Evaluation of New Urinary Tract Infection Screening Devices

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Several new methods for detection of bacteriuria were studied to evaluate their usefulness as screening procedures. A new filter paper device incorporating dehydrated media and tetrazolium was found to be reliable when compared with the standard pour plate method in the laboratory and with the dip-slide method in a field test. It failed to detect yeasts and slowly growing streptococci. Antibiotics blocked the test when susceptible organisms were present. An agar-cup method was found to be quite reliable, but could be improved by use of differential media. The Griess test was confirmed in a small trial to be highly specific when used in conjunction with a first morning specimen, but of little value with random specimens. Phenzopyridine was found to give false positive reactions. The subnormal glucose test, although highly sensitive and specific, gave too many false positive tests to be useful other than as a screening method.

The availability of numerous simple quantitative bacteriological methods suitable for office and survey work should enhance the ability of physicians to screen high-risk populations for bacteriuria, aid in diagnosis of cases, and improve follow-up of patients with recurrent infection.

In addition to bacteriological methods (2, 3, 5, 7, 8, 10–12), chemical screening tests have been devised based upon reduction of nitrate to nitrite (the Griess test) by bacteria (4, 6, 9, 18–20), removal of residual amounts of glucose in the urine by bacterial action (7, 13, 15), presence of enzymes in urine derived from bacteria or inflammatory cells such as catalase, and reduction of tetrazolium salts by bacteria after standardized incubation of voided urine (4, 16). In general, most culture methods have the advantage of being both highly sensitive and specific, providing presumptive identification and permitting subculture to obtain antimicrobial susceptibility information. The chemical tests have the advantage of providing more rapid results, but are often less reliable. The ideal test would incorporate the best features of both classes of test and provide rapid antimicrobial susceptibility information as well.

This report deals with clinical and laboratory evaluation of four new tests designed to estimate quantitatively the number of bacteria in urine or to detect nitrite or low levels of glucose in urine.

MATERIALS AND METHODS

Filter paper culture-tetrazolium test combined with nitrite indicator. A clear plastic strip (3.25 by 0.4 in, about 8 × 1 cm) with one chemical reagent pad and two pads containing dehydrated culture media (1 cm²) (Ames Co., Elkhart, Ind.) attached in series near one end (Fig. 1) was used for the filter paper culture-tetrazolium test with nitrite indicator. The chemical reagent pad, designed to detect traces of nitrite in urine, turns bright pink within a few seconds of contact. The proximal medium pad contains an inhibitor of gram-positive organisms; the distal pad supports growth of both gram-positive and -negative bacteria commonly found in urinary tract infections. The nature of the formulations used in the culture and reagent pads has not as yet been released and is considered proprietary at this time. The media pads contain colorless tetrazolium which, when reduced in the presence of bacterial multiplication, produces discrete red spots on the pad. The density of the spots is then used to indicate the number of bacteria originally inoculated onto the pad. The actual test consists of dipping the device in urine, placing it in a plastic envelope, and expressing air from the envelope, followed by overnight incubation at 37 C.

Filter paper urinary nitrite indicator. A strip of filter paper impregnated at one end with a proprietary formulation of reagents (Bac-U-Dip, Warner-Chilcott Laboratories, Morris Plains, N.J.) to detect traces of nitrite was used. As with the previously
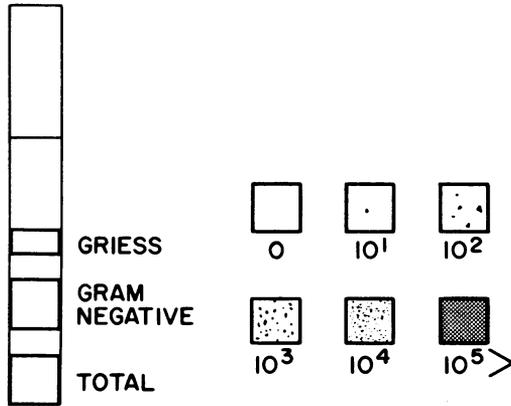
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FIG. 1. *Filter paper-tetrazolium culture and Griess test dip-stick. Density of reduced tetrazolium precipitates shown at the right are used to quantitate bacteria present in the urine.*

described test, a pink color appears within a few seconds of contact.

Low urinary glucose indicator stick. A commercial adaptation (Uriglox, KABI Laboratories, Stockholm, Sweden) of the method described by Schersten and co-workers (17) was used. It consists of a strip of filter paper impregnated at the proximal end with reagents which detect concentrations of glucose greater than 1 to 1.5 mg per 100 ml of urine. The paper strip is contained within a clear plastic envelope. For use, the plastic is stripped and the distal end is placed into the urine. The plastic allows contact of fluid with only the distal end. Fluid then ascends the strip by capillary action and interfering substances are removed by absorption to the treated paper. When sufficient glucose is present, a blue color is developed when the fluid reaches the reagent impregnated end in about 6 to 10 minutes. A positive test is evidenced when the paper fails to change color, indicating subnormal concentrations of glucose presumably due to consumption by bacteria of the small amount of glucose normally present in urine.

Agar-cup quantitative culture method. The agar-cup system (Speci-Test bacteriuria test, Ross Laboratories, Columbus, Ohio) consists of a plastic cup .75 in (about 1.9 cm) tall and 2 in (about 5 cm) at the base (Fig. 2). Nutrient agar is contained in a 1.5 in (about 3.7 cm) shallow well and held in place by a circular plastic rim. The cup is sealed by a metal foil at the top and by an internal clear plastic shield which covers the agar surface. For use, the seals are removed and urine is poured into the cup. The cup is then drained, set on its side to permit excess urine to drain away, and incubated overnight at 37 C. The density of colonies on the agar surface is then compared with photographic standards to judge the colony count.

Dip-slide method. A commercial adaption (Uricult, Orion Laboratories, Helsinki, Finland) of the method of Guttman and Naylor (7), with a recent model which substitutes a plastic surface for glass, was used. The medium on one side is McConkey agar and on the other is nutrient agar.

Pour plates. Serial dilutions of urine in physiological NaCl solutions were prepared by using serologic pipettes. Then 0.1 ml was added to petri dishes and thoroughly mixed with molten Trypticase soy agar. The plates were incubated at 37 C overnight and colonies were counted the next day. The final dilutions tested were 10^{-1}, 10^{-3}, and 10^{-5} per ml of urine.

Definitions. These are the standard definitions used by the World Health Organization (21): sensitivity = diseased persons with positive test/all persons in population with disease; specificity = nondiseased persons with negative test/all persons in population without disease.

Evaluation of the various methods for detecting bacteriuria was conducted with urine specimens submitted for routine culture at the University of Wisconsin and Madison Veterans Administration Hospitals and during the course of a field study of the prevalence of bacteriuria among young women in Kampala, Uganda. The hospital studies were designed to permit comparison of the rapid culture methods with the standard pour plate technique. The field study utilized first morning specimens to enable evaluation of
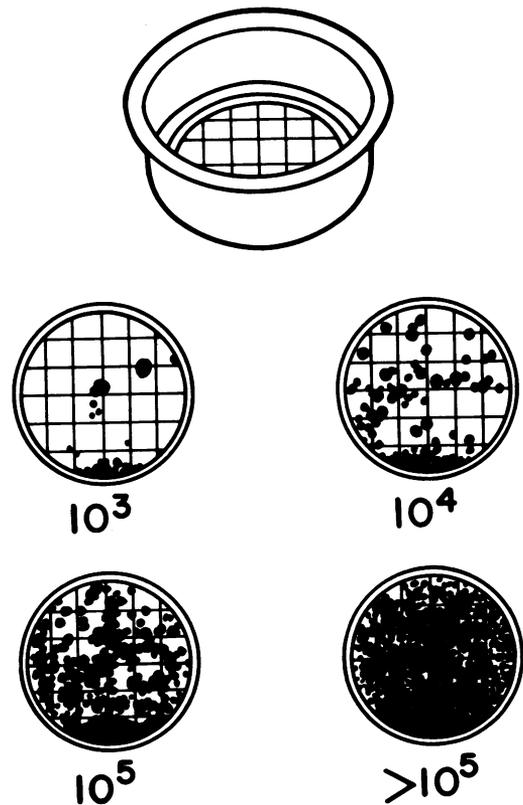

FIG. 2. *Illustration of the agar-cup quantitative culture method. Only one medium is used. Counts are estimated by comparison of colony densities obtained after incubation.*

the chemical methods in relation to the dip-slide method. Both the nitrite (Griess) and glucose tests require that urine be incubated overnight in the bladder so that bacteria have sufficient time to reduce diet-derived nitrate to nitrite or metabolize the small amount of glucose normally present in urine.

In the hospital study, all urine specimens brought to the clinical diagnostic laboratory were immediately refrigerated and then tested within 24 h of receipt. In the field study, clean, voided urine specimens were tested within 30 min to 1 h of collection without refrigeration.

RESULTS

Chemical methods: the Griess test. Results were identical with both of the nitrite test methods studied. Accordingly, results are considered together. First morning specimens were obtained from 405 young women, 78 of East Indian extraction and the rest indigenous Africans. Repeat urine specimens were obtained from all individuals whose first specimen contained 100,000 or more bacteria per ml of urine by the dip-slide method. Subjects were considered to have significant bacteriuria only if this count was obtained on two consecutive specimens. The relation between positive tests for nitrite and significant bacteriuria by this definition is shown in Table 1. The nitrite test was positive in the initial test for six cases in whom significant persistent bacteriuria was documented and in five of the six when the test was repeated. There were no false positives.

In the hospital study, the nitrite test was positive in 136 (42.0%) of 324 specimens with 10^5 or more colonies per ml by the pour plate method. The majority of samples submitted to the hospital laboratories, however, were not first morning specimens. There were six patients that had a positive nitrite test in the absence of significant bacteriuria. All six patients were receiving phenzopyridine (Pyridium) when these specimens were submitted for routine culture. Two additional patients were repeatedly studied while receiving phenzopyridine, and their urine samples consistently gave posi-

TABLE 1. *Sensitivity and specificity of nitrite indicator dip-sticks (Griess test)*[a]

Test results	Actual results	
	Positive	Negative
Positive	6	0
Negative	0	399

[a] Two consecutive positive urine cultures containing 10^5 or more bacteria in the first morning specimens were taken from 405 individuals. Both sensitivity and specificity are 100%.

TABLE 2. *Sensitivity and specificity of dip-stick test for low levels of glucose in urine*[a]

Test results	Actual results	
	Positive	Negative
(A) Initial study		
Positive (no detectable glucose)	7	17
Negative (glucose)	0	418
(B) Repeat study[b]		
Positive (no detectable glucose)	6	2
Negative (glucose)	0	10

[a] Cultures were obtained as in Table 1. For test A, sensitivity = 100%, specificity = 96.1%. For test B, sensitivity = 100%, specificity = 83.3%.

[b] Performed on cultures from 18 of the 24 subjects whose initial test revealed no glucose.

tive nitrite tests in the absence of bacteriuria. Commonly used antimicrobial agents had no effect on the nitrite test. Urine from six patients receiving various preparations of nitrites for angina pectoris gave a negative test.

The Griess test reagent pad was found to detect as little as 0.6 μg/ml of $NaNO_3$ diluted in water. Pads tended to discolor when left in room air overnight. Pads inoculated in urine tended to turn pink after a few minutes; therefore reading was not found to be valid if delayed beyond this point.

The glucose test. The glucose test was evaluated in a slightly larger sample of the same population in Uganda studied with the Griess test (Table 2A). Both sensitivity and specificity of the method were good. The large number of false positive tests, however, prevented definitive diagnosis in any individual case. Even though sensitivity and specificity continued to be good when those with initially positive tests were restudied on a second occasion (Table 2B), two individuals continued to give a false positive test. The value of the glucose test as a reliable screening method was greatly diminished by the relatively high proportion of false positive tests.

Culture methods: filter paper culture-tetrazolium test. A comparison of bacterial counts obtained with this and the pour plate method in 1,000 consecutive urine specimens submitted for routine culture is shown in Table 3. Identical counts were obtained in 780 samples (78.0%). By using the criterion of less than 10^5 as negative and 10^5 or more colonies per ml as positive, the sensitivity of the new method was 90.7 and specificity was 99.1%. There were 30 instances, however, in which counts of 10^5 or more by the pour plate method were accom-

TABLE 3. *Comparison of total bacterial counts obtained with the standard dilution pour plate method and the filter paper culture-tetrazolium test[a]*

Filter paper (colonies/ml)	Pour plate (colonies/ml)[b]							
	<10	10	10^2	10^3	10^4	10^5	10^6	$\geq 10^7$
<10	<u>265</u>	28	23	18	4	5 (1)	4	1
10	4	<u>34</u>	21	8	3	0	0	0
10^2	1	4	<u>53</u>	19	4	0 (2)	0	1
10^3	1	1	4	<u>69</u>	22	1	2	0
10^4	0	0	0	9	<u>63</u>	6 (9)	6	4
$\geq 10^5$	0	0	0	2	2	<u>34 (2)</u>	63	197

[a] Counts were taken from 1,000 urine specimens. ( ) indicates number of specimens with counts of 50,000 to 90,000 colonies per ml.

[b] Genera and species encountered in 324 cultures containing 100,000 colonies or greater on pour plate included *Escherichia coli* (54.5%), *Klebsiella-Enterobacter* (22.1%), *Proteus* (11.9%), *Pseudomonas* (4.5%), *Staphylococcus* (1.3%), *Enterococcus* (3.2%), *Candida* (2.2%), and others (0.3%). A similar distribution was encountered in the other studies.

panied by lower counts with the filter paper test. Fourteen of these were clearly missed by the new method (counts were 10^3 or less). Examination of the identity of the organisms missed revealed most of them to be yeasts whose growth is not supported by the filter paper media, or small colonies of slowly growing streptococci. Enteric bacteria commonly encountered in urinary infection were rarely missed.

Field studies were also conducted comparing the filter paper with the dip-slide method. Data on the relative ability of the two methods to detect gram-negative bacteria are given in Table 4. The methods correlated well with a relative sensitivity of 83.3 and specificity of 99.6% for the filter paper method.

Effect of antibacterial agents on filter paper culture-tetrazolium test. An overnight culture of a strain of *Escherichia coli* susceptible to a wide variety of antibiotics was diluted to a concentration of 10^5 colonies per ml and incubated with antibiotics at a final concentration of 50 µg/ml in Trypticase soy broth. Samples were removed immediately and at intervals up to 24 h and cultured by the filter paper and pour plate methods (Fig. 3). Major differences were observed between the two culture methods. All of the drugs except sulfamethoxazole (SMZ) and trimethoprim (TMP) were found to completely block growth of bacteria in the filter paper culture. Pour plate culture, however, revealed the expected gradual reduction in bacterial counts with time for most drugs and a bacteriostatic effect in these media with SMZ and TMP.

This effect is believed to be due to retention of antibiotics in the filter paper as opposed to dilution to subinhibitory levels in the pour plates. The notion is supported by the observa-

TABLE 4. *Comparison of counts of gram-negative bacteria obtained with the dip-slide method and the filter paper-tetrazolium test[a]*

Filter[b] paper (colonies/ml)	Dip-slide (colonies/ml)					
	<10^3	10^3	10^4	10^5	10^6	10^7
<10	362	1	1			
10	61	2	1			
10^2	71	5	1			
10^3	17	<u>12</u>	2			
10^4	3	1	<u>10</u>	3	1	
$\geq 10^5$	1		1	2	5	13

[a] A total of 577 first morning clear urine specimens were collected under field conditions.

[b] One specimen was difficult to interpret.

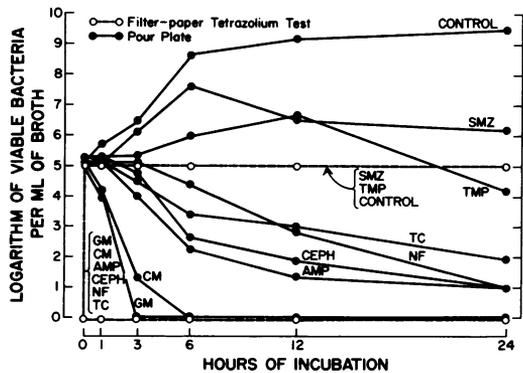

FIG. 3. *Effect of various antibiotics at concentrations of 50 µg/ml on bacterial counts obtained with a sensitive strain of Escherichia coli with the filter paper and pour plate culture methods. Cultures were incubated at 37 C and samples were removed at intervals after addition of the antibiotics.*

tion that pour plates in which urine was diluted only 1:10 often gave lower total counts than plates diluted to 1:100 or 1:1,000.

TABLE 5. *Comparison of total bacterial counts obtained with the standard dilution pour plate method and the agar-cup test*[a]

Agar-cup (colonies/ml)	Pour plate (colonies/ml)				
	$<10^3$	10^3	10^4	10^5	$\geq 10^6$
$<10^3$	<u>209</u>	16	3	0	0
10^3	20	<u>22</u>	10	0	1
10^4	5	15	<u>24</u>	3 (1)	3
10^5	0	1	13	<u>10 (4)</u>	10
$\geq 10^6$	1	1	3	17	<u>108</u>

[a] Counts were obtained from 500 urine specimens. ( ) indicates five specimens with counts of 50,000 to 90,000 colonies per ml.

Agar-cup culture method. A comparison was made of total bacterial counts in urine obtained with this method and the standard pour plate method (Table 5). The overall correlation was good. Identical results were obtained in 74%. The sensitivity and specificity of the method in distinguishing counts of 10^5 or more from lower levels was 95.4 and 93.4%, respectively. All of the false negatives with this test were with common enteric organisms rather than unusual organisms missed by the filter paper test. The three markedly false positive tests were due to spreading colonies of *Proteus* species. Preliminary identification of species was not possible with the single medium used.

DISCUSSION

The development and availability of many new, inexpensive urine culture devices should improve the ability of physicians to detect and manage urinary tract infections. One can now choose between pour and streak plate methods or use the dip-slide or one of its modifications, the pipette, spoon, paddle, agar cup, or similar methods with about equal confidence. All appear to give results which correlate quite well with standard quantitative methods used in the diagnostic bacteriology laboratory. Those methods containing differential media should be preferred because gram-positive organisms are often contaminants. As with any culture method, the validity of the results will largely depend on how the specimen was obtained and stored before testing. Much of the problem of delay in culture may be avoided, however, by inoculating the specimens in the office or on the wards and then transporting the growing cultures to the laboratory for incubation and definitive interpretation. Subcultures are readily made for identification and antimicrobial susceptibility tests. The ideal culture method would provide simultaneous information on both the quantitative count and antimicrobial susceptibilities.

The filter paper-tetrazolium culture method differs from most of the other tests in that individual colonies cannot be subcultured. It does, however, have the advantage of good storage properties and very little bulk. It may prove to be particularly popular with physicians for routine screening of large numbers of patients or for follow-up of known cases. It must be emphasized, however, that yeasts and slowly growing streptococci will not be detected by this method. The effect of most antimicrobial agents in blocking growth of bacteria in this system should not often lead to erroneous tests, because only agents active against the organism block the test. Nevertheless, this phenomenon must be taken into account for proper interpretation of results in patients receiving antibacterial drugs. The Griess test must also be recognized to give false positive tests in patients receiving phenzopyridine, but the commonly used antimicrobial agents do not appear to interfere.

The use of the filter paper-Griess test together with a culture method (either incorporated together or separately) should be helpful in screening programs and follow-up of patients. As shown in this and other studies (4, 6, 9, 18–20), when used with a first morning specimen which the patients can test or bring to the office, a positive Griess test strongly suggests the presence of urinary infection. The Griess test is clearly not useful for testing randomly collected specimens where the duration of incubation in the bladder is unknown. It must also be emphasized that the test requires that sufficient dietary sources of nitrite be available. This is usually not a problem when a customary American diet is consumed. Hospitalized patients receiving parenteral nutrition without a nitrogen source may not have nitrate in their urine. The concentration of bacteria must be well in excess of 100,000 per ml. Dilution of the urine or slow growth of organisms will give a negative test. Most of the organisms which commonly produce urinary infections (aerobic enteric bacteria and staphylococci) are able to reduce nitrate, but streptococci, gonococci, and *Mycobacterium tuberculosis* will not (9).

The test for residual glucose in the urine seems much less promising because of the rather large number of false positive tests. This requires extra effort to rule out individuals who eventually will turn out to have negative cultures. Some authors report no false positive tests with the glucose method (15), but seven false positives were observed among 304 subjects with no significant bacteriuria in the

original study of Schersten and Fritz (17). Thus, this phenomenon is not unique to the population of indigenous Africans or East Indians examined in this study.

The rapid culture and chemical methods lead themselves to self-screening by patients and may greatly decrease the logistical problems of mass screening programs. The most efficient screening program might be one in which the patient bathes carefully at home, passes a first morning specimen, and tests the urine with both a Griess reagent strip and one of the simple culture methods. This procedure would theoretically weed out most of the nonbacteriuric patients and permit concentration of efforts on confirmation of the positives. It remains to be determined by actual field trial, however, whether this can be effectively done and whether self-reading of the Griess test by the patient is practical.

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