Evaluation of Miniature Test for Bacteriuria Using Dehydrated Media and Nitrite Pads

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A new dip-inoculum method for detecting bacteriuria which utilizes dehydrated media pads and a nitrite pad attached to a small plastic strip was evaluated in hospitalized patients. Discrepant interpretations were made by independent observers in 9.3% of the specimens with > 10⁴ colonies per ml. The media pads failed to support growth of yeast and gave variable results with Staphylococcus epidermidis and non-group D streptococci. False-negative culture results commonly occurred if the patients were receiving antibiotics. The nitrite test occasionally remained positive for brief periods after the elimination of bacteriuria by antibiotics. Conditions and drugs (especially phenazopyridine) which discolor urine interfered with reading both the culture and nitrite tests.

Although not suitable for hospital use, or for monitoring therapy, the test strip is probably as reliable as the calibrated loop-streak plate culture for office screening.

The diagnosis of urinary tract infection is established by laboratory methods to detect the presence of bacteria in urine, since only about half of those individuals with bacteriuria have urinary complaints, and only about half of those complaining of dysuria are found to have bacteriuria. Quantitative bacterial culture methods have been widely accepted for distinguishing true bacteriuria from bacterial contamination of the urine specimen with urethral flora during voiding (9).

The calibrated loop-streak plate culture method is used by most diagnostic microbiology laboratories. However, this method has some practical disadvantages: it requires prompt delivery of the specimen to the laboratory, or refrigeration of the specimen until it is tested, and personnel trained in bacteriological skills; furthermore, it is relatively costly. A usual charge for a single urine culture, which includes identification and antimicrobial disk susceptibility testing, may be as high as $18.

The availability of reasonably cheap, efficient, and accurate dip-inoculum urine culture methods has made screening for bacteriuria potentially a standard part of the health examination. The prototype of the dip-inoculum method is a simple glass slide coated on one end with selective agar media (2). A variety of modified dip-inoculum tests have been developed, including agar-filled cups and paddles and agar-coated pipettes (3, 6). These methods compare favorably with the standard pour plate and may, in certain circumstances, be even more accurate than the calibrated loop-streak plate culture (7, 8). The disadvantages of presently available dip methods are the need for refrigerator storage of the test materials and the time required for incubation.

Thus there is still a need for a rapid, cheap, and reliable test for bacteriuria which could be read while the patient is still present. Chemical screening tests give almost immediate results but are not as reliable as direct microscope examination of stained urine (9).

We have evaluated a novel test for bacteriuria which appears to have several advantages over presently available methods, as it combines both chemical and bacteriological methods in a small plastic strip which is capable of indefinite storage and can be mailed in a single plain envelope. This test, called the Dip-Incubate-Read (DIR) method during investigational work and recently marketed as Microstix (Ames Co., Elkhart, Ind.), consists of a clear polystyrene strip with two dehydrated culture media pads and a nitrite reagent pad attached in series near one end. This report presents direct compari-
sons of results from both quantitative cultures and the DIR test in 1,661 urine specimens.

MATERIALS AND METHODS

Bacteriuria test strips. The DIR strips (Fig. 1) were individually wrapped and supplied in brown glass bottles with desiccant packs. The distal media pad consists of a 1-cm² area of absorbent paper impregnated with essential nutrients and a tetrazolium indicator which, when rehydrated with urine, supports growth of both gram-positive and gram-negative bacteria. The gram-negative media pad, which is the same size and is adjacent to the "total" bacteria pad, contains inhibitors of gram-positive organisms. The tetrazolium indicator in both pads is colorless and, when reduced in the presence of bacterial multiplication, produces discrete red spots on the pad. The density of the spots is then used to estimate the number of bacteria in the urine. The proximal pad, measuring 0.5 cm by 1 cm, is impregnated with chemical substances to detect traces of nitrite in the urine and turns bright pink within 30 s. The strips are stable in the dry state and refrigeration is not recommended by the manufacturer. The test is performed by immersing the test pads in urine, reading the nitrite test, and placing the strip in a plastic envelope which is then incubated at 37 °C for 12 to 18 h.

Clinical material. From June to August 1972, urine specimens were collected daily from hospitalized patients with indwelling urinary catheters by aseptic needle puncture of the catheter and from the drainage bag spigot; thus, 1,359 samples were obtained from 146 patients. In addition, 302 urine specimens (from 260 patients) submitted to the hospital diagnostic bacteriology laboratory were studied. The hospital records of all patients were reviewed to determine the usage of antimicrobial agents and drugs which are known to produce changes in urine color.

All specimens from the study of catheterized patients were delivered to the laboratory within 1 h of collection and refrigerated at 4 °C until tested. All specimens submitted to the laboratory were refrigerated immediately upon arrival. Each urine sample was cultured simultaneously by a calibrated loop-streak plate procedure (using blood, Levine eosin methylene blue, and bile-esculin agar) and by a DIR test strip. In addition, for samples obtained by monitoring catheterized patients, a 0.1-ml inoculum was pipetted onto the surface of a blood agar plate and then streaked with a wire loop, to provide increased sensitivity for low colony counts.

Two individuals independently interpreted the density of growth on the dip test media pads for specimens submitted to the diagnostic laboratory. When these interpretations differed, both readings were noted but the lower count was used for subsequent analysis. All DIR tests were interpreted without knowledge of the results of the flood/streak plate cultures. Standard bacteriological methods for identification of species were carried out.

RESULTS

Observer variation. Both readers reported the same results of the DIR tests for 255 (84.4%) of 302 specimens submitted to the diagnostic laboratory. Discrepant results were reported for both media pads in 23 tests, for the total pad alone in 13 and for the gram-negative pad in 11. The majority of the discrepancies (35 of 47) were by a factor 10-fold, but differences of 10³-fold occurred in four instances. However, 19 of the 47 discrepant readings occurred when one of the readers reported 10⁴ colonies per ml on one or both pads. Of 75 specimens with 10⁴ colonies per ml or more by streak plate, seven (9.3%) discrepant readings occurred with DIR tests (one of the readers reporting 10⁴ colonies per ml).

Overall comparison of total bacterial counts. Among the 1,661 specimens, 697 yielded growth of a single microbial species, and 86 yielded mixed growth of two or more species. Comparative total bacterial colony counts between the DIR test strips and standard flood/streak plate cultures among routine specimens submitted to the diagnostic laboratory and, separately, specimens obtained from the monitoring of patients with indwelling urinary catheters are shown in Tables 1 and 2. Identical results were obtained by both methods in 1,256 (75.6%) of 1,661 specimens. The DIR test detected 293 (67.8%) of 432 specimens with colony counts of 10⁴/ml or greater by standard cultures. However, there was a 5.3% (13 of 245) occur-
Table 1. Comparison of total bacterial colony counts obtained with standard streak plate method and the DIR test in specimens submitted to the diagnostic bacteriology laboratories

| DIR method (colonies/ml) | Streak plate (colonies/ml)* | <10 10 10 10 10 | >10 10 |
|-------------------------|-----------------------------|-----------------|---------|---------|---------|
| <10                     | 106                         | 16 20 19 8      |         |         |
| 10                      | 3                           | 4 3 2 0        |         |         |
| 10³                     | 1                           | 6 6 2 1        |         |         |
| 10⁴                     | 0                           | 5 7 6 1        |         |         |
| 10⁴                     | 0                           | 4 5 7 1        |         |         |
| 10⁵                     | 0                           | 0 2 9 53       |         |         |
| Unreadable              | 0                           | 0 0 0 0        |         |         |

* 100 specimens were obtained from patients with indwelling urinary catheters.

** Intermediate counts were rounded to the lower log.10.

Thirty-one specimens from patients with urinary catheters.

Table 2. Comparison of total bacterial colony counts obtained with standard flood plate method and the DIR test in specimens from catheterized patients

| DIR method (colonies/ml) | Flood plate (colonies/ml)* | <10 10 10 10 10 | >10 10 |
|-------------------------|-----------------------------|-----------------|---------|---------|---------|
| <10                     | 762                         | 11 44 55 33 72  |         |         |
| 10                      | 3                           | 6 0 0 1        |         |         |
| 10³                     | 1                           | 11 0 1 7       |         |         |
| 10⁴                     | 0                           | 1 3 7 3        |         |         |
| 10⁴                     | 0                           | 0 6 29 28      |         |         |
| >10⁴                    | 0                           | 0 0 0 2 240     |         |         |
| Unreadable              | 3                           | 0 0 0 0 8       |         |         |

* Intermediate counts were rounded to the lower log.10.

rence of false-positive DIR test reports (DIR = 10⁵/ml) among specimens with 10⁴ to 10⁵ colonies per ml by standard cultures. No false-positive DIR tests occurred with "true" colony counts of 10⁴/ml or less.

Of the 139 false-negative DIR tests among the 432 specimens with 10⁴ colonies per ml by standard cultures, the majority showed no growth on the DIR pads and these occurred more often with specimens from catheterized patients. All except 23 of the false-negative tests could be explained by the failure of the DIR test pads to support growth of certain species (which were isolated more frequently from catheterized patients) or by the use of systemic antimicrobial agents (which also was more frequent in catheterized patients). Unreadable DIR pads accounted for 8 of the 23 remaining false-negative tests, and these occurred only with specimens from catheterized patients. All of the unreadable tests occurred when the test pads were discolored by grossly bloody urine or by drugs, such as phenazopyridine, which impart an orange color to the urine.

False-negatives: effect of microbial species. Among 139 false-negative DIR tests with specimens yielding 10⁴ colonies per ml or greater, 43 occurred with pure cultures of yeast and 7 occurred with Staphylococcus epidermidis or diphtheroids. Overall, the DIR test failed to support growth in any of 83 specimens which contained varying colony counts of yeast.

The presence of gram-positive cocci is determined by growth on the "total" pad and absence of growth on the selective gram-negative pad. Enterococci could not be distinguished from other gram-positive cocci. However, growth of S. epidermidis was inconsistent. S. epidermidis did not grow in four of five specimens with colony counts of 10⁴/ml by the streak plate. Streptococci (not group A or D) also failed detection by the DIR test in two instances.

The correlation between standard quantitative cultures and the DIR test in specimens obtained from patients who had not received antimicrobials is shown in Table 3. The DIR test detected colony counts of ≥10⁴/ml in 89 (95.7%) of 93 specimens with a single species of either gram-negative bacilli or enterococci. Enterococci could always be distinguished from gram-negative bacilli, although growth around...
the edges of the gram-negative pad occasionally occurred.

**False-negatives: effect of antimicrobial agents.** Sixty-six false-negative DIR tests occurred in specimens from patients who had received systemic antimicrobial agents or whose medication history was uncertain. The suspected obfuscation of the DIR test was confirmed by direct comparison of the culture methods in relation to the antibiotic status of the patients (Table 4). The DIR test and the streak plate yielded colony counts of 10⁵/ml or greater in 157 (96.3%) of 163 specimens obtained from patients not receiving antimicrobial agents. Furthermore, among 144 specimens with colony counts of 10⁵/ml of gram-negative bacilli or enterococci obtained from patients not receiving antimicrobials, the DIR test results were negative in only two and 10³ colonies per ml in 10.

Frequent false-negative DIR tests occurred in specimens obtained from patients who had received antimicrobial agents but were not still receiving them on the day the specimens were collected. However, the effect of antibiotics was evident only within 2 days after the antibiotics had been stopped, and 111 of 119 (93.3%) specimens obtained from 3 to 13 days after antibiotics had been discontinued showed agreement when gram-negative bacilli or enterococci were isolated with colony counts of 10⁵/ml or more.

Poor correlation between the DIR test and the streak plate cultures was obtained with virtually all antimicrobials used, although differences presumably reflect the sensitivity of the bacterial species to urinary levels achieved with each of the antimicrobials, as well as the small numbers in each group (Table 5).

**Correlation between two culture pads.**

| Antibiotic use | Counts ≥ 10⁵/ml by both methods | DIR colony count lower | <10 colonies/ml | 10-10³ colonies/ml |
|----------------|---------------------------------|------------------------|-----------------|------------------|
|                | No. %                           |                        | No. %           | No. %            |
| Current        | 15 26.8                         | 33 58.9                | 8 14.3          |
| Prior          | 130 85.5                        | 18 11.9                | 4 2.6           |
| None           | 157 96.3                        | 5 3.1                  | 1 0.6           |
| Unknown        | 15 88.2                         | 1 5.9                  | 1 5.9           |

*Includes only specimens which yielded gram-negative bacilli or enterococci (single or mixed species) with total colony counts ≥ 10⁵/ml by standard method.

**TABLE 5. Relation of use of specific antimicrobial agents to total colony counts obtained with standard quantitative methods and the DIR test***

| Antimicrobial agent used | Counts ≥ 10⁵/ml by both methods | DIR colony count lower | <10 colonies/ml | 10-10³ colonies/ml |
|-------------------------|---------------------------------|------------------------|-----------------|------------------|
|                         | No. %                           |                        | No. %           | No. %            |
| Tetracycline            | 1 100.0                         | 0 0                    | 0 0             |
| Amoxicillin             | 4 28.6                         | 9 64.3                 | 1 7.1           |
| Nafcillin               | 0 0                            | 3 100.0               | 0 0             |
| Cephalosporins          | 6 46.2                         | 4 30.8                 | 3 23.0          |
| Gentamicin              | 1 7.7                          | 12 92.3                | 0 0             |
| Sulfonamides            | 3 42.9                         | 0 0                    | 4 57.1          |

*Includes only specimens which yielded gram-negative bacilli or enterococci with colony counts ≥ 10⁵/ml by standard method.

Among 72 specimens which yielded 10⁵ colonies per ml or greater of gram-negative bacilli and which had been collected from patients not receiving antibiotics, both the total and gram-negative pad were read as 10⁶ in 64 (88.9%). A variety of discrepancies occurred in the remaining ones; in four instances only one of the pads was read as 10⁶ whereas the other was negative. In three of these, the gram-negative pad was negative, and these could have been misinterpreted as enterococci.

**Nitrite tests.** The relation between positive tests for nitrite and significant bacteriuria (defined as colony counts greater than 10⁵/ml) by streak plate cultures is shown in Table 6. The low sensitivity of the test (39.1%) reflected in part the frequent isolation of organisms which do not reduce nitrate. Thus, the sensitivity of the nitrite test was 51% in this study when only gram-negative bacilli were considered. No differences in sensitivity of the nitrite test were found by comparing catheterized to clean-voided specimens or by comparing specimens from the drainage bag to those from the catheter itself. However, 16 specimens with greater than 10⁴ colonies per ml were detected only by the nitrite test, and all were from patients who had received antibiotics.

**False-positive nitrite tests occurred in 23 specimens; in 20 of these instances, the patients had received systemic antibiotics before the**

| Nitrite test | Streak plate dip test |
|--------------|-----------------------|
|              | >10⁴                  | <10⁴                  |
| Positive     | 169 23                |
| Negative     | 263 1,206             |

**TABLE 6. Sensitivity and specificity of nitrite dip test**
specimens were obtained. In 12 of these instances the specimens were obtained from patients who had documented bacteriuria with colony counts greater than $10^4$ ml on the previous day and in whom antibiotics had been ordered subsequently, with a decrease in colony counts to less than $10^3$ ml. Discolored urine, usually due to drugs, was responsible for two unreadable nitrite tests.

**DISCUSSION**

The DIR test strips have several advantages over previously available dip-inoculum culture methods: compactness and ease of mailing, capability for prolonged storage without need of refrigeration, and the requirement for a smaller urine sample volume. The nitrite portion of the test allows for an immediate result for approximately one-half of the bacteriuric patients.

Major difficulties were recognized when the test strip was evaluated in a hospitalized population. Complete failure to detect yeast and irregular growth of *S. epidermidis* and non-group D streptococci are important limitations, since these microorganisms are more frequently isolated from nosocomial infections, especially those secondary to indwelling bladder catheterization.

The effect of antibiotics in causing false-negative DIR tests (positive standard culture-negative DIR media pads) had been previously unsuspected, and has been confirmed by Craig et al. (4) by simultaneous pour plate and DIR test strip cultures using a variety of antimicrobials added directly to Trypticase soy broth containing $10^6$ colonies of *Escherichia coli* per ml. This blocking effect of antibiotics has not been demonstrated with other dip-inoculum methods, which have shown nearly identical results with standard methods (1, 5). It appears likely that the absorption of a large volume of urine relative to the small size of the dehydrated pads results in a larger local concentration of the antimicrobial than occurs with other dip tests. Similarly, the antimicrobial would be considerably diluted in conventional pour plate and loop-streak cultures.

When antimicrobials were responsible for false-negative DIR culture pads, the nitrite test commonly remained positive. However, false-positive nitrite tests also occurred within 24 h after antimicrobial treatment had been given. In these cases, the standard loop-streak culture was also negative, making the nitrite test unreliable as an early indicator of effective therapy.

The DIR test strips correlated less well with conventional cultures at lower colony counts, since 21 specimens (60%) showed identical colony counts among 35 specimens with 10 to $10^3$ colonies of gram-negative bacilli or enterococci per ml, when the patients had not received antibiotics. Although not important in screening clean-voided urine specimens, these disagreements may be important in specimens from indwelling urinary catheters for which “significant” colony counts have not been established.

The small discrepancy with colony counts greater than $10^4$ ml of gram-negative bacilli or enterococci (patients not receiving antibiotics) could be entirely due to observer variation. The most frequent difficulty in reading the test pads occurred at the $10^4$ to $10^5$ colony per ml level. It is likely that errors in interpretation might be even more frequent with untrained observers.

The selection of the calibrated loop-streak plate culture method as the standard of comparison is a limitation of the present study. Although this method is regularly compared with pour plate controls in our laboratory, others have reported false-negative and false-positive results in 2 to 10% of samples (9). Therefore, it is probable that the DIR test strip is at least as accurate as the loop-streak plate culture method, if the important effects of antimicrobial agents and the failures to detect certain microbial species are considered.

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