Evaluation of three commercial agar preparations for the presumptive identification of significant urinary isolates

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Accepted: 25 October 2005

ABSTRACT

Three commercially available pre-poured chromogenic preparations – chromogenic urinary tract infection (UTI) medium (chromogenic UTI, Oxoid), CHROMagar Orientation (Becton Dickinson) and CPS ID2 (bioMérieux) – are evaluated in comparison to routine urine microbiology using cysteine lactose electrolyte-deficient (CLED) medium and conventional methods of identification and susceptibility testing by Vitek 1 for the majority of isolates. Most isolates were *Escherichia coli*, and a chromogenic medium has been shown to be a reliable, rapid and more economic medium on which to presumptively identify these organisms due to the substrates the strain utilises in the plate and the chromogen subsequently produced. However, the opacity of chromogenic UTI made the medium difficult to inoculate and read, although the colours were clear and strong. Although there was no statistical difference between CHROMagar Orientation and CPS ID2, the colours observed on the former were stronger. This meant that colony counting was possible at significant concentrations of 10^4 and 10^5 colony-forming units (cfu)/mL and it may be easier to detect mixtures that would indicate contamination. Chromogenic media are richer than CLED and a number of *Lactobacillus* spp. (normally regarded as normal flora) grew on this medium. These were not considered to be significant.

KEY WORDS: Chromogenic media.
Escherichia coli.
Urinary tract infection.
Vitek

Introduction

Chromogenic media have been introduced recently for the improved isolation and presumptive identification of urinary isolates.[1–11] These media have been used in laboratories where identification of Gram-negative pathogens by conventional methods has been reduced, or eliminated completely, due to cost pressures. The use of these media has the theoretical advantage of allowing reasonably accurate identification of the common urinary pathogens after overnight incubation at a much reduced cost. In this study, three commercially produced chromogenic preparations are evaluated in parallel for accuracy of identification, speed and relative cost.

Materials and methods

In the microbiology laboratory at University College London Hospitals (UCLH), urine samples were screened using the reverse cone technique[12] on cysteine lactose electrolyte-deficient (CLED) medium.[13] The majority of urinary isolates were identified by Vitek 1[14] and the most frequent organisms were *Escherichia coli*, enterococci and those of the Klebsiella-Enterobacter-Serratia (KES) and Proteus-Morganella-Providencia (PMP) groups.

As the majority of urinary isolates were *E. coli* it was considered appropriate to evaluate several commercial chromogenic media – chromogenic urinary tract infection (UTI) medium (chromogenic UTI, Oxoid), CHROMagar Orientation (Becton Dickinson) and CPS ID2 (bioMérieux) – available for urine microbiology. Table 1 lists the substrates used for the identification of bacteria using these media. Table 2 describes the colours of the various organisms isolated.

Organisms that appear as pink/purple colonies can be identified further using the spot indole reagent (para-dimethylaminocinnamaldehyde, Pro-Lab Diagnostics). *Proteus* species (colourless/ brown) can be identified using TDA reagent, and then differentiated further by the spot indole test. Colonies appearing blue are likely to be the KES group and these organisms need to be differentiated using conventional methods (Vitek, bioMérieux).[14] Organisms growing as tiny white colonies, which may be Gram-positive cocci or *Candida* spp., can be identified using a Gram stain and appropriate identification protocols.

Retrospective sampling of 171 positive routine urine samples using the reverse cone technique were tested on the three chromogenic media and assessed in comparison to growth on CLED medium. The results obtained were compared to a laboratory computer database search for a 12-month period to obtain the proportion of the species isolated in urinary tract infections (Table 3).

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Results

Results of this study revealed that 61% of isolates were *E. coli*, which is a common finding in most hospital laboratories. However, the different appearance of organism growth as coloured colonies on the chromogenic media means that it is easier and quicker to detect mixtures.

Table 4 shows the number of urines that grew a mixture of organisms on the chromogenic media, but were reported as pure growth using CLED medium. Table 5 shows the number of isolates that gave discrepant results with the different chromogenic media compared to the routine combination of CLED medium and Vitek to identify the organisms.

Table 1. Enzymes detected by chromogenic media.

CHROMagar Orientation	B-galactosidase	B-glucosidase
CPS ID2	B-glucuronidase	B-glucosidase
Chromogenic UTI	B-galactosidase	B-glucosidase

Table 2. Colour of pigment on chromogenic media.

Organism	Colour
Escherichia coli	Pale pink/beige
KES group	Dark blue
PMP group	Beige
Pseudomonas spp.	Pale green-colourless, feathery edge
Enterococcus spp.	Blue, small
Group B streptococci	Light blue, small
Staphylococcus aureus	Gold, small
Staphylococcus saprophyticus	Pink, small

Discussion

Recent work[1–11] has demonstrated the possible use of chromogenic agar for the presumptive identification of *E. coli* in patients with urinary tract infection. Three commercial companies supply pre-poured media in the UK.

Initial results showed that all the media evaluated in the present study support luxuriant growth of the common urinary pathogens. However, colour development on both CHROMagar Orientation and chromogenic UTI appeared stronger under independent examination by the majority of staff. Colours appeared more intense on chromogenic UTI, due to its opaque white background, but the medium was more difficult to use as a result of this opacity. Organisms growing as white colonies were difficult to observe and

Table 4. Detection of mixtures on chromogenic media reported as pure on CLED.

Chromogenic medium	Number of mixtures detected
CHROMagar Orientation	7
CPS ID2	9
Chromogenic UTI	14

Table 5. Analysis of discrepancies on chromogenic media.

CLED (identified result)	Discrepant number	Orientation	CPS ID2	Chromogenic UTI
Escherichia coli	11	4	7	5
KES group	9	0	4	9
Gram-positive cocci	1	1	1	1

Table 3. Comparison of trial isolates.

Organism	Number of isolates/year	Percentage	Number in trial	Percentage in trial
Acinetobacter baumannii	8	0.13	1	0.058
Candida spp.	77	1.27	1	0.58
Citrobacter freundii	2	0.03	2	1.1
Citrobacter koseri	79	1.3	2	1.1
Coagulase-negative staphylococci	57	0.94	1	0.58
Escherichia coli	3932	64.94	106	61
Enterococcus spp.	509	8.41	12	7
Klebsiella spp.	1	0.02		
Klebsiella oxytoca	88	1.45	1	0.58
Morganella morganii	29	0.48	1	0.58
Proteus mirabilis	212	3.5	8	4.6
Pseudomonas aeruginosa	192	3.17	5	2.9
Pseudomonas spp.	4	0.07		
Serratia marscencens	21	0.35	2	1.1
Serratia spp.	2	0.03		
Staphylococcus saprophyticus	63	1.04		

interpret. Although the colony colours were less intense on CPS ID2, it proved easier to use for overall differentiation of the common organisms.

Pseudomonas aeruginosa showed easily recognisable colonial morphology on CHROMagar Orientation and chromogenic UTI but produced smaller colonies on CPS ID2, similar to its appearance on CLED. Many Gram-positive organisms appear as small, pinpoint colonies on CLED, and there was no difference in the growth of these organisms on any of the chromogenic media evaluated. All staff that observed the media noted that the colours were clear and that mixtures were easier to detect than on CLED medium.

Mucoid organisms may cause a problem, as they can on CLED, in that it is impossible to determine whether or not the mucoid colonies are a mixture. This was often resolved by examination of follow-up and identification purity plates. The KES group of organisms, however, contributed only 4% of isolates in the present trial.

Proteus species appeared pale to dark brown on all the chromogenic media studied. Although spreading over the medium was inhibited, colony count proved difficult on chromogenic UTI because of merging. Some *Proteus* spp. may be multiresistant to antibiotics and therefore full identification and susceptibility testing is appropriate. Furthermore, in this study, 17 *Lactobacillus* species (presumed normal vaginal flora) were isolated on chromogenic agar. These were not considered to be clinically significant.

Prior to the introduction of a chromogenic medium, all urinary isolates were fully identified using the Vitek 1 system, following the manufacturer's instructions. The list price for suitable Vitek cards is £3.80 for the identification of each urinary isolate. As 106 *E. coli* strains were identified correctly using chromogenic media, the equivalent cost using Vitek would have been £402.80. Presumptive identification on a chromogenic medium using the reverse cone technique on quarter plates[12] would involve the use of 27 plates at a cost of approximately £20-£25, depending on the medium used. In 2002 the UCLH laboratory processed 3932 strains of *E. coli* from urine culture on Vitek 1, at an annual cost of £14,942. Had a chromogenic medium been used the equivalent cost would have been between £587 and £934.

Kass[15] showed the value of enumeration to determine the significance of urinary isolates (i.e., 10^5/mL in urine microbiology), and this was revised subsequently by Stamm and colleagues to 10^4 organisms/mL.[16] The present study showed that colony counting on chromogenic media was possible at significant concentrations of 10^4 and 10^5 colony-forming units.

In conclusion, urine microbiology can be made simpler, cheaper and more efficient by use of a chromogenic medium. In this study, CHROMagar Orientation proved to be the medium of choice to replace CLED for the isolation and presumptive identification of *E. coli* and other common urinary tract pathogens. □

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