Technique for the culture and direct sensitivity testing or large numbers of urine specimens

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SUMMARY A technique was required for batch culturing and direct sensitivity testing of urine specimens to reduce increasing demands on laboratory services. A comparison was made between the filter paper strip method and multipoint inoculator technique using a Denley inoculator (Denley Ltd, Billingshurst, Sussex). The results showed that multipoint inoculation was a rapid, economical, and reliable method for the handling of large numbers of urine samples.

The full investigation of all urine samples received by hospital microbiology departments has led to increasing demands on laboratory resources. Recent budgetary restraints have emphasised the need to save staff time and materials without adversely affecting the speed and accuracy of reporting.

Leigh and Williams's blotting paper strip method for detecting significant bacteriuria¹ indicated an advance in the batch culturing of urine specimens. The selection of specimens, however, for direct sensitivity testing on microscopic evidence remained a problem, and mixed cultures were not always easy to identify when growth was confluent.

The introduction of a Denley multipoint inoculator into the laboratory provided an opportunity to evaluate a more cost effective and time saving technique for both batch culturing and direct sensitivity testing of all urine samples. The ease of inoculation and the saving in media by using the 21 pin head of the inoculator allows a combination of media to be chosen to help isolate a wide range of organisms and to recognise mixed cultures. The colony counts obtained using the stainless steel pins for inoculation were calibrated with the bacterial count by the Miles and Misra method,² and the two methods were compared.

Material and methods

CALIBRATION

Initially it was essential to calibrate the inoculum delivered by the steel pins of the multipoint inoculator. The correlation between the number of organisms/litre and the number of colonies/pin inoculum was determined as follows: overnight broth cultures of *Escherichia coli*, *Klebsiella aerogenes*,

Pseudomonas aeruginosa, *Streptococcus faecalis*, and *Staphylococcus saprophyticus* were diluted to extinction in Ringer's solution (Oxoid Ltd). Tests for total viable counts were performed, within 20 minutes of the dilutions being made, by the Miles and Misra technique. Fifty replicate cultures of the middle ranges of dilutions were performed for each organism on cystine lactose electrolyte deficient agar (CLED, Oxoid Ltd) using the multipoint inoculator. All plates were incubated aerobically at 37°C overnight.

Counts were made, and the correlation between the colony count achieved with the multipoint inoculator and the viable count was determined for each organism.

SPECIMENS AND MEDIA

Urine samples were collected by ward staff into 30 ml sterile plastic universal containers, transported to the laboratory, and refrigerated immediately on receipt. The samples were examined by the routine method of microscopy and filter paper strip inoculation on to CLED agar. Urine samples with a count of > 50 leucocytes $\times 10^6/l$ were examined by a direct disc sensitivity test using diagnostic sensitivity test agar (Oxoid Ltd) with an *E coli* control (NCTC 10418). After completing routine testing one of us (SEB) batched the urine samples. Aliquots of urine were dispensed, using disposable glass pipettes, into plastic auto-analyser cups placed in Denley 21 well carriers for use with a multipoint inoculator and inoculated on to the selected media. All plates were incubated at 37°C overnight and read independently the next morning. Bacterial counts were assessed on CLED agar. A total of 350 urine specimens were cultured by the two methods and the results were then compared.

The following media were inoculated with multipoint

Table 1 Concentration of antibacterial agents used in sensitivity plates

Antibiotic	Medium	Final concentration (g/l)
Ampicillin	Diagnostic sensitivity test agar	0.01
Nitrofurantoin	Diagnostic sensitivity test agar	0.05
Sulphadimidine	Diagnostic sensitivity test agar + 2.5% lysed horse blood	0.05
Trimethoprim	Diagnostic sensitivity test agar + 2.5% lysed horse blood	0.025
Cephadrine	Diagnostic sensitivity test agar	0.025
Carbenicillin	Diagnostic sensitivity test agar	0.1
Gentamicin	Diagnostic sensitivity test agar	0.008

Table 2 Relation between average colony count achieved by multipoint inoculation and total viable count

Total viable count	Average colony count (organisms/l)		
	10 ⁸	10 ⁷	10 ⁶
<i>E coli</i>	36	9	3
<i>Staph saprophyticus</i>	26	6	2
Faecal streptococcus	25	6	2
<i>Klebsiella</i> spp	20	3	1
<i>Ps aeruginosa</i>	30	8	3

inoculation to determine the optimal combination for the identification of mixed cultures: horse blood agar (BA Oxoid Ltd), nutrient agar (NA Oxoid Ltd), CLED agar, MacConkey agar (MAC Oxoid Ltd), nalidixic acid blood agar (NAL BA Oxoid Ltd), aesculin bile agar (local formulation), and DNAase agar (Oxoid Ltd).

Sets of diagnostic sensitivity agar (DST Oxoid Ltd) plates containing different antibiotics (Table 1) were included with each batch of urine samples to determine the sensitivity pattern of all pure cultures.

Table 3 Results of culture of 350 urine specimens on CLED agar

Multipoint inoculation (No of organisms/l)	Filter paper strip (No of organisms/l)	Occurrence (%)
> 10 ⁸	> 10 ⁸	99 (28.3)
10 ⁷ -10 ⁸	10 ⁷ -10 ⁸	16 (4.6)
< 10 ⁷	< 10 ⁷	55 (15.7)
No growth	No growth	120 (34.3)
> 10 ⁸	10 ⁷ -10 ⁸	5 (1.4)
> 10 ⁸	< 10 ⁷	8 (2.3)
> 10 ⁸	No growth	1 (0.3)
10 ⁷ -10 ⁸	< 10 ⁷	6 (1.7)
10 ⁷ -10 ⁸	No growth	3 (0.9)
< 10 ⁷	No growth	25 (7.1)
< 10 ⁷	10 ⁷ -10 ⁸	1 (0.3)
No growth	< 10 ⁷	11 (3.1)

Results

Table 2 gives the results of average colony counts achieved by multipoint inoculation. The 50 replicates of each dilution showed little variation in the number of colony forming units.

Table 3 shows the results of the 350 duplicate urine cultures. Comparison of the two methods gave a total of 60 (17.1%) discrepant results. Fourteen (4%) samples examined gave a count of greater than 10⁸ organisms/l by the multipoint inoculation method and a lower count by the filter paper strip method. Of the samples that gave a count of greater than 10⁸ organisms/l by the filter paper strip method, none gave a lower count by the multipoint inoculation method. As can be seen from the analysis of discrepant results in Table 4 these consisted of both Gram positive and Gram negative organisms.

The following combination of selective and non-selective media were chosen as routine isolation media:

CLED agar

This non-inhibitory diagnostic agar gave good growth of most urinary pathogens.

MacConkey agar

A valuable medium for counting *Klebsiella* spp. Many strains are less mucoid on this medium, and mixed cultures of coliform organisms tend to be more easily recognised on this medium than CLED agar.

Aesculin bile agar

This allowed the prompt recognition of faecal streptococci, particularly in mixed cultures.

Nalidixic acid blood agar

This is an inhibitory medium that prevented the growth of most coliform organisms, and is an excellent medium for detecting *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and β haemolytic streptococci.

Table 4 Analysis of discrepant culture results

Organism	Bacterial count		Occurrence (%)
	Multipoint inoculation	Filter paper strip	
Coliform	$> 10^8$	10^7-10^8	5
Coliform	$> 10^8$	$< 10^7$	4
Enterococci	$> 10^8$	$< 10^7$	2
β Haemolytic streptococci	$> 10^8$	$< 10^7$	2
Coliform	$> 10^8$	No growth	1
Coliform	10^7-10^8	$< 10^7$	6
Coliform	10^7-10^8	No growth	2
Enterococci	10^7-10^8	No growth	1
<i>E. coli</i> , staphylococci, enterococci	$< 10^7$	No growth	5
Coliform	$< 10^7$	No growth	8
Staphylococci, enterococci	$< 10^7$	No growth	12
Coliform	$< 10^7$	10^7-10^8	1
Staphylococci, enterococci	No growth	$< 10^7$	6
Staphylococci	No growth	$< 10^7$	5

Discussion

The use of more than one medium for the isolation of urinary pathogens by the filter paper strip method is not feasible without the test becoming unwieldy. The ease of culturing specimens, however, on to a wide range of media using the multipoint inoculation technique permits flexibility in the choice of media used, and isolation patterns of the various media may be used to obtain a preliminary identification.

Of the 350 urine specimens examined by the two methods, results for 290 (86%) showed complete agreement. Of those that gave discrepant results, 48 (80%) gave higher counts by multipoint inoculation. The discrepancies may be due to the difference in physical properties of the stainless steel pins and filter paper strips. In a few specimens (3.4%) the filter paper strip method appeared more sensitive. Reasons for this are unclear, and in one case only (Table 3) was a borderline significant count shown by the filter paper strip method and not by multipoint inoculation. Repeat culture of the urine samples to check discrepant results was not feasible due to aging of the specimen.

A drawback of both systems was the difficulty in counting mucoid strains of *Klebsiella* spp. This was partly overcome by using several different culture media in the multipoint inoculation technique as some media seem to reduce the amount of free slime produced by *Klebsiella* spp. The combination of culture media chosen gave optimal isolation of organisms and recognition of mixed cultures of bacteria.

As a direct sensitivity test was performed on all urine specimens it was possible to issue a complete report the day after receipt of the specimen. The need to make arbitrary decisions as to which urine samples should be tested by direct sensitivity tests was re-

moved, and if mixed cultures were thought to be significant an indication of the most appropriate agent to be used could be given. No major problems were found in reading plates inoculated by the multipoint system despite the small area that each point covered, but use of a template was essential.

In comparison with the filter paper strip method, the batch culturing of urine samples described here proved to be an easy and rapid method of handling specimens. As all the media incorporated with antibiotics were prepared by the media department using pure powders the introduction of this technique has led to considerable savings in time and money.

The method described in conjunction with microscopical examination has been used in this laboratory for three years and has proved to be a reliable and economical technique for culture and direct sensitivity testing of large numbers of urine specimens. During this period we noted that not all strains of *Staph saprophyticus* grow on CLED agar on primary plating and over an 18 month period 160 such strains have been collected for further investigations.

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References

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