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Screening of Urine Cultures by Three Automated Systems

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A study was conducted to compare three automated systems and the Gram stain for their ability to detect significant bacteriuria. A total of 1,000 urine specimens were evaluated by Autobac MTS (General Diagnostics), AutoMicrobic system (AMS; Vitek Systems, Inc.), and MS-2 (Abbott Laboratories) and compared with a semiquantitative culture plate method. Two hundred thirty-nine (23.9%) specimens had colony counts of $>10^5$ colony-forming units (CFU)/ml by the culture plate method (group I). Of these, 204 (85.3%) were positive by Autobac, 198 (82.8%) were positive by AMS, and 179 (74.9%) were positive by MS-2. When pure cultures of diphtheroids, lactobacilli, and viridans streptococci not group D were considered contaminants and therefore excluded, there were 118 specimens containing pure cultures of probable pathogens. The percentage of significant isolates detected was 97.4% (115 of 118) by the Gram stain, 96.6% (114 of 118) by Autobac, and 95.8% (113 of 118) by AMS and MS-2. The average detection time for all organisms was 2.2 h by Autobac, 6.1 h by AMS, and 1.8 h by MS-2; therefore, all three methods were more rapid than the 18- to 24-h standard plate culture method. One hundred sixty-one (16.1%) specimens had colony counts of 10^4 to 10^5 CFU/ml (group II). The probable pathogens not detected in this group were two (1.2%) by Autobac and MS-2 and three by AMS (1.9%). The average detection time for group II was 4.2 h by Autobac, 8.9 h by AMS, and 3.8 h by MS-2. Six hundred specimens had colony counts of $<10^4$ CFU/ml. Of these, 188 had colony counts equal to 10^3 and $<10^4$ CFU/ml (group III), and 412 cultures were below detectable limits by the standard plate method (group IV). Less than 37 and 15% of groups III and IV, respectively, were detected by instrumentation. Average detection times for groups III and IV were 4.6 and 4.8 h by Autobac, 10 and 11 h by AMS, and 4.2 and 4.4 h by MS-2. The cost of supplies and technical time with Gram stain, Autobac, and MS-2, when used as screening methods, were comparable and considerably less expensive than for the reference method. The AMS was the least expensive system when the cost for identifying probable pathogens was included.

Urine cultures are the most common type of specimens processed in the diagnostic microbiology laboratory. Several variables are taken into account when processing these specimens. Among these is the presence or absence of growth. The criterion of a single organism at a concentration of $\geq 10^5$ colony-forming units (CFU)/ml in a clean-voided urine is the generally accepted definition of significant bacteriuria (10). Until recently, the detection of growth required a standard culture method using two or more types of plated media. Inoculation of media was followed by overnight incubation. Approximately 80% of all urine specimens submitted for culture are negative for probable pathogens; thus, much time and effort is spent processing negative cultures (11).

Many screening methods have been used to detect bacteria in urine. Some of these procedures are more rapid to perform than the standard plate culture method, and the presence or absence of growth may be determined within a few hours. Screening methods include direct microscopy of stained smears and a variety of chemical and physical methods (2, 4, 5, 9, 13, 15).

The Gram stain is a rapid, accurate, and inexpensive procedure to perform and has long been used as a guide to diagnose urinary tract infections. Correlations have been made between the number of organisms present in the Gram stain of uncentrifuged urine and infection (10). Microscopic examination of unstained urinary sediment has also been reported to be a valuable, sensitive procedure, especially in the diagnosis of staphylococcal urinary tract infections (12). Most of these microscopic methods have been reported to correlate with quantitative culture in 80 to 90% of the cases. Other rapid screening methods include measurement

of bacterial ATP by luciferase (2), detection of the potential generated by growing organisms by using a calomel and platinum electrode (electrochemical) (13), measurement of heat generated by metabolizing organisms (microcalorimetry) (4), and changes in electrical impedance (5).

More recently, three automated systems, Autobac (General Diagnostics), AutoMicrobic system (AMS; Vitek Systems, Inc.), and MS-2 (Abbott Laboratories), have been introduced that have the capability of detecting and/or identifying bacteriuria. All can detect bacteria in urine; in addition, the AMS has the ability to identify most organisms causing urinary tract infection. The detection of growth is based on changes in light transmission (photometry). Previous studies have reported the ability of each system alone to detect bacteriuria (1, 6, 7, 8, 11, 16); however, none has compared all three systems simultaneously. The purpose of this investigation was to compare these three systems under the same laboratory conditions with a standard semiquantitative culture plate method for accuracy, detection time, and cost.

MATERIALS AND METHODS

Specimens. One thousand clean-voided urine specimens from both in-patients and out-patients at the University of California Irvine Medical Center, Orange, were studied. Specimens were refrigerated at 4°C immediately after collection and were processed within 8 h.

Gram stain. Five microliters of well-mixed uncentrifuged urine was Gram stained and examined for the presence or absence of microorganisms. At least five oil immersion fields were examined. The presence of at least one organism per oil immersion field was considered a positive smear.

Reference procedure. A semiquantitative procedure, as described by Barry et al. (3), was used as the reference method. By using a calibrated platinum loop, 0.001 ml of a well-mixed urine specimen was inoculated onto a 5% sheep blood agar plate and a biplate consisting of MacConkey agar and polymyxin B-nalidixic acid blood agar (Cal Labs, North Hollywood, Calif.). Cultures were incubated at 35°C aerobically overnight and examined for the number and types of organisms present. Significant isolates were identified by standard biochemical procedures (14). Organisms considered contaminants were diphtheroids, lactobacilli, viridans streptococci not group D, or mixed cultures of two or more species.

All urines were divided into four categories based on the CFU per milliliter obtained by the reference method. Group I included all urines with colony counts of $>10^5$ CFU/ml; group II were those with colony counts of 10^4 to 10^5 CFU/ml; group III included specimens with colony counts equal to 10^3 to $<10^4$ CFU/ml; and group IV were those that were not detected by the reference method ($<10^3$ CFU/ml).

Automated procedures. Urine specimens were inoculated into the three systems, Autobac, AMS, and MS-2, according to the manufacturers' instructions. Autobac readings were taken at hourly intervals up to 5 h. At 1-h intervals, readings on the AMS were taken, and results were recorded when the system indicated that the specimen was positive and also at the time of final identification. At the time of final identification, overall quantitation with AMS ($\geq$70,000 CFU/ml) was provided. MS-2 automatically printed results when the specimen was positive.

Time and cost analysis. A study was conducted to determine the time necessary to achieve a positive urine by each instrument. The amount of time necessary to detect bacterial growth in urine specimens was determined for each of the four groups.

An analysis was done based on the cost of materials and technologists' time. Technologists' time was calculated based on the average time required to process 20 specimens by each system. The cost of materials was based on the actual costs of purchase for our laboratory. The cost of the instruments was not included in this analysis: Autobac, $28,500 (photometer and incubator shaker); AMS, $59,500 (diluent dispenser, filling module, reader-incubator, and a computer module with data terminal); and MS-2, $32,600 (contact module and analysis module).

Predictive value. Predictive values were calculated according to the method of Ransohoff and Feinstein (17).

RESULTS

Classification of the urine specimens. A total of 1,000 clean-voided urine specimens were evaluated (Table 1). Of these, 239 (23.9%) specimens were positive with colony counts of $>10^5$ CFU/ml by the reference method. Group II included 161 (16.1%) specimens with colony counts of 10^4 to 10^5 CFU/ml. The remaining 600 (60.0%) specimens had colony counts of $<10^4$ CFU/ml. Of these, 188 (18.8%) had colony counts equal to 10^3 to $<10^4$ CFU/ml (group III), and 412 (41.2%) specimens were below detectable limits by the standard plate culture method (group IV).

Group I. Of the 239 specimens in group I, 172 (72.0%) were detected by all methods, and 154 (64.4%) were pure cultures. Of these, 23.4% (36 of 154) were isolates of diphtheroids, lactobacilli, or viridans streptococci not group D. The 76.6% (118 of 154) cultures remaining contained probable pathogens which included gram-negative bacilli, group D streptococci, and *Staphylococcus* species. All systems were positive for 94.9% (112 of 118) of these cultures. Autobac detected 96.6% (114 of 118), whereas AMS and MS-2 detected 95.8% (113 of 118) of the isolates.

Twenty (8.4%) of these 239 isolates were not detected by any of the three systems. Sixteen (80%) of those not detected by the automated systems were mixed cultures containing two or more species. The remaining four contained pure cultures of probable pathogens.

Each of the systems did not detect three gram-negative bacilli (one *Escherichia coli* and two *Klebsiella pneumoniae*) and one *Streptococcus agalactiae*. In addition, AMS was unable to

TABLE 1. Number and percentage of positive and negative urine cultures detected by each method

Group (no. of specimens)	Concn (CFU/ml)	No. (%) of cultures that were					
		All systems positive	All systems negative	Autobac positive	AMS positive	MS-2 positive	Gram stain positive
I (239)	$>10^5$	172 (72.0)	20 (8.4)	204 (85.3)	198 (82.8)	179 (74.9)	207 (86.6)
II (161)	10^4–10^5	59 (36.6)	74 (46.0)	72 (44.7)	83 (51.5)	64 (39.7)	82 (50.9)
III (188)	$\geq 10^3 < 10^4$	19 (10.1)	108 (57.4)	32 (17.0)	68 (36.2)	28 (14.9)	44 (23.4)
IV (412)	$<10^3$	3 (0.7)	345 (83.7)	15 (3.6)	59 (14.3)	6 (1.4)	41 (10.0)

detect one group D streptococcus, and MS-2 missed a second strain of *E. coli*.

(i) Autobac positive cultures. The Autobac system detected 85.3% (204 of 239) of the urine specimens in group I. Of the 35 urine specimens not detected, 4 (11.4%) were pure cultures. When only probable pathogens were considered, the Autobac was able to detect 96.6% (114 of 118) of positive cultures.

(ii) AMS positive cultures. AMS detected 82.8% (198 of 239) of the urinary isolates in group I. Of the 41 specimens not detected, 5 (12.2%) contained pure cultures of probable pathogens. When only probable pathogens were considered, AMS detected 95.8% (113 of 118) of the positive cultures.

(iii) MS-2 positive cultures. If all isolates are considered, the MS-2 detected 74.9% (179 of 239) of cultures in group I. Of the 60 specimens not detected, 5 (8.3%) contained pure cultures of probable pathogens. Considering probable pathogens only, the MS-2 system detected 95.8% (113 of 118) of the isolates.

(iv) Gram stain. The Gram stain detected 86.6% (207 of 239) of the isolates in group I. Of the 32 specimens not detected by the Gram stain, 3 (9.4%) were pure cultures of probable pathogens (one *E. coli*, one group D streptococcus, and one *S. agalactiae*). The Gram stain detected 97.4% (115 of 118) of the urine cultures with significant isolates.

(v) Detection times. In group I, the detection times for Autobac and MS-2 were similar. The Autobac detected 16 cultures more rapidly than the other systems, whereas AMS detected one culture more rapidly. The average detection time for MS-2 was less than that of the other systems, and it detected 27 cultures more rapidly than either Autobac or AMS.

Table 2 lists pure cultures of probable pathogens in group I. *E. coli*, the most common cause of urinary tract infection, represented 75.4% (89 of 118) of the pure culture isolates. The remaining 24.6% (29 of 118) of the isolates included other gram-negative bacilli, staphylococci, and streptococci. The average detection times for group I ranged from 1.3 h for detection of *E. coli* by MS-2 to 8.7 h for detection of *Pseudomonas aeruginosa* by AMS.

The majority of probable pathogens were detected within 2 h with Autobac (79.8%) and MS-2 (78.8%) (Table 3). At this time, the percentage of contaminants detected was <43% for both systems. A 4-h reading increased the number of true positive urines to 100% with Autobac and 98.2% with MS-2. Along with the greater number of true positive urines at 4 h, the contaminants detected at 4 h increased to >75%. In contrast, AMS identified 76.1% of the true positives and 44.7% of the contaminants by 5 h, and it was not until ≥9 h that all of the probable pathogens were detected. Therefore, with all systems, although an early reading (2 h for Autobac and MS-2; 5 h for AMS) would identify the majority of positives with a minimum number of false-positives, approximately 20% of the true positives would be missed.

Group II. Group II comprised 161 (16.1%) specimens and included those urine cultures with colony counts of 10^4 to 10^5 CFU/ml (Table

TABLE 2. Group I: range and average detection times of probable pathogens

Organism	No. of isolates	Avg detection time (h)					
		Autobac		AMS		MS-2	
		Range	Mean	Range	Mean	Range	Mean
Escherichia coli	89	1.0–3.0	1.7	2.0– 8.0	3.9	0.5–3.2	1.3
Klebsiella pneumoniae	12	1.0–4.0	1.8	2.0–13.0	5.6	0.5–2.8	1.6
Enterobacter species	5	2.0–3.0	2.4	4.0–12.0	5.6	1.3–4.5	1.8
Pseudomonas aeruginosa	3	4.0	4.0	2.0–11.0	8.7	3.2–4.4	3.5
Staphylococcus species	6	2.0–4.0	3.1	2.0– 9.0	5.0	3.2–4.5	3.5
Enterococci	3	2.0–3.0	2.3	3.0– 1.0	5.3	1.3–4.5	2.8

TABLE 3. Group I: detection times of pathogens[a] versus contaminants[b]

Hour	Autobac				MS-2				AMS			
	Pathogen		Contaminant		Pathogen		Contaminant		Pathogen		Contaminant	
	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
≤2	79.8	91	36.7	33	78.8	89	42.4	28				
3	94.7	108	48.9	44	95.6	108	60.6	40				
4	100.0	114	75.5	68	98.2	111	84.8	56				
5			100.0	90	100.0	113	100.0	66	76.1	86	44.7	38
6									84.1	95	49.4	42
7									88.5	100	55.3	47
8									92.9	105	61.2	52
≥9									100.0	113	100.0	85
Total	114		90		113		66		113		85	

[a] Pure culture of a probable pathogen.
[b] Pure culture of viridans streptococci, diphtheroids, lactobacilli, or a mixed culture.

1). Forty-six percent (74 of 161) of these cultures were negative by all three automated systems, whereas all the test methods detected 59 (36.6%) of isolates in this group. In addition, Autobac detected 72 (44.7%) of the specimens in this group; 83 (51.5%) were positive by AMS, 64 (39.7%) were positive by MS-2, and 82 (50.9%) were positive by Gram stain. Of the 161 isolates in this group, 22 (13.7%) were pure cultures. Pure cultures represent 37.3% (22 of 59) of the cultures detected by all test methods. The Autobac and MS-2 systems detected 90.9% (20 of 22) and AMS detected 86.4% (19 of 22) of pure cultures of probable pathogens in this group. Autobac did not detect one *P. aeruginosa* and one *S. agalactiae*; MS-2 failed to detect one *P. aeruginosa* and one enterococcus; and AMS did not detect two *S. agalactiae* and one enterococcus. The average detection times for all isolates in group II ranged from 3.8 h with MS-2 to 8.9 h by AMS. The range of detection times of probable pathogens was from 2.8 h with MS-2 to 12.3 h with AMS (Table 4). All of the pure cultures of gram-negative bacilli were detected within 4 h by Autobac and MS-2.

Group III. Group III included 188 specimens with colony counts equal to 10^3 to $<10^4$ CFU/ml (Table 1). Since the reference method can detect organisms at a level of $\geq 10^3$ CFU/ml, this group was established. Of the 19 (10.1%) cultures detected by all three systems, 9 (47.4%) represented pure cultures of gram-negative rods or streptococci. In this group, the least number of false-positives was detected by MS-2.

Group IV. The reference method cannot detect colony counts of $<10^3$ CFU/ml. However, all three automated systems detected 3 (0.7%) of the 412 cultures in this group.

The majority of positive cultures in groups III and IV were detected within 5 h by MS-2 and Autobac and more than 5 h by AMS.

Predictive value. Table 5 shows the positive and negative predictive values for the four screening methods. The data shown were calculated in two ways, depending on the definition of significant bacteriuria. One definition treats all positive urines as those with colony counts of $>10^5$ CFU/ml regardless of the type or variety of bacteria present. This category was necessary since the urine screens cannot identify or differentiate between all of the organisms present. The alternate way of viewing the data is to consider as positive only those urines with a pure culture of a probable pathogen with a colony count of $>10^5$ CFU/ml.

When all urines containing $>10^5$ CFU/ml,

TABLE 4. Group II: range and average detection times of probable pathogens

Organism	No. of isolates	Avg detection time (h)					
		Autobac		AMS		MS-2	
		Range	Mean	Range	Mean	Range	Mean
Escherichia coli	10	1.0–5.0	3.4	4.0– 9.0	7.1	1.5–4.1	3.0
Klebsiella pneumoniae	1	3.0		10.0		3.1	
Proteus spp.	1	4.0		12.0		3.8	
Serratia spp.	1	3.0		9.0		2.8	
Pseudomonas aeruginosa	3	4.0–5.0	4.3	4.0–12.0	8.3	3.8–4.8	4.5
Streptococcus spp.[a]	6	4.0	4.0	7.0–13.0	12.3	2.8–3.9	3.4

[a] Groups B and D.

TABLE 5. Predictive values for the urine screens[a]

Determination	Predictive value (%)							
	Autobac[b]		AMS[c]		MS-2[b]		Gram stain	
	All organisms	Pure pathogens	All organisms	Pure pathogens	All organisms	Pure pathogens	All organisms	Pure pathogens
Sensitivity	85.3	96.6	82.8	95.8	74.9	95.8	86.6	97.4
Specificity	84.4	76.4	72.4	66.6	87.1	81.4	78.0	70.6
Predictive positive	63.1	35.2	48.5	27.7	64.6	40.8	55.3	30.7
Predictive negative	94.8	99.4	93.1	99.2	91.7	99.3	94.9	99.5

[a] Data are calculated based on two definitions of a positive culture: all urines with organisms of $>10^5$ CFU/ml (all organisms); and urines with one probable pathogen of $>10^5$ CFU/ml (pure pathogens).
[b] Final reading at 5 h.
[c] Final reading at 13 h.

TABLE 6. Cost analysis for detection of growth

Determination	Cost ($/specimen)				
	Culture method	Autobac	AMS	MS-2	Gram stain
Cost of supplies	0.67	0.13	2.93	0.40	0.03
Cost of technologists' time[a]	0.80	0.40	0.30	0.20	0.44
Total cost	1.47	0.53	3.23	0.60	0.47

[a] At $12.00/h, assuming 4.0, 2.0, 1.5, 1.0, and 2.2 min/sample for the standard culture method, Autobac, AMS, MS-2, and Gram stain, respectively.

regardless of the type or variety of organisms present, are considered, the Gram stain was the most sensitive method of detection (86.6%), whereas MS-2 was the least sensitive (74.9%). However, if specificity is taken into account, MS-2 was the most specific (87.1%), and AMS was the least specific (72.4%). When only those urines containing a pure culture of a potential pathogen of $>10^5$ CFU/ml are considered as positive, then all of the urine screens had a sensitivity >95%. MS-2 was as sensitive as the other methods and more specific in predicting a positive, for it did not detect as many false-positive urines. All urine screens detected a negative urine >99% of the time if only pure cultures of pathogens are considered as true positives. However, if a urine is positive by one of the screening methods, approximately 50 to 60% of them will actually be a false-positive.

Cost analysis. The total cost of growth detection and identification for each system, including supplies and time, was determined (Tables 6 and 7). In addition to detection of growth, the AMS provides a limited identification with no additional technologist time or material. In determining the cost for identifying a positive urine (Table 7), the Gram stain was the least expensive screening method. Autobac and MS-2 were the least expensive screening systems, whereas AMS was the least expensive identification system.

DISCUSSION

Clean-voided urines with bacterial counts of $>10^5$ CFU/ml of a pure culture are most likely to represent an infection, whereas those with counts of $<10^5$ CFU/ml may signify contamination (10). Based on these guidelines, we found all

TABLE 7. Cost analysis for identification of positive urine cultures

Determination	Cost ($/specimen)			
	Culture method	Autobac	AMS	MS-2
Total cost of growth detection	1.47	0.53	3.23	0.60
Supplies for plating positive cultures	0.00	1.47	0.00	1.47
Cost of identification	1.25	1.25	0.00	1.25
Cost of technologists' time[a]	1.20	1.20	0.00	1.20
Total cost of detection and identification	3.92	4.45	3.23	4.52
Difference between negative and positive urine	2.45	3.92	0.00	3.92

[a] At $12.00/h.

three automated systems to be comparable in their ability to detect significant bacteriuria.

In this study, the sensitivity of the automated methods for detection of all organisms with colony counts of $>10^5$ CFU/ml was $\geq$75%, and the specificity was >72%. In addition, we calculated the sensitivity, specificity, and predictive value of cultures containing only pure pathogens of $>10^5$ CFU/ml. Although this information has not been presented in previous studies, we feel that it is of value to clinical laboratories. The sensitivity of all automated methods for the detection of pure pathogens was >95%, whereas the specificity ranged from 67 to 81%. These findings correlate with the study of Kelly and Balfour, who found a sensitivity of 97% for Autobac (11).

Considering only pure cultures of probable pathogens, all methods had a >99% chance of predicting a negative urine, whereas the ability to predict a positive urine ranged from 28 to 41%. It is obvious from these data that the screening methods excel in their ability to detect a negative urine; therefore, they are extremely useful in the clinical laboratory, where 70% of urine cultures are negative.

Although it has been reported that 10^5 CFU/ml is the dividing line between contamination and infection, in certain instances pure cultures of probable pathogens with counts between 10^4 and 10^5 CFU/ml may cause infection (10). For this reason, it may be important for these instruments to have the capability of detecting pure cultures of probable pathogens in urine specimens with low counts. The instruments detected about 50% of the cultures with colony counts of 10^4 to 10^5 CFU/ml (group II). Of the cultures detected, approximately 85% either were mixed or contained a pure culture of a contaminant. The remaining 15% were pure cultures of probable pathogens of which the majority (>95%) were detected by all instruments. Our findings of a high positive rate in this group agrees with previously published data (11).

Urine cultures with colony counts of $<10^4$ CFU/ml are usually obtained from individuals without urinary tract infections (12). In this study, 60% of the urines tested had colony counts of $<10^4$ CFU/ml (groups III and IV). AMS detected the highest number of false-positives, whereas the least number (6%) of false-positives was detected by MS-2.

The Gram stain proved helpful as a urine screen in this study, as has been reported by others (9, 10, 12, 15). Kass reported that Gram-stained smears of uncentrifuged urines, with at least one organism per oil immersion field, were positive in 80% of urine specimens with colony counts of $>10^5$ CFU/ml (10). In this study, 97% of the pure cultures of probable pathogens in group I were positive by the Gram stain. The sensitivity and specificity of the Gram stain were comparable to those obtained by the automated instruments for groups II, III, and IV.

Our findings on the detection time with Autobac agree with those previously reported by Jenkins et al. (8). In their study, a 3-h reading with Autobac detected up to 75% of positive urines. From our data, if a reading was taken with Autobac and MS-2 at 2 h, the majority (80%) of potential pathogens of $>10^5$ CFU/ml would be detected; however, certain pathogens, especially *P. aeruginosa*, would be missed. Increasing the detection time to 4 h would eliminate the majority of false-negatives. However, since the average detection times for groups II to IV ranged from 3.8 to 4.8 h, a 4-h reading would also increase the number of false-positives to >75%. Detection of the majority of positive urines was slower with AMS. By 5 h only 76% of pathogens would be detected, and an 8-h reading would have to be included to increase the detection rate to 93%.

Cost effectiveness is an important factor in laboratories, especially when considering instrumentation. The initial cost of the instruments is high, but is recovered by eliminating the plating of negative urines. We found a cost savings of approximately $0.50/specimen by screening urine cultures with Autobac and MS-2. The cost of AMS was approximately 30% greater than that of the reference method; however, this includes final identification of most positive specimens. This is in agreement with Nicholson and Koepke, who found AMS to be more costly than the conventional method (16).

In conclusion, the most important contribution of automated screening methods to patient care is in their ability to obtain rapid results. In our study, the results of the three systems were available in 1 to 13 h compared to 18 to 24 h by the standard procedure. When considering pure pathogens of $>10^5$ CFU/ml, all systems had sensitivities >95% and could predict a negative urine in >99% of the cases. Autobac and MS-2 are time-saving and cost-effective methods compared with the reference method. The advantage of the AMS is that it can simultaneously identify and detect isolates in positive urines in much less time than the reference method, but it is still more costly. Using an automated system to screen urines provides an overall time savings to clinical microbiology laboratories; however, each institution should evaluate its needs before making a commitment to any one system.

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