The Rapid Diagnosis of Urinary Tract Infection
A Side-Room Method

F. D. DORNFEST

SUMMARY
A controlled trial of a rapid method for microscopic examination of the urine for organisms is reported. The method achieved results as accurate as those of semi-quantitative culture, at minimal cost. It is easy enough to be learned by medically untrained staff, and results are available within 10 minutes. This rapid availability of results makes it possible to prescribe more appropriately at the initial consultation.


There has been a long-felt need for a rapid and reliable diagnostic test for urinary tract infection, as the history and physical examination have repeatedly proved grossly unreliable. The 'classical clinical picture' of acute symptomatic urinary tract infection (dysuria, frequency, backache and fever) only proves to be true in about 50% of cases. All side-room tests devised to improve the reliability of diagnosis and to avoid the delay of 18 - 24 hours before results of urine culture become available have in some way fallen short of their objectives. Chemical side-room tests such as that for nitrite (BM Nitrite) and microscopic examination of the spun deposit for pyuria are rapid but not accurate enough to be of much value. Results from dipslides (Uricult) and dipsticks (Microstix), although more accurate, involve the same delay as laboratory culture, and there are obvious advantages to both the clinician and his patient in having an accurate result available immediately.

The work of Van Trommel indicated that a dried and stained smear of the spun deposit examined microscopically for organisms was very much more accurate in the diagnosis of urinary tract infection than an unstained spun deposit examined for pyuria. His work, although poorly controlled, also indicated that methylene blue was perfectly adequate as a staining material and had many advantages over the traditional Gram stain.

It was decided to perform a controlled trial using a modification of the method described by Van Trommel. Modification was introduced to simplify and speed up the method so that a result could be obtained in less than 10 minutes.

METHOD

The trial was conducted in a 3-member, suburban general practice over a period of 6 months.

Patient Selection
During the trial period, urine was collected from the following three groups of patients: (i) all patients presenting with symptoms of urinary tract infection; (ii) all asymptomatic patients presenting for routine life insurance examination; and (iii) patients presenting for routine antenatal examination. Urine specimens with a high probability of infection or contamination were selected, as the trial was not concerned with the incidence of urinary tract infection. Patients in the first group were considered to have a high probability of infection, and those in the second group a low probability. They were matched by the antenatal group, which consisted of an approximately equal number of patients who were thought to have the highest incidence of contamination owing to the vaginal discharge of late pregnancy and the difficulty of collecting a clean-catch midstream specimen from a patient with a pendulous abdomen.

Specimen Collection
All patients were instructed how to collect a clean-catch midstream specimen. Stress was placed on separation of the labia or retraction of the prepuce, and allowing the first portion of the stream to be discarded before the last portion was voided into the specimen container. Disposable plastic containers were used.

Method under Investigation
Immediately upon receipt, each specimen was mixed by inverting the plastic collection container a few times. A 4 ml aliquot was then poured into a graduated conical centrifuge tube. The remainder of the specimen was placed in the refrigerator at 4°C and used for control of the trial, as described later. The 4 ml aliquot was centrifuged at 3 000 rpm for 2 minutes or more. After centrifugation, the supernatant was decanted by inverting the centrifuge tube. The tube was then righted and held near the top between the thumb and index finger of one hand. The deposit was resuspended in the supernatant trapped at the bottom of the tube by flicking the lower end of the tube vigorously with the index fingernail of the other hand. One drop of the spun deposit was tapped out of the tube onto each of two glass slides. These slides were numbered for that
specimen. One slide was covered with a coverslip and examined within 2 hours under low power as a wet preparation. A wax circle was drawn on the underside of the second slide, which was stained as follows: it was placed on the sterilizer, which was usually at boiling point, or if the sterilizer was cold and a rapid result was required the slide was placed on the lid of a kettle which could be brought to the boil very much more rapidly.

The dried preparation was fixed by spraying with methylated spirits from an aerosol can or by dipping into a container of methylated spirits.

The dried and fixed smear was covered with a 1% aqueous solution of methylene blue. This was washed off immediately with tap water and the smear blotted dry with filter paper. The dry-stained preparation was examined immediately only if the patient had symptoms of urinary tract infection, in order to aid diagnosis. When this was necessary it was re-examined after 2 weeks, under high power or preferably oil immersion, with all the other dry preparations. This was done to avoid observer bias. Slides were examined by the author only.

Controls

All the specimens in the trial were number-coded and processed by the practice receptionist, who had no medical or scientific training. She was given minimal instruction and no subsequent supervision. The code remained in her possession and was not broken during the trial.

The urine remaining after removal of the 4 ml aliquot was used for control purposes. It was kept in the refrigerator at 4°C in the original plastic container. The specimens were called for at 2-day intervals and transported to the laboratory in their containers, which were placed in a polystyrene cold-box. A dummy specimen was also kept in the refrigerator and was included in the cold-box at each collection. The temperature of this dummy specimen was measured and recorded both before leaving the surgery and on arrival at the laboratory, which was 10 km away. The average temperature on leaving was 2.3°C and on arrival 5.1°C. No specimen showed a rise in temperature to more than 11°C. At the laboratory a semiquantitative count by the filter-paper method of Leigh and Williams was performed, this being the routine method employed there. The entire trial was controlled by a surface viable count performed by the laboratory as a reference method.

One hundred and nine specimens were processed by all three methods: (i) the rapid-dried, methylene blue-stained, spun-deposit (RMSD) method at the surgery; (ii) the routine hospital laboratory method of Leigh and Williams (LW); and (iii) the surface viable count (SVC) laboratory reference method. Criteria for the diagnosis of urinary tract infection based on the hospital laboratory methods are given in Table I and those for the RMSD method in Table II.

TABLE I. DIAGNOSTIC CRITERIA FOR RESULTS OBTAINED BY THE RMSD METHOD

| Positive result | more than 100 000 known pathogenic organisms per ml in pure culture |
| Doubtful result | (a) more than 10 000 but less than 100 000 known pathogenic organisms per ml in pure culture, (b) more than 100 000 known pathogenic organisms per ml in mixed culture of no more than two organisms |
| Negative result | one which meets neither the criteria for a positive nor those for a doubtful result |

Sensitivity and specificity of the LW and RMSD methods were determined using the SVC method as a reference. Sensitivity and specificity were defined according to a modification of the standard definitions of the World Health Organization as set out below:

Sensitivity = \( \frac{\text{number with UTI who had a positive test}}{\text{number in study population who had UTI}} \times 100 \)

Specificity = \( \frac{\text{number who did not have UTI and had a negative test}}{\text{number in study population who did not have UTI}} \times 100 \)

RESULTS

Thirty-one specimens were found to be positive by the SVC reference method (Table III). Of these, 29 were positive by the RMSD method and 27 by the LW method—sensitivities of 93.5% and 87.1% respectively.

TABLE III. RESULTS OBTAINED BY THE SVC REFERENCE METHOD PLOTTED AGAINST THE RESULTS OBTAINED FOR THE SAME SPECIMEN BY THE RMSD METHOD AND THE LW ROUTINE LABORATORY METHOD

<table>
<thead>
<tr>
<th>SVC</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doubtful (RMSD)</td>
<td>10</td>
</tr>
<tr>
<td>Doubtful (LW)</td>
<td>8</td>
</tr>
<tr>
<td>Negative (RMSD)</td>
<td>5</td>
</tr>
<tr>
<td>Negative (LW)</td>
<td>7</td>
</tr>
<tr>
<td>Positive (RMSD)</td>
<td>5</td>
</tr>
<tr>
<td>Positive (LW)</td>
<td>5</td>
</tr>
</tbody>
</table>

* = Specificity RMSD 91.4%.
† = Specificity LW 93.1%.
‡ = Sensitivity RMSD 93.5%.
§ = Sensitivity LW 84.4%.
Fifty-eight specimens were negative by the SVC reference method. Of these 53 were detected by the RMSD method and 54 by the LW method — specificities of 91.4% and 93.1% respectively.

Twenty specimens were doubtful by the SVC reference method. The RMSD method detected 10 of these and the LW method 8.

**DISCUSSION**

In terms of sensitivity and specificity, the RMSD method yielded results which were as good as, if not slightly better than, the LW method routinely used in the hospital laboratory. Investigation of a 4 ml specimen by the RMSD method was possible, which was useful because experience has shown that patients complaining of frequency are often unable to produce the 10 ml required for many other methods. Emphasis was placed on rapidity and ease of method, usually the determining factors for acceptance of a method in clinical practice. For this reason centrifugation was performed at 3 000 rpm for 2 minutes rather than at 1 000 rpm for 5 minutes as is suggested in standard texts; the spun deposit was resuspended in the residual supernatant trapped in the tube after inversion rather than in a measured volume; rapid drying of the specimen at up to 100°C was employed, rather than drying at room temperature or 37°C; and methylene blue stain was used, a one-step procedure, rather than Gram stain.

The disintegration of organisms and cells when rapidly centrifuged predicted by most standard texts was not a problem, as evidenced by the results. To the author's knowledge the rapid drying of smears has never been documented. This method is important as it alone produced a saving of about 35 minutes — there was no change in the appearance of organisms or squamous cells, but in many instances the rapid drying did make an appreciable difference to the appearance of leucocytes, pus cells and red cells. This difference is no real disadvantage, as these features are all easily seen on the wet preparation. In any event it is normal laboratory procedure to prepare both wet and stained preparations when examining the urine.

Van Trommel's assertion that methylene blue stain appears to be as satisfactory as Gram stain is borne out by this work. Gram staining is not necessary, as no attempt is made to identify the organisms seen; only the number of organisms seen is important. Despite the fact that organisms were not identified, contamination could be detected with great accuracy; it was revealed by the features mentioned in Table I. Methylene blue has additional advantages. It is used in a one-step, drop-on, wash-off procedure, and the reagent has a longer shelf-life than Gram stain.

**CONCLUSION**

A methylene blue stain of the rapidly dried spun deposit examined microscopically gives a diagnostic accuracy for urinary tract infection equivalent to the method of Leigh and Williams routinely used by most laboratories. The RMSD method has the following advantages: (i) results can be obtained in less than 10 minutes — the patient can therefore be asked to wait the result before leaving the premises and before therapy is commenced; (ii) it is a semiquantitative direct measure of bacteriuria, not an indirect measure of infection like the nitrite test or microscopy for pyuria; (iii) the method is well within the scope of practitioners of all disciplines and, apart from the actual interpretation of slides, can even be carried out by staff with no medical training; (iv) the method is far less costly to the patient than culture methods.

For these reasons the method can be recommended for general practice and other disciplines for routine use. Even where the facilities of a laboratory are readily available, this method has definite advantages.

I should like to thank Dr A. Forder for his advice and for arranging for control tests to be performed by the Department of Bacteriology of the University of Cape Town in the Groote Schuur Hospital laboratories; Drs G. Futeran and C. Rabino-witz for providing specimens from their patients and for their assistance while this trial was in process; and Boehringer Mannheim and their medical representative Mr L. Viljoen for providing transport of specimens from the practice to the laboratory.

**REFERENCES**