# Screening for urinary tract infection with the Mastascan Elite

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*Accepted: 7 October 2002*

**ABSTRACT**

Mastascan Elite urine screen (MUS) is compared to a conventional method to determine significant growth of urinary pathogens and determine identification and susceptibility. MUS can detect at least $10^3$ colony-forming units (cfu)/mL of commonly isolated pathogens and in this study identified 161 true positive bacterial growths (15.2%), 840 true negatives (79.2%), 54 false positives (5.1%) and six false negatives (0.6%). Overall performance in determining susceptibility to nine antibiotics directly from urine was 10.5% true resistance, 82.4% true susceptibility, 5.3% false resistance and 1.8% false susceptibility. Using limited tests, the system correctly identified 80.7% of isolates, 11.8% could not be identified at all and 7.5% were incorrectly identified. In this study, MUS provided 94.7% of results in 24 h, compared to 78.4% produced by the conventional method. Thus, MUS proved an effective and efficient method for processing urines, saving significant time, materials and human resources.

KEY WORDS: Screening. Urinary tract infection.

## Introduction

Urinalysis in the microbiology laboratory is a high-volume procedure that despite new approaches[1-4] is labour-intensive, time-consuming and laborious. One approach to this problem is to inoculate urine directly onto agar using a multipoint technique[5,6] and subsequently to use microtitre trays[7] and image analysis.[8,9]

The Mastascan Elite system (Mast Group, Bootle, UK) is a computerised, automated image-analysis system that can read disc-diffusion tests and agar-incorporation tests, as well as perform microtitre urine screening. The REDIPREP urine screen (Mast) comprises media for the determination of antibiotic susceptibility and bacterial identification, contained in 96-well microtitre trays. The number of antibiotics tested and the level of isolate identification are determined by the user. Plates are inoculated directly with urine using a multipoint inoculator and are read after overnight incubation.

This study compares the performance of Mastascan Elite and REDIPREP with a calibrated-loop culture technique[10] and a validated agar-incorporation method for identification and the determination of susceptibility.[11]

## Materials and methods

In the first part of this study, the ability of the Mastascan Elite urine screen (MUS) to correctly differentiate significant from non-significant urinary tract infection (UTI) was assessed. In the second, the ability of MUS to determine organism identification and antimicrobial susceptibilities was assessed. Urine was used as the inoculum for MUS, whereas comparative methods employed pure isolated cultures.

*Minimum detection levels*

A Miles and Misra[12] surface-viable counting technique was performed to determine the minimum detection levels of MUS. Suspensions (equivalent: 2 MacFarland) of *Escherichia coli, Staphylococcus aureus, S epidermidis, Enterobacter cloacae, Klebsiella pneumoniae, Enterococcus faecalis* and *Pseudomonas aeruginosa* were diluted 10-fold to a final $10^{-8}$ concentration.

Suspensions were inoculated using multipoint inoculation onto a microtitre tray containing CLED agar (CLED DM110; Mast). The plates were read after 18 h incubation at 35-37°C using the Mastascan Elite.

*Specimens*

Consecutive urine samples (1120) were obtained from the routine workload of the Royal Hallamshire Hospital, Sheffield, and comprised both mid-stream and catheter specimens.

*Conventional method*

A calibrated (10 µL) nichrome loop was used to streak one half of a CLED plate (CM423; Oxoid, Basingstoke, Hampshire, UK), which was incubated in air for 18 h at 35-37°C. Significant growth was interpreted as ≥ $10^3$ colony forming units (cfu)/mL urine. Using multipoint technology, a pure growth was used to determine susceptibility to a range of antibiotics incorporated in Isosensitest agar (Oxoid) at concentrations recommended by the BSAC.[13] Both sets of plates were read using an interfaced Mastascan Elite.

REDIPREP plates contained media for the determination of β-glucuronidase, phenylalanine deaminase, indole production and aesculin hydrolysis. Although not part of the evaluation, plates containing Chromogenic Urinary Tract

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Medium (CM949; Oxoid) were also examined. Susceptibility plates comprised Isotonic Sensitivity Test Agar (Mast) that contained antibiotics at concentrations recommended by the BSAC. Wells were inoculated with 0.3 µL urine using a multipoint inoculator and were read with a stand-alone Mastascan Elite.

*Data analysis*

Positive predictive value was calculated from the formula TP/(TP+FP), negative predictive value from the formula TN/(FN+TN), sensitivity from the formula TP/(TP+FN) and specificity from the formula TN/(FP+TN) where TP is true positive, FP is false positive, TN is true negative and FN is false negative.

**Table 1.** Comparison of culture results ($n$=1120) obtained using MUS and conventional culture techniques

| | | Conventional | | |
|---|---|---|---|---|
| | | No significant pathogen | Mixed bacterial growth | Significant pathogen |
| MUS | No significant pathogen | 840 | 0 | 6 |
| | Mixed bacterial growth | 11 | 24 | 24 |
| | Significant pathogen | 27 | 27 | 161 |

## Results

Preliminary experiments revealed that MUS could detect at least $10^3$ cfu/mL of *Escherichia coli, Proteus mirabilis, Enterobacter cloacae, Pseudomonas aeruginosa, K. pneumoniae, Staphylococcus aureus, S. epidermidis* and *Enterococcus faecalis*.

Of the 1120 specimens processed by both systems (Table I), 840 were deemed to be negative and 161 positive by both systems (89.4%). MUS detected 846 non-significant growths, 840 of which were deemed non-significant by the conventional method. MUS detected 59 mixed bacterial growths, 24 of which were deemed to contain a significant pathogen by the conventional method. Clearly, these specimens (5.3%) required further work before they could be interpreted with confidence and were omitted from further calculations. MUS detected 215 significant growths, 54 of which were deemed to be not significant or mixed by the conventional method.

Identification and antimicrobial susceptibility of the 161 'true positives' were determined using conventional methodology and MUS (Table 2). In total, the antimicrobial results for 1185 organism were compared, with 125 resistant by both methods (10.5%), 975 susceptible by both methods (82.4%), 63 falsely resistant by the MUS (5.3%) and 22 falsely susceptible by the MUS (1.8%). Major errors (false susceptibility) occurred more often with organisms other than *Escherichia coli* (17 versus 5) and more often with β-lactam antibiotics than others (17 versus 5).

Identification media evaluated in the study correctly identified 130/161 pathogens after overnight incubation (*E.*

**Table 2.** Comparison of antibiotic susceptibility results obtained using MUS and a conventional breakpoint antibiotic susceptibility testing technique

| | True resistance | False resistance | True susceptibility | False susceptibility |
|---|---|---|---|---|
| *E. coli* | 70 | 38 | 719 | 5 |
| *Klebsiella* spp. | 22 | 7 | 96 | 3 |
| *Proteus* spp. | 11 | 6 | 44 | 3 |
| Other Gram-negatives | 12 | 8 | 54 | 6 |
| Pseudomonads | 0 | 0 | 6 | 0 |
| Enterococci | 0 | 4 | 26 | 0 |
| Staphylococci | 10 | 0 | 30 | 5 |
| | | | | |
| Gram-positives | 10 | 4 | 56 | 5 |
| Gram-negatives | 115 | 59 | 919 | 17 |
| | | | | |
| Ampicillin | 65 | 8 | 76 | 9 |
| Co-amoxyclav | 7 | 7 | 126 | 3 |
| Cephalexin | 5 | 15 | 122 | 1 |
| Ciprofloxacin | 5 | 2 | 139 | 0 |
| Cefuroxime | 3 | 5 | 131 | 4 |
| Nitrofurantoin | 13 | 12 | 129 | 4 |
| Gentamicin | 2 | 5 | 139 | 0 |
| Trimethoprim | 25 | 9 | 108 | 1 |
| Vancomycin | 0 | 0 | 5 | 0 |
| | | | | |
| Total | 125 | 63 | 975 | 22 |

**Table 3.** Respective results for urine screening methods compared to conventional quantitative cultures during evaluation

| Urine screening system | Sensitivity | Specificity | Positive predictive value | Negative predictive value |
|---|---|---|---|---|
| MUS | 96.4% | 94.0% | 74.9% | 99.3% |
| Sysmex UF-100[3] | 83.1% | 76.4% | 62.0% | 90.7% |
| Yellow Iris[1] | 92.8% | 60.1% | 47.9% | 95.5% |
| Clinitek 200[2] | 96.9% | 75.7% | 52.7% | 98.9% |
| Uriscreen[4] | 65.2% | 85.7% | 57.7% | 89,2% |
| Gram's stain/unspun urine[14] | 96.0% | 99.2% | 97.6% | 98.7% |

*coli* 87; *Klebsiella* spp. 15; *Proteus* spp. 8; enterococci 15 and staphylococci 5), and failed to identify 19 strains (*E. coli*: 10; *Hafnia* sp.: 1; *Serratia* sp.: 1; *Enterobacter agglomerans*: 1; *Citrobacter diversus*: 3; pseudomonads: 3) and misidentified a further 12 (*Klebsiella* spp.: 1; *E. cloacae*: 2; *Escherichia coli*: 7; *Serratia* spp.: 1; *C. freundii*: 1).

On average, it took one person approximately five min to inoculate a set of 96 urines using MUS and approximately 15 min to read them. With conventional culture, one person took 1 h to inoculate 96 urines, 30 min to read the microscopy[14] and 40 min to read the plates. In this study, MUS produced 94.7% of results in 24 h, compared with 78.4% produced by the conventional method.

## Discussion

Preliminary experiments established that MUS could detect at least $10^3$ cfu/mL of the common pathogens likely to be isolated from urine. The majority of specimens (89.4% [14.4% positive, 75.0% negative]) were correctly interpreted by MUS after overnight incubation. Six specimens were interpreted as no significant growth by MUS, but as harbouring a potential pathogen by the conventional method. This could have been caused by sampling error, the presence of antibacterial agents or the subjective manner in which conventional cultures were interpreted.

Fifty-nine (5.3%) specimens were interpreted as mixed bacterial growth by MUS; however, on conventional culture, 24 grew a significant pathogen, 24 were mixed and 11 did not grow a pathogen. We feel that such specimens would require further processing before a report is issued and, in practice, these should have a full culture or repeat urines requested.

Of 161 specimens interpreted as containing a significant pathogen by MUS, 27 contained no significant pathogen and 27 yielded a mixed bacterial growth. Taking the conventional method as the gold standard, MUS identified 161 true positives (15.2%), 840 true negatives (79.2%), 54 false positives (5.1%) and six false negatives (0.6%).

Thus, MUS had a sensitivity of 96.4%, a specificity of 94.0%, a positive predictive value of 74.9% and a negative predictive value of 99.3%. Respective results for urine screening methods compared to conventional quantitative urine culture are shown in Table 3.

Antimicrobial susceptibility of the 161 true positives was determined using conventional methodology and MUS. Concordance was seen in 92.9% of 1185 results, with 5.3% minor errors and 1.8% major errors. Major errors occurred with organisms least likely to be isolated from urine (i.e., isolates other than *E. coli*, and mainly with β-lactam antibiotics). We felt that this error rate could be improved if a Knowledge Expert System[15] was used and that major errors could be eliminated if urines were screened for antibacterial activity.

Antibacterial activity detection plates are easily available from commercial sources and could be incorporated into the system if required. The majority of isolates used in this evaluation (82.3%) proved susceptible to urinary antibiotics; however, other workers[16,17] have also used MUS successfully to determine antimicrobial susceptibilities.

Identification media evaluated in this study correctly identified 80.7% of isolates to genus level, 11.8% could not be identified at all, and 7.5% were incorrectly identified. This was not unexpected as only a few biochemical tests are utilised in MUS. Adding more identification tests might increase the accuracy of identification achieved at the genus level.

Although not formally evaluated, Chromogenic Urinary Tract Medium was examined and found to be particularly useful for detecting mixed cultures. Overall, we felt that it would be as useful as the identification set evaluated in the current study. Chromogenic Urinary Tract Medium is available in a REDIPREP plate form.

At a time when Association of Clinical Pathologists (ACP) best practice guidelines suggest that, to influence treatment, results of urine culture should be available within 24 h of specimen receipt in greater than 90% of cases,[18] MUS proved efficient in this respect. In this study, MUS produced 94.7% of results in 24 h, compared with 78.4% by the conventional method.

The MUS instrument evaluated was a stand-alone item and would have benefited from the optional interface to a laboratory computer system. The facility to access microscopy results at the time of reading cultures on the Mastascan Elite would have been valuable. Alternatively, stratification of urines based on microscopy results would be useful. Furthermore, extension of the identification would provide the opportunity to automatically flag mixed cultures.

We would suggest that users inoculate a microtitre tray containing chromogenic medium for identification and to more readily detect mixed cultures. Detection of antibacterial activity in urine may prevent reporting of false-negative bacterial growths and false susceptibility to antibiotics. Adoption of a simple rules base would also improve antibiotic reporting.[15]

In conclusion, the Mastascan Elite MUS proved to be an effective screening method for UTI, saving time, materials and labour. □

## References


1 Barlett RC, Zern DA, Ratkiewicz I, Tetreault JZ. Screening for urinary tract infection with the Yellow IRIS. *Lab Med* 1992; **23**: 599-602.

2 Bowman RA, Riley TV. Evaluation of Clinitek 200 and RapimatII/T for screening for urinary tract infection. *J Clin Pathol* 1991; **44**: 58-60.

3 Okada H, Sakai Y, Miyazaki S, Arakawa S, Hamaguchi Y, Kamidono S. Detection of significant bacteriuria by automated urinalysis using flow cytometry. *J Clin Microbiol* 2000; **38**: 2870-2.

4 Palmer LS, Richards I, Kaplan W. Clinical evaluation of a rapid diagnostic screen (Uriscreen) for bacteriuria in children. *J Urol* 1997; **157**: 654-7.

5 Kerfoot P, McGhie D, Cahill E, Fountain T. A mechanised batch screening method for bacteriuria. *J Clin Pathol* 1983; **36**: 1318-9.

6 Cheetham P, Brown SE. Technique for the culture and direct sensitivity testing of large numbers or urine specimens. *J Clin Pathol* 1986; **39**: 335-7.

7 Tuck AC. An improved multipoint technique for the routine microbiological examination or urine specimens. *Med Lab Sci* 1987; **44**: 290-3.

8 Ridout PR. Urine screening using the Mastascan 2 system. *Mast Matters* 1988; **29**: 1-2.

9 Adcock S, Mole OJ, Adams H, Faiers M. Manual and semi-automatic multipoint urine methods. *BSMT Newsletter* 1989; **10**: 23-41.

10 McGeachie J, Kennedy AC. Simplified quantitative methods for bacteriuria and pyuria. *J Clin Pathol* 1963; **16**: 32.

11 Pease AA, Wheat PF, Harris DM. Antimicrobial susceptibility testing and biochemical identification using multipoint inoculation: 5 years' experience. *Med Lab Sci* 1988; **45**: 28-33.

12 Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. *J Hyg Camb* 1938; **38**: 732

13 Andrews JM, BSAC Working Party on Susceptibility Testing. BSAC standardized disc susceptibility testing method. *J Antimicrob Chemother* 2001; **48** (Suppl. S1): 43-57.

14 Cardoso CL, Muraro CB, Siqueira VLD, Guilhermetti M. Simplified technique for detection of significant bacteriuria by microscopic examination of urine. *J Clin Microbiol* 1998; **36**: 820-3.

15 Livermore DM, Winstanley TG, Shannon P. Interpretative reading: recognising the unusual and inferring resistance mechanisms from resistance phenotypes. *J Antimicrob Chemother* 2001; **48** (Suppl. S1): 87-102.

16 Kerfoot P. Routine screening of urines by multipoint inoculation. *Mast Matters* 1986; **27**: 7-10.

17 Henrichsen C, Moyes A. A semi-automated method for the culture, identification and susceptibility testing of bacteria direct from urine specimens. *Med Lab Sci* 1987; **44**: 50-8.

18 Graham JC, Galloway A. ACP Best Practice No 167: The laboratory diagnosis of urinary tract infection. *J Clin Pathol* 2001; **54**: 911-9.