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Simple Methods for Detection of Bacteriuria In the Physician's Office

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Simple Methods for Detection of Bacteriuria
In the Physician’s Office

E. G. Heinze, Jr.,* A. Hodari,** T. Connolly,* D. Jones,*
F. Cox,* and E. L. Quinn*

Two simplified quantitative urine culture techniques, suitable for use in the
physician’s office, were shown to be reliable and practical. The miniature
culture method (Testuria®) and flood-plate technique gave comparable quantita­
tive results, and the latter also yielded prompt information of organism
specificity which was helpful in judging the significance of the culture findings.

The standard colony-counting technique for determining the presence of bacteriuria¹
is not well adapted for screening test purposes in the physician’s office. Most alternate
methods lack the simplicity and reliability needed for this purpose.¹⁺⁻ Ryan and co­
workers² and Vejlsgaard,⁴ however, described modified culture techniques which were
reported to be satisfactory screening tests. We evaluated these two methods in patients
attending the Infectious Diseases Clinic and in patients attending the Obstetrical Clinic
of the Henry Ford Hospital, and the results are herein reported.

Materials and Methods

Urine specimens were collected by the clean midstream voiding technique. Each
specimen was immediately refrigerated,³ and the culture was performed later the
same day. Specimens from the Infectious Diseases Clinic patients were tested by both
a modified miniature culture method and quantitative flood-plate method in the
Infectious Diseases Clinic Laboratory. Specimens from the Obstetrical patients were
tested by the miniature culture method and a standard colony-counting method in the
hospital diagnostic bacteriology laboratory (Dr. J. Truant).⁷

The miniature culture method (MCM) described by Ryan and coworkers⁵ was
used as standardized by Leigh and Williams.⁴ Materials employed were commercially
available.⁶ A sterile filter paper strip provided by the manufacturer was dipped into
the urine to the designated mark and removed from the urine after 5 seconds. The
wetted end of the strip was then applied with slight pressure for 10 seconds to the
surface of a small culture tray containing sterile trypticase soy agar. The trays were

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†Ayerst Testuria® Dip Test; Ayerst Laboratories, Inc., New York, N.Y.
sealed with a cellophane cover and incubated inverted at 37° C for 18-24 hours. The filter paper was calibrated to deliver a volume of urine that would yield 0-2 colonies if <25,000 bacteria/ml, 3-25 colonies if 25,000 to 100,000 organisms/ml were present and >25 colonies for urines containing >100,000 bacteria/ml.

The quantitative flood-plate screening method, adapted from Vejlsgaard’s technique, employed disposable material and commercially prepared media. This method which required a minimal number of dilution steps, was found to correlate consistently with the standard colony-counting technique. One-tenth ml of urine was transferred with a sterile disposable 1 ml pipet to 10 ml of a sterile diluent (a vial of sterile saline for injection is satisfactory). Using a second pipet, a 0.1 ml aliquot of this dilution was transferred to the surface of a blood agar plate and to the surface of Eosin Methylene Blue Agar (Levine). This inoculum represented a 1 x 10^3 dilution of the urine specimen. The inoculum was allowed to spread over the surface of the plates. In order to prevent spreading to the sides of the plates, they were not inverted for 15-20 minutes after inoculation. Thereafter, the plates were inverted and incubated at 37° C for 18-24 hours. The number of viable colonies on the blood agar medium were counted and multiplied by the dilution factor (x 1000). Tentative identification of the organisms was made on the basis of morphology on the blood agar medium and on the basis of morphology and lactose reaction on Eosin Methylene Blue Agar.

Results

A total of 322 urine specimens collected in the Infectious Diseases Clinic were tested. In Table I, the results of the quantitative flood-plate method and miniature culture method are compared. By the criteria described, the two methods were in agreement in 304 of 322 (94%) of the urine specimens. The MCM gave a definite false positive result in only one specimen and no false negative.

In addition to the quantitative information obtained by the flood-plate method, it was possible to make a tentative identification of the isolates on the basis of colony morphology and lactose reaction. Table II lists the frequency of isolation of various organisms by these criteria. These data were in agreement with subsequent definitive identification of these organisms for most of the isolates.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Results of 322 Urine Cultures in Symptomatic and Asymptomatic Infectious Diseases Clinic Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative Flood-Plate Culture Method</td>
<td>Miniature Culture Method</td>
</tr>
<tr>
<td>No. of Colonies per ml</td>
<td>0-2</td>
</tr>
<tr>
<td>&lt;25,000</td>
<td>176 (55%)</td>
</tr>
<tr>
<td>25,000 - 100,000</td>
<td>4 (1%)</td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>0</td>
</tr>
</tbody>
</table>

1Plastic pipets, 1 ml graduated in 0.01 ml, from Falcon Plastics, Div. of B.D. Laboratories. Diluent, sterile saline for injections, 10 ml vial. Colab Double Poured Plates containing blood agar and Eosin Methylene Blue Agar (Levine).
Detection of Bacteriuria

TABLE II
Identification* of Isolates From 109 Positive Urine Cultures Utilizing the Quantitative Flood-Plate Method.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>50.8%</td>
</tr>
<tr>
<td>Klebsiella-Enterobacter group</td>
<td>16.9%</td>
</tr>
<tr>
<td>Proteus (swarming)</td>
<td>11.0%</td>
</tr>
<tr>
<td>Other non-lactose fermentors</td>
<td>3.3%</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>1.7%</td>
</tr>
<tr>
<td>Mixed</td>
<td>6.7%</td>
</tr>
<tr>
<td>Streptococci</td>
<td>9.3%</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

*Based on tentative identification by colony morphology and lactose reaction on the blood agar and Eosin-Methylene Blue agar (Levine).

Urine specimens were collected from 200 consecutive obstetrical patients. Twelve women had symptoms of urinary tract infection and were not included in this analysis of urine cultures for asymptomatic bacteriuria of pregnancy. There was agreement of the MCM and standard colony-counting technique in 186 of 188 specimens. (Table III). The MCM gave no definite false positive or false negative results. Four of six miniature culture colony counts in the intermediate range grew 10,000 to 100,000 organisms in the pour-plate and the other two intermediate counts were definitely positive by the standard method.

Discussion

A number of techniques have been proposed as office screening methods for bacteriuria. The Greiss Nitrate Test and its modifications were reported to correlate poorly with the standard colony-counting technique, i.e., 17% to 37% of bacteruric specimens were not detected. The TTC (Triphenyltetrazolium Chloride) test was positive in only 65% to 80% of urine specimens with >100,000 organisms/ml. Thirdly, the microscopic examination of the urine for cells, bacteria or both was advocated by several authors, who reported greater than 90% correlation with urine cultural methods. However, Brumfitt and Percival reported poor results using pyuria as a screening test for bacteriuria and Kass stated “the usual manner of studying pyuria is not trustworthy as a means for excluding the presence of infection of the urinary tract.” Recently, a culture technique using agar-coated glass microscope slides was reported to yield reliable correlation with the standard colony-counting technique.

The miniature culture method was reported to yield greater than 90% correlation with the colony-counting culture method. The results reported here substantiated the accuracy and usefulness of this technique. In addition, the MCM fulfills the main

TABLE III
Results of 188 Cultures in Asymptomatic Pregnant Patients

<table>
<thead>
<tr>
<th>Standard Colony-Count Culture Method</th>
<th>Miniature Culture Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Colonies per ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0-2</td>
</tr>
<tr>
<td>&lt;10,000</td>
<td>168 (89%)</td>
</tr>
<tr>
<td>10,000-100,000</td>
<td>0</td>
</tr>
<tr>
<td>&gt;100,000</td>
<td>0</td>
</tr>
</tbody>
</table>
criteria of low cost and simplicity with respect to time, materials, performance and interpretation which are desirable for an office screening procedure.

In order to identify the organisms on the agar blocks from specimens with positive or borderline MCM results, usual bacteriologic procedures must be employed. This can be accomplished by subculturing directly from the agar block or by reisolating the organisms from the original urine specimen which was refrigerated until the results of the screening test were read.

The flood-plate variant of the standard colony-counting culture method is another reliable screening test for bacteriuria. Agreement of the flood-plate method and the MCM was found in at least 94% of specimens tested. Moreover, this flood-plate technique was considered to be workable in a physician's office since it utilizes readily available, inexpensive, easily disposable materials. Although more time consuming than the MCM, each culture can be processed for incubation in less than five minutes. Since differential media are utilized, preliminary information regarding the probable species of organisms isolated is also obtained, and is helpful in judging the significance of the culture findings. Identification of isolates by this technique is easily made by the physician or his office technician.

Summary

The quantitative flood-plate and miniature culture methods for the detection of bacteriuria were tested in our office and found to agree in at least 94% of urine specimens cultured. An in-use clinical trial of the miniature culture methods in the prenatal clinic also showed a high correlation with the standard colony-counting technique. It is concluded that these two simplified techniques are practical office bacteriological methods in terms of cost, time, and reliability.

REFERENCES