Comparison between microscopical examination of unstained deposits of urine and quantitative culture

It has been our practice for some years to examine urine for infection by microscopy of the fresh unstained centrifuged deposit in the outpatient clinic, in addition to the standard bacteriological culture. The relation between this microscopical examination and the subsequent quantitative laboratory results is the subject of this report.

Material and methods

189 children attending a general paediatric outpatient clinic were studied. Their ages ranged from 2 weeks to 14 years and none was receiving antibiotics. The perineum was only cleansed if there was obvious contamination. If toilet was necessary the area was washed with aqueous Savlon (ICI) and then carefully dried.

Urine was collected as suprapubt, midstream, clean-catch, or bag specimens and then thoroughly mixed and divided into two parts. One part was sent for formal laboratory examination in containers with boric acid as a preservative (Porter and Brodie, 1969). The remaining urine was examined immediately in the clinic side-room.

Laboratory examination. Surface bacterial counts were performed on well dried plates of CLED medium (Oxoid). Each plate was inoculated with 1 ml of a dilution of urine prepared in nutrient broth. The dilutions used were tenfold, spanning the range $1 \times 10^{-2}$ to $1 \times 10^{-6}$. Cultures were incubated at $37^\circ$C for 18 hours and the dilution growing nearest to 200 colonies was counted.

Side-room examination. Labstix (Ames) were used among other methods to determine the presence of protein. The turbidity of the uncentrifuged specimens was gauged by holding a glass tube of urine to the daylight and was recorded. The deposits of centrifuged specimens of urine were examined microscopically in parallel with uncentrifuged samples. The deposits were prepared by centrifuging 10 ml urine for 5 minutes at 2500 rpm and discarding the supernatant. The deposit was shaken and two or three drops poured on to a glass slide and examined under a coverslip.

The centrifuged deposits and the uncentrifuged urine samples were examined unstained at a magnification of $\times 400$ by one of us (J.M.L.). The nature of the formed elements was noted, the number of pus cells per field recorded, and the number of organisms present was quantitated on the following scale: $0 =$no organisms; $\pm =$occasional organisms; $+=1-10$ organisms per field; $++=11-100$ organisms per field; $+++=$innumerable, loosely packed organisms; $++++=$innumerable, densely packed organisms.

Results

Table 1 shows that 38 of the 189 samples of urine contained $\geq 10^6$ organisms/ml urine as determined by the surface viable count. The centrifuged deposits of 33 (87%) of the 38 infected urines contained more than 10 organisms per field on microscopical examination; 25 of these (66%) contained innumerable bacteria. In contrast, only 6 (4%) of the 151 urines that were sterile or grew fewer than $10^6$ organisms/ml urine on culture contained more than 10 bacteria per field, but none was considered to include innumerable organisms.

<table>
<thead>
<tr>
<th>No. of organisms/ml</th>
<th>No. of samples</th>
<th>++++</th>
<th>+++</th>
<th>++</th>
<th>+</th>
<th>0</th>
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</thead>
<tbody>
<tr>
<td>$10^6$</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>$10^7$</td>
<td>2</td>
<td>1</td>
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<td>0</td>
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<tr>
<td>$10^8$</td>
<td>27</td>
<td>2</td>
<td>19</td>
<td>5</td>
<td>1</td>
<td>0</td>
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<tr>
<td>$10^9$</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
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<tr>
<td>$10^4$</td>
<td>11</td>
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<td>0</td>
<td>2</td>
<td>3</td>
<td>6</td>
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<tr>
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<td>0</td>
<td>0</td>
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<td>9</td>
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<tr>
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<td>1</td>
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<tr>
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<tr>
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<td>1</td>
<td>3</td>
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</table>
The differences between the microbiological observation on the spun deposits and uncentrifuged urines among the 38 samples growing $\geq 10^5$ organisms/ml are summarized in Table 2. This shows that more than 10 organisms per field were seen on 33 (87%) occasions when the centrifuged deposit was examined, but in only 29 (76%) specimens when the unspun urine was used. Table 3 shows that only 14 (36%) of the 38 urines that grew $\geq 10^5$ organisms/ml also contained more than 5 pus cells in each microscopic field, while in 8 (21%) of the samples no pus cells were seen.

Six (16%) of the 38 urines growing $\geq 10^5$ organisms/ml of urine gave a positive reaction for protein and 19 (50%) were judged to be significantly turbid when viewed against the light, but 9 (24%) were recorded as crystal clear.

**Discussion**

These results show that infected urine may be normal on naked eye examination, negative for protein, and contain no excessive pus cells. In marked contrast, however, the unstained urinary sediment of infected urine invariably is seen to be abnormal using a simple side-room procedure. The present observations confirm that seeing more than 10 organisms per field on microscopy of the centrifuged deposit correlates closely with the subsequent laboratory culture, for 87% of the infected urine included more than 10 organisms per microscopic field while only 4% of the urines growing fewer than $10^5$ organisms/ml were recorded as containing more than 10 bacteria per field on microscopic examination. If no organisms or only occasional bacteria are seen in a deposit, then it is most unlikely that there will be a subsequent significant growth, for only 1 of 129 urines with these microscopic findings yielded a culture of $\geq 10^5$ organisms/ml.

It is notable that 4 of the 6 'false positives' occurred in girls over the age of 12 years and that among younger children of both sexes such spurious findings were remarkably uncommon. Further, in these 4 urine specimens from older girls epithelial squames were frequent, all contained 1-5 pus cells per field, were cloudy on naked eye examination, and were regarded from the beginning as likely to be contaminated. Thus, seeing more than 10 bacteria per field in unspun deposits of centrifuged urine provides significant evidence that antibacterial therapy should be started at once particularly in young children, while the failure to see organisms suggests that such treatment is unnecessary. Only 1 infected child in 9 will be missed by this screening procedure and only 1 child in 25 will be given antibiotics unnecessarily.

Ten organisms per microscopic field can be seen by inexperienced workers and can be appreciated at a glance after some training. Below this number, however, organisms are not recognized as readily or consistently. In our investigation, taking more than 10 organisms per field as the recommended separation point in identifying the urines likely to grow $\geq 10^5$ organisms/ml, 87% were recognized among the deposits of centrifuged urine but only 76% with the uncentrifuged samples. We find examination of the centrifuged deposit preferable to examination of uncentrifuged urine.

Our findings confirm those of Kunin (1961) who noted a good correlation between bacterial microscopic examination of urine deposits and their subsequent bacterial count. Likewise, Robins et al. (1975) obtained an 88% agreement between microscopic examination of unspun samples and dip-slide culture of urine. Because of the ease with which 10 organisms per field can be identified we prefer to centrifuge the specimens before microscopy. The technique is simple and reliable for use in the ward and outpatient department, school and child health clinics, or in general practitioner surgeries, to facilitate early diagnosis and treatment of urinary tract infection. Clinical students and house officers should receive more training in the technique of urine microscopy.

**Summary**

Side-room examination of fresh samples of urine was compared with the results of surface viable bacterial counts. Examination of centrifuged deposits of urine
for bacterial content was shown to compare very well with subsequent culture results. 87% of infected urines were detected, and only 6% of noninfected urines were wrongly identified. Evaluation of the uncentrifuged samples was less easy. There was poor agreement between the naked eye appearance, the presence of protein, and the pus cell count and the ultimate laboratory bacterial count. Microscopy of urinary sediments after centrifuging is recommended to assist in the rapid diagnosis of urinary tract infections particularly in young children.

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References


J. M. LITTLEWOOD, S. I. JACOBS, and C. H. RAMSDEN

*St. James's Hospital, Leeds LS9 7TF.*

Correspondence to Dr. J. M. Littlewood.

Bladder emptying in neonates

It has been suggested that neonates may not empty their bladders completely when micturating (Sertel and Scopes, 1973). At school age, girls have a higher incidence of urinary tract infection than boys (Mair, 1973) while in neonates the sex incidence is more equal, though some authors have found a higher incidence in males (Lincoln and Winberg, 1964). It has been suggested that incomplete bladder emptying occurs only in the male neonate, and that this explains the sex incidence in urinary infection (Johnston, 1976). In addition, residual urine in the bladder would invalidate timed urine collections.

Ultrasound scanning is a safe noninvasive investigation already widely used in obstetrics and for prenatal diagnosis. Although the fetal bladder can easily be shown using ultrasound (Campbell et al., 1973), no technique has previously been described to show the bladder in the newborn. The aim of this study is to determine the effectiveness of bladder emptying in the neonate.

Subjects

Sixteen normal newborn infants (9 males, 7 females) were studied. Their ages ranged from 2–13 days. Informed consent was obtained from the mother who was encouraged to come with her child to the ultrasound department. Babies were selected only if their mothers had had an ultrasound scan during pregnancy, and thus were familiar with the apparatus.

Methods

A Diasonograph NE 4102 with a 2–5 MHz probe was used for the ultrasound scanning. Most examinations were carried out after a scan converter had been added to the original machine, thus providing greyscale visualization.

A 'water bath' method was used as follows. The baby's skin was coated with warm olive oil. The water bath consisted of a sheet of plastic held in a metal frame which was held over the baby's abdomen. It was noted that when the water bath was held over the baby's abdomen it helped to calm the baby so that he was less active. Cooling was avoided by the addition of warm water to the water bath. The babies were scanned in the supine position at frequent intervals shortly after feeding until they passed urine. They were scanned again immediately after micturition. When the total quantity of urine passed was caught, its volume was measured in a volumetric measuring cylinder.

Scans were made in two planes. The initial scan was always in the sagittal plane. The second scan was made transversely in a plane at 90° to the long axis of the bladder, through its widest diameter. Further scans were made to determine the shape of the bladder. Where quantitation was possible, electronic calipers preset at 2 cm were drawn on each film and from this the scale of measurements could be determined. The volume of the bladder was calculated using the formula \( \frac{1}{2}abc \) where \( a \), \( b \), and \( c \) are the three diameters of the bladder (Campbell et al., 1973). The amount of urine passed was estimated by subtracting the postmicturition volume from the premicturition volume.

Results

Both pre- and postmicturition scans support the view that the shape of the bladder is most accurately described as an ovoid. Of the 16 babies studied, 15 did not completely empty their bladders. In the remaining infant, who was female, the bladder could not be detected on the postmicturition scan.