Simplification of procedures used to test urine samples for Chlamydia trachomatis

B J Thomas, C Gilchrist, P E Hay, D Taylor-Robinson

Abstract

Two methods of simplifying the procedure for examining urine samples for Chlamydia trachomatis were investigated. When 73 urine samples from 56 men with acute non-gonococcal urethritis were examined by direct immunofluorescence (MicroTrak), centrifuging 1 ml volumes of urine at 13 000 rpm for five minutes was at least as efficient for detecting C. trachomatis as centrifuging larger volumes at 3000 rpm for 30 minutes. Furthermore, examination of urine produced during a visit to a sexually transmitted disease clinic was at least as efficient as examination of early morning urine for detecting C. trachomatis by MicroTrak, or by an enzyme immunoassay (IDEIA). Both modifications have practical advantages and should encourage the use of urine samples for diagnosing chlamydial infections in men.

Recently published results have indicated that examination of an early morning urine specimen might be useful for detecting Chlamydia trachomatis in the male urethra.

The results of a study of our own have shown that detection of C. trachomatis in urine by the immunoassay IDEIA (Novo Nordisk Diagnostics, UK) is slightly less sensitive than by MicroTrak (Syva, UK Ltd) which, in turn, is somewhat less sensitive than staining C. trachomatis in urethral smears by MicroTrak.

The handling technique for urine samples is cumbersome and time-consuming, involving centrifugation of 20 ml of urine for 30 minutes. We therefore addressed the question of whether it is possible to simplify the technique by testing a fraction of this urine centrifuged at a greater speed for a shorter time.

Previous investigations have also suggested that the first 20 ml of an early morning urine specimen should contain more chlamydial elementary bodies than the first passed 20 ml of urine produced later in the day—for example, during a visit to a sexually transmitted disease (STD) clinic. An early morning urine has therefore been recommended as the sample of choice for detecting C. trachomatis. We tested corresponding early morning urine and first passed urine specimens by MicroTrak and by IDEIA and compared the numbers of elementary bodies found in each type of specimen by the former method to determine whether the more easily obtained and reliable urine specimen collected during a clinic visit is as good a source of C. trachomatis as an early morning urine.

Methods

Men with acute non-gonococcal urethritis (NGU) were seen at the Jefferiss Wing of St Mary's Hospital, Paddington. Urethral swabs were taken as described previously.

The first 20 ml of urine passed after the urethral swab had been taken were collected in a sterile container; this constituted the first passed urine. Patients were asked to return the following day with the first 20 ml of the first urine passed that morning before antibiotic treatment was started.

Urethral smears were made by rolling swabs on MicroTrak slides, and these were processed as described previously. Urine samples were stored at 4°C for a maximum of three days and were warmed at 37°C before processing to redissolve any deposit which had formed. After vigorous mixing, 1 ml of urine was removed and centrifuged at 13 000 rpm in a microfuge (MSE Micro Centaur) for five minutes. The remaining 19–22 ml was divided into three aliquots, each of which was centrifuged at 3000 rpm (MSE Mistral 2000) for 30 minutes. The deposit from the 1 ml urine sample was resuspended in 10 µl of distilled water which were dried on a MicroTrak slide and fixed in acetone. The deposits from the remainder were treated as follows: one was resuspended in 100 µl of distilled water and 10 µl dried on a MicroTrak slide. For many urine samples this produced a very thick smear, so that further dilutions of the deposit were necessary to obtain a satisfactory sample; one was resuspended in 1 ml of IDEIA transport medium and stored at −70°C; the third was resuspended in 200 µl of distilled water and stored at −70°C for future reference.

The MicroTrak and IDEIA procedures were carried out according to the manufacturer's instructions. In view of extensive experience of the MicroTrak technique, any specimen containing a single, typical fluorescing elementary body was considered positive and the number of elementary bodies in a smear was recorded as described previously.

Results

Fifty six men with acute NGU provided urethral swabs and 73 urine samples (56 first

<table>
<thead>
<tr>
<th>No of 1 ml urine samples</th>
<th>Positive</th>
<th>Negative</th>
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<tbody>
<tr>
<td>No of RV samples</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>41</td>
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</tbody>
</table>

Table 1 Comparison of 1 ml urine samples and remaining volume (RV) samples (6–7 ml) for detection of C. trachomatis by MicroTrak.
Simplification

and all six occasions; and in their results contained urine) being positive by MicroTrak agreement, 30 positive results: of seven sample on volume bodies in elementary volume bodies in clinic visits the results

during this study.

After the diagnosis of C trachomatis, the results obtained for the 1 ml and the remaining volume urine samples were in good agreement, 30 of the former (23 first passed urine; seven early morning urine) and 28 of the latter (24 first passed urine; four early morning urine) being positive by MicroTrak (table 1). In all, there were 26 concordant positive results: of these, the number of elementary bodies in corresponding 1 ml and remaining volume samples was similar on 20 occasions; was more in the 1 ml sample on two occasions; and more in the remaining volume sample on four occasions. The C trachomatis positive urine in all six samples with discrepant results contained \( \leq 10 \) elementary bodies, and all six men had \( \leq 10 \) elementary bodies in their urethral smears.

**Table 2. Comparison of early morning urine and first passed urine samples for detection of C trachomatis by MicroTrak and IDEIA**

<table>
<thead>
<tr>
<th>First passed urine by MicroTrak</th>
<th>First passed urine by IDEIA</th>
</tr>
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<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Early morning urine by MicroTrak</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
</tr>
</tbody>
</table>

Comparison of early morning urine and first passed urine deposits for detecting C trachomatis by MicroTrak and IDEIA

Thirty seven men each provided an early morning urine and a first passed urine. Eighteen had positive urethral smears and 15 early morning urine samples, and 19 first passed urine samples were positive by MicroTrak (table 2). Of the 15 corresponding early morning urine and first passed urine samples that were C trachomatis positive in both, six contained similar numbers of elementary bodies, and in only two did the early morning urine sample contain more than the first passed urine. All four discrepant urine samples contained \( \leq 10 \) elementary bodies and one of these four patients also had a negative urethral test.

Compared with MicroTrak, 16 early morning urine and only 15 first passed urine samples were positive by IDEIA (table 2). In the case in which there was a discrepant result, the positive first passed urine, which was undetected by IDEIA, contained \( \leq 10 \) elementary bodies, as judged by MicroTrak, as did the urethral sample.

**Discussion**

Although the demand for chlamydial diagnosis in men is less than that in women, a non-invasive technique for acquiring specimens from the genital tract is attractive if the test is required. Our own work, and that of others, has indicated that in men with acute NGU, urine samples are an acceptable alternative to urethral swabs for this purpose. In the present study methods of simplifying the collection and handling of urine samples have been shown not to decrease the sensitivity of this approach to C trachomatis detection while improving it practically. The advantages of centrifuging 1 ml samples at high speed for a short time are particularly apparent when MicroTrak is the detection method of choice; the problem of producing smears of deposits which are too thin for microscopical examination is overcome without the extra step of further diluting the deposit.

In a study in the United States of America 25% of men attending an STD clinic in whom C trachomatis was detected by culturing a urethral specimen had no signs or symptoms of urethritis. It is not known whether such a figure applies to the United Kingdom and there is clearly a need to determine the prevalence of C trachomatis positive NGU in asymptomatic men, and C trachomatis in men without NGU. Both of these are low prevalence groups in which MicroTrak, rather than IDEIA, would be the test of choice because of its superior sensitivity. Under these circumstances the modification we describe would save considerable time. In high prevalence populations, however, such as men with acute NGU, where IDEIA could be used with greater confidence, a larger volume of urine is recommended for this test to provide as much antigen as possible for detection.

In clinic practice urethral specimens from a small proportion of patients are inadequate or swabbing is not undertaken at all because of the discomfort experienced or anticipated. Moreover, such an experience sometimes discourages patients from reattending. It is clear that having a urine sample from recalcitrant patients is incomparably preferable to having an inadequate urethral swab or none at all. Furthermore, the observation that testing first passed urine samples by either MicroTrak or IDEIA was about as sensitive for detecting C trachomatis as testing early morning urine samples suggests that the former are just as suitable as the latter. This is of practical advantage in that men who present at an STD clinic can provide a urine sample then, rather than having to return the following day with an early morning urine. This, and the other modification we describe, should encourage the use of urine samples for diagnosing chlamydial infections in men.