependent in part on the efficacy of the vacuum system supplied in the filling station, it is significant that by the introduction of a scanning detector the automated system acquired the capability to differentiate between the artificially accumulating air bubbles and the turbidity and color changes produced by the presence of microbial particles in specific wells.

Initially, it was thought that this multilaboratory collaborative investigation would provide the opportunity to compare the routine microbiological analysis of urine performed in each laboratory with the method which served as the manual standard for AMS performance. The latter approach used materials produced at a single source and analyzed several dilutions of each specimen in a prescribed fashion followed by each participant (3). Any microorganism isolated in any dilution was identified and reported, i.e., no judgment concerning the clinical finding was exercised. On the other hand, the routine methods of each laboratory were constrained by guidelines such as the significant number of CFU leading to further studies, the acceptability of a specimen yielding more than two microbial species, classification of specimens on the basis of CFU/ml, the identity of the microorganism isolated, and the available history of patients. These judgmental considerations made a meaningful comparison between the two manual modalities impossible. By far, the most frequent difference arose when the routine clinical approach did not pursue identification of an isolated protist further because it was present in the specimen in numbers less than the accepted criterion. This appeared as a negative result when compared to the standard manual method, which identified all organisms regardless of number in accordance with its expressed purpose as a manual control of the enumerating and recognition functions of the AMS.

It must be emphasized as well that the ability of the instrument to categorize the microorganisms in a specimen is independent of the number of particles established by the nonspecific enumeration wells. The latter is a 1:10 dilution of the inoculum introduced into the differentiating wells. The ability of each organism to grow in its specific compartment is influenced, of course, by the original number present and the time allowed to reach a preset threshold by multiplication. Thus, counts indicating a microbial load of less than 70,000 do not interfere with the ability of the instrument to report the presence of certain bacteria within a specimen. It must also be kept in mind that the time/CFU ratio is different for each well. The enrichment-selective media employed do not permit the same number of multiplications for each organism in its specific well in the same time interval. Thus, the time report does not necessarily indicate the number of particles present. Instead, final counts in the enumeration wells must be taken into consideration. This lack of distinguishing between component microorganisms in a polymicrobic specimen may represent a limitation of the AMS in its present configuration. Studies now in progress are designed to alleviate this problem.

In a previous study (3), the ability of other organisms to grow and manifest within the urine Identi-Pak has been evaluated. Organisms rarely encountered in clinical urine specimens do not proliferate in the enrichment-selective compartments for specific organisms. The growth control medium, provided in a specific compartment within the card and identical to the medium employed in the enumeration wells, will permit the growth of organisms other than those specifically separated in the AMS. The AMS reports the presence of such organisms as an unidentified microorganism. Aspiration of the growth control well contents and analysis by manual techniques can be used for their identification.

The AMS is the first automated procedure which addresses clinical specimens directly (2); it detects, enumerates, and identifies the most frequent and significant bacteria and yeasts commonly found in urine in a fully automated mode. Heretofore, instrumentation has, for the most part, provided a mechanized approach to the postisolation functions of the clinical microbiology laboratory. Whereas the AMS procedures described so far apply only to the analysis of urine specimens, other applications, such as antibiotic susceptibilities, biochemical tests for identification, the determination of minimal inhibitory concentrations, as well as other types of clinical laboratory specimens, are under active investigation and should aid in bringing this capability to many of the routine procedures of the clinical microbiology laboratory.

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