Detection of Urinary Tract Infections in Pregnant Women

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CONSIDERABLE EVIDENCE exists that asymptomatic infections of the urinary tract are much more frequent than was realized only a few years ago (*1–13*). Among pregnant women in particular, prevalence rates have ranged as high as 12 percent (*14*). Such infections are a hazard to mother and fetus in that particular pregnancy and may predispose to hypertension, chronic renal disease, and other medical problems of later life (*3, 4, 6–8, 10–12*). It is obvious that if an abnormality is common and potentially serious, it should be sought routinely. Such detection demands simple, reliable, and inexpensive techniques. Several such methods have been evaluated and compared in this study. One of them fulfills the desired criteria for mass or routine screening for bacteriuria.

In recent years many papers have appeared on methods for assessing the presence of urinary tract infection (*1, 2, 15, 16*). Likewise, methods for collecting urine which are easier and safer but as reliable as catheterization have been investigated. Evidence is strong for the validity of the quantitative culture of a clean voided specimen (*1, 5, 9, 16–20*). The standard reference method for quantitative urine-culturing is the pour plate made with appropriate dilutions of properly collected urine. In our study the pour plate was compared with the gram-stained smear of a drop of uncentrifuged urine, the streak plate made with a calibrated platinum bacteriologic loop, and with the catalase filter-paper-disk-flotation test.

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Materials and Methods

Population surveyed. Patients were surveyed at five of the prenatal stations operated by the Chicago Board of Health. Women attending these clinics were almost all pregnant, chiefly Negro, and from the lower income strata. All women registering in the five stations from July 13, 1963, to September 23, 1963, were tested on their initial visit. From 986 women, 1,156 urine specimens were collected.

Collection of urine specimens. The patient, disrobed from the waist down, is seated on a bedpan placed on an examining table. After the patient has spread her labia majora manually, the nurse cleans the vestibule with Phisohex (R) solution and sterile gauze sponges and rinses it with sterile water. The patient then begins to urinate. The first portion of urine is allowed to pass into the bedpan; the next 3 to 4 ounces are collected in a sterile plastic container, which is then capped and immediately placed in an ice chest for transportation to the central laboratory.

Initially, some difficulty occurred in inducing patients to urinate under these conditions. This difficulty was overcome by two simple changes in procedure. Those women with the urge to urinate were taken first. The others were given a glass of water, and if the persons collecting the urine were matter of fact about the whole procedure, adequate specimens could be collected from almost all the women shortly thereafter.

Bacteriological procedures. The four bacteriological procedures being compared were initiated within 3 hours of the collection of specimens.

1. Pour plates of trypticase soy agar were made in the usual manner using 0.01 and 0.001 ml. of urine. After incubation for 20 hours at 37° C. the colonies were counted with a Quebec colony counter.

2. Streak plates of trypticase soy agar were made, using platinum bacteriologic loops calibrated to deliver 0.01 and 0.001 ml. of urine. The flamed loops were dipped in the urine and a drop streaked on each half of the agar plate. Incubation and colony count were as described for the pour plates.

3. Gram-staining was done in the usual manner; that is, 0.05 ml. of uncentrifuged urine was transferred to a slide and air dried, providing an area of about 12 mm.^{2} of sediment. Ten oil-immersion fields were examined and the slides classified as to whether they showed 0, 1 to 5, or more than 5 organisms per oil-immersion field. The urine samples were also evaluated for pyuria by counting the number of leucocytes per oil-immersion field. Although this procedure is not the best one for evaluating pyuria, it was deemed worthwhile in view of the minimal added expense and effort.

4. The catalase flotation test was performed according to published directions (*21*). Disks of filter paper were saturated with 10 drops of urine and placed in 16 mm. internal-diameter test tubes which had been acid washed and made chemically clean. Five ml. of 3 percent hydrogen peroxide was added, and the time required for the filter-paper disk to float to the surface was recorded. The test was read as negative if flotation had not occurred by 90 minutes.

Additional cultures. Second and occasionally third cultures were obtained from women who showed 10,000 or more organisms per milliliter of urine on any of the agar plates.

Reducing bias. To minimize bias in comparing the gram stain, streak plate, and catalase tests with the standard pour plate, different persons read the sets of each test on a given day. Each person was unaware of what the other tests had shown on the same urine specimens. On successive days these persons rotated in reading the gram stains, streak plates, catalase tests, and pour plates.

Results

Pour plate. A total of 1,156 urine specimens from 986 women were evaluated. The distribution of bacterial counts using the 0.001 ml. pour plate as the standard of reference is presented in the chart. The bimodal pattern, in which few urine specimens show intermediate numbers of organisms and most fall at either end of the spectrum, suggests that use of 100,000 organisms per milliliter as the dividing line between contamination and significant bacteriuria is probably valid.

Distribution of urine culture findings with the pour-plate method

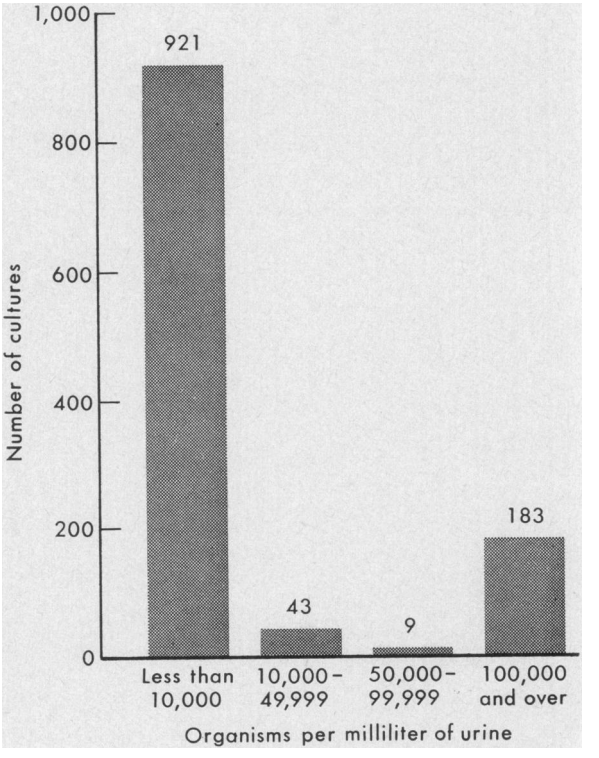

Table 1. Comparison of findings with 0.001 milliliter pour plate and with gram stain

Gram strain—organisms per oil-immersion field	Pour plate—organisms per milliliter of urine			
	Less than 10,000	10,000–99,000	100,000 or more	Total
	Number of specimens tested by both methods			
0	749	23	8	780
1–5	150	24	19	193
6 or more	13	5	146	164
Total	912	52	173	1, 137
	Rate per 1,000 specimens tested by both methods			
0	659	20	7	686
1–5	132	21	17	170
6 or more	11	4	129	144
Total	802	45	153	1, 000

It should be noted that when more than 300 colonies are present on a plate, they tend to run together, making an accurate count impossible. Therefore, with a pour-plate specimen of 1:100 dilution, more than 30,000 organisms per milliliter cannot be counted accurately. With a 1:1,000 dilution the dividing line of 100,000 organisms per milliliter of urine is readily surpassed before counting errors begin to occur. Since with counts below 30,000 organisms per milliliter of urine an almost perfect correlation was found between the two dilutions, the 1:100 pour plate was considered to be superfluous and was no longer used after the study was half completed. For the same reasons and with similar correlative data, the 0.01 ml. quantitative loop for making streak plates was also abandoned.

Gram stain versus pour plate. Results of the gram stain of the uncentrifuged urine compared with the 0.001 ml. pour plate are presented in table 1. If one or more organisms per oil-immersion field is used as the criterion for a positive gram stain, 165 urine specimens had a positive gram stain from the total of 173 specimens showing 100,000 or more organisms per milliliter of urine on the pour plate. Eight of 173 (4.6 percent) were falsely negative. The positive correlation was 95.4 percent. Of the 964 urine specimens which showed less than 100,000 organisms per milliliter, 192 had 1 or more organisms per oil-immersion field on the gram stain, giving 19.9 percent falsely positive gram stains. If 6 or more organisms per oil-immersion field is used as the criterion for a positive gram stain, 146 of the gram stains of the 173 urine specimens showing 100,000 or more organisms per milliliter on the pour plate were positive. Twenty-seven of 173 (16.6 percent) were falsely negative. The positive correlation was 84.4 percent. Eighteen of the gram stains of the 964 urine specimens which had less than 100,000 organisms per milliliter on the pour plate showed 6 or more organisms per oil-immersion field, giving 1.9 percent false-positive results.

On examination of the gram-stained slides for pyuria (table 2), 1 or more leucocytes per oil-immersion field were found in 51 of the 170 specimens which had 100,000 or more organisms per milliliter; 119 gram stains showed less than 1 leucocyte per oil-immersion field when the pour plate was positive.

Streak plate versus pour plate. Results of

Table 2. Comparison of findings with 0.001 milliliter pour plate and examination of the gram stain for leucocytes

Gram stain—1 or more leucocytes per oil-immersion field	Pour plate—organisms per milliliter of urine			
	Less than 10,000	10,000–99,999	100,000 or more	Total
	Number of specimens tested by both methods			
No	850	39	119	1, 008
Yes	59	8	51	118
Total	909	47	170	1, 126
	Rate per 1,000 specimens tested by both methods			
No	755	35	106	896
Yes	52	7	45	104
Total	807	42	151	1, 000

the streak plate made with the loop calibrated to deliver 0.001 ml. are compared with results when the 0.001 ml. pour plate was used (table 3). If the two methods are compared simply by correlating those plates showing less than 100,000 organisms per milliliter and those showing 100,000 or more organisms per milliliter, a close relation holds. The rate of false positives for the streak plate is 1 percent (10 of 972); the rate of false negatives, 4.9 percent (9 of 183). Others have also found a good correlation between these two methods (*22*, *23*).

Significant bacteriuria was found in 195 urine specimens when the results of both the pour plate and the streak plate were combined. Results of the two methods were in agreement on 173 positive specimens. Nine specimens were positive on the pour plate when the streak plate was negative, and 10 specimens were positive on the streak plate when the pour plate was negative.

A total of 165 urine specimens were positive by all three methods, that is, the gram stain showed 1 or more organisms per oil-immersion field, and the 0.001 ml. streak plate and the 0.001 pour plate each showed 100,000 or more organisms per milliliter of urine.

Table 3. Comparison of findings with 0.001 milliliter pour plate and 0.001 milliliter calibrated-loop streak plate

Streak plate—organisms per milliliter of urine	Pour plate—organisms per milliliter of urine			
	Less than 10,000	10,000–99,999	100,000 or more	Total
	Number of specimens tested by both methods			
Less than 10,000	881	13	3	897
10,000–99,000	34	35	6	75
100,000 or more	6	4	174	184
Total	921	52	183	1,156
	Rate per 1,000 specimens tested by both methods			
Less than 10,000	762	11	3	776
10,000–99,000	30	30	5	65
100,000 or more	5	3	151	159
Total	797	44	159	1,000

Table 4. Recurring costs for the field collection of 1,000 urine specimens

Item	Actual cost	Estimated routine cost
Supplies	$483.33	$334.73
Urine specimen cups, sterile, 4 oz	75.00	75.00
Isopropyl alcohol, 70 percent	50.00	10.00
Phisohex (R)	50.00	50.00
Gauze pads, sterile, 4″ x 4″	73.33	73.33
Labeling tape	15.00	6.40
Polyethylene gloves	200.00	100.00
Miscellaneous (toilet paper, plastic sheets, paper cups, and such items)	20.00	20.00
Labor	2,100.00	750.00
Total	$2,583.33	$1,084.73

Catalase flotation test. Results of this test were so irregular and so at variance with the other tests that it was abandoned early in the study. Initially, even blanks run with tapwater were positive. With acid-washing of the glassware and scrupulous cleanliness, this difficulty was eliminated. Of the 68 urine specimens tested, 37 were catalase positive and 31 catalase negative. Of 8 urine specimens showing more than 100,000 organisms per milliliter on the pour plate, 4 were catalase positive and 4 catalase negative. Of the 60 urine specimens showing less than 100,000 organisms per milliliter, 33 were catalase positive and 27 catalase negative. Although there is evidence that catalase may be present in noninfected urine during pregnancy (personal communication from Dr. A. I. Braude, professor of medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pa.), its presence would explain only the false positives, 33 of 37, and not the false negatives, 4 of 8.

Cost data. Expenses for the field collection of 1,000 urine specimens from pregnant women are summarized in table 4. The table shows the actual costs and estimated costs of future collections. Collecting the urine specimens cost about $2.50 per specimen. As experience ac-

cumulated, it became apparent that this figure could be decreased considerably. The chief expense was labor, and more than half of this cost represented the time required to take a lengthy and detailed medical history for research purposes. This history is unnecessary when the quantitative urine culture is performed as a routine test. The projected small savings in material shown in the second column are due to a more conservative approach gained with experience in collection techniques. These various savings can bring collection costs down to about $1 per specimen. Not included in the costs of collections are nonrecurring items such as expenditures for the purchase of bedpans, aluminum cups, and portable ice chests. No portion of the basic overhead of a functioning prenatal clinic with its accompanying personnel is included in the cost data.

The actual recurring costs entailed with the three bacteriological procedures per 1,000 specimens are enumerated in table 5. It is evident that the streak plate is the least expensive of the three methods. The comparative bacteriological costs do not encompass the overhead of a bacteriological laboratory, including cost and upkeep of such standard equipment as incubators, autoclaves, microscopes, pipettes, and the like. Also not included are the clerical expenses in contacting patients, transmitting data, ordering supplies, and so forth. The cost of the technical labor, however, is included in the comparative costs.

Table 5. Comparison of recurring costs of pour plate, streak plate, and gram stain per 1,000 specimens

Item	Cost
Pour plate	$723.00
Sterile petri dishes	160.00
Trypticase soy agar	5.00
Preparation of media and so forth (120 hours)	360.00
Making plates (16 hours)	48.00
Reading plates (50 hours)	150.00
Streak plate	370.50
Sterile petri dishes	80.00
Trypticase soy agar	2.50
Preparation of media and so forth (30 hours)	90.00
Making plates (16 hours)	48.00
Reading plates (50 hours)	150.00
Gram stain	510.00
Tape	5.00
Slides	15.00
Reagents	10.00
Making slides (10 hours)	30.00
Staining, reading slides (150 hours)	450.00

Discussion

The highest correlation with the quantitative pour plate was attained using the streak plate made with the calibrated platinum loop. The streak plate procedure has many advantages. A streak plate can be made with a minimum of effort and skill; the pour plate is considerably more difficult to prepare. Although about 24 hours is needed before a result is obtained with the streak plate, if a culture is positive, further studies such as identification of the organism and assessment of the antibiotic sensitivity can be initiated promptly. The expense, skill, and effort required to make a streak plate are no greater than for preparation of a gram stain or for use of other methods to be discussed subsequently. Moreover, since it is a highly accurate, definitive bacteriological procedure, the streak plate appears to be the method of choice in screening for bacteriuria.

Comparison of results with the pour plate and the streak plate shows a high degree of correlation. The number of false negative results with the streak plate is minimal, a criterion of major importance in any screening test. Although the calibrated-loop streak plate is an excellent and reliable technique, experience with it is not yet wide enough to permit routine use without making periodic checks using the pour plate.

The results of the gram stain and significant bacteriuria on the pour plate correlate well in this study. The degree of correspondence is about the same as has been noted by many others (*1*, *8*, *22*). The high rate of false positivity, however, means that many of the followup pour plates done to confirm the positive gram stain and to provide further study of the organism would show insignificant numbers of organisms. This followup represents needless work that would be avoided by use of the streak plate as described previously. In addition, the processing of a large number of gram stains must be accomplished quickly so that more definitive procedures can be initiated when indicated. Leucocytes in the urine correlated poorly with

bacteriuria, as shown by the high rate of false negativity. Use of techniques other than gram staining to search for leucocytes might improve the results of correlating pyuria and bacteriuria, but it is unlikely that the information yielded would justify the effort.

Comparative costs for laboratory procedures are revealing. As might be expected, the standard pour plate is the most expensive method, costing about 72 cents per specimen processed. The quantitative streak plate costs about half as much, 37 cents, and requires considerably less equipment. The greater economy of the streak plate over the pour plate is due to the ease with which streak plates can be made ready for use; pour plates must be prepared right at the time of plating. Moreover, considerable technical skill is necessary for even distribution of the inoculum and maintenance of the narrow temperature range in the pour plate; only elementary skill is needed to make and inoculate a streak plate. The cost of the gram stain is intermediate between the two cultural methods. Materials for the gram stain are inexpensive, but reading the slides is time consuming and requires a skilled technician. As mentioned, implicit in the expense of the gram stain is the requirement for additional definitive culture methods in a sizable proportion of specimens.

Although the cost and the degree of technical skill required for making pour plates are not excessive when compared with other routine or mass screening procedures, the greater economy and simplicity of the quantitative loop streak plate are strong points in its favor. These advantages enable the streak plate to be adapted to small-scale programs or even to office use. The loops cost only a few dollars; the plates can be purchased ready for use at little expense. Small and inexpensive incubators are available, and with experience a hand lens can be used for colony counting. It should also be recognized that when public health programs are carried out on a large scale and literally thousands of persons are screened, use of more economical procedures achieves a sizable saving of money, which is then available for expanding or prolonging the testing programs.

Neither the Griess test nor the tetrazolium test were evaluated, since, as with the gram stain, correlation of results for these two tests with results on the pour plate is approximately 70 to 90 percent (*24–26*). This degree of correspondence means a significant frequency of false negativity, a result which use of the quantitative streak plate obviates. Furthermore, when a positive result does occur with the Griess and the tetrazolium tests, the usual bacteriological procedures must be pursued anyway. Such procedures can be initiated promptly with the Griess test but must await several hours of incubation in the tetrazolium test.

Summary and Conclusions

Several laboratory methods for assessing the presence of bacteria in the urine have been compared. The quantitative streak-plate method showed an outstanding degree of correlation with the standard bacteriological pour-plate method and was considerably less arduous and less expensive to perform. The chief expense continues to arise from the procedure of collecting aseptically noncatheterized urine specimens from women. At present no way to curtail this expense is apparent. Justification for a relatively expensive detection procedure such as the quantitative, noncatheterized urine culture obviously depends not only on the wide prevalence of bacteriuria in some populations but also on its recognized deleterious effect on mother and fetus. Considerable evidence now available also indicates that asymptomatic bacteriuria is frequent and potentially dangerous in adult women whether or not they are pregnant.

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EQUIPMENT REFERENCE

(A) Platinum rhodium bacteriologic inoculating loops calibrated to deliver 0.01 and 0.001 ml. urine, catalog no. 7433–C, Arthur H. Thomas Co., Vine & 3d St., P.O. Box 779, Philadelphia 5, Pa.