

HiCrome UTI Agar : An Alternative To Conventional Media For Presumptive Identification And Isolation Of Uropathogens



Microbiology

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ABSTRACT

The increase in resistance of uropathogens to antimicrobial agents, especially in hospitalized patients, demands rapid identification of the pathogen. Current detection methods including culture and identification are time-consuming. The chromogenic media offers simultaneous presumptive identification of gram-positive and gram-negative bacteria and yeasts on a single medium by means of distinct colony colours produced by reactions of genus- or species-specific enzymes with a suitable chromogenic substrate. The present study was undertaken to validate the usefulness of HiCrome UTI agar as a primary urine culture medium for its rate of isolation and presumptive identification of uropathogens in comparison to CLED agar, BA and MA agar. This study included 1500 midstream and/or catheter-catch urine samples from clinically suspected UTI patients of different age and sex groups. HiCrome UTI agar, MacConkey agar, Blood agar and CLED agar media were obtained as a dehydrated powder from HiMedia laboratories. Urine culture was done on above said media and presumptive identification of bacterial growth was done. Out of total 1500 urine samples, 552 urine samples showed significant growth of a single isolate and 13 urine samples showed mixed growth of two organisms and 50% samples were sterile. Among all the bacterial isolates *E. coli* were maximum in number ($n=195$), followed by *Klebsiella* spp. ($n=81$). *Staphylococcus aureus* and *Candida* spp. constitute 12.97% and 7.61% of the total urinary isolates, respectively. Maximum number of urinary isolates was presumptively identified on HiCrome UTI agar (88.06%) and there is no significant difference in the rate of presumptively identification from CLED agar and Blood agar and MacConkey agar in combination (62.97% and 61.24, respectively). Presumptively identification of mixed growth of organisms on HiCrome UTI agar was 100%, but CLED agar, Blood agar and MacConkey agar in combination failed to presumptively identify 100% mixed growth of organisms. The overall findings of this study suggests that though expensive, chromogenic media like HiCrome UTI Agar media, is an acceptable alternative to traditional media for the isolation of urinary pathogens.

Introduction:

Urinary tract infection (UTI) is the most common type of infection and continues to be a major health problem. The increase in resistance of microorganisms to antimicrobial agents, especially in hospitalized patients, demands rapid identification of the pathogen. Early information enables the selection of the appropriate antibiotics prior to the results of standard susceptibility tests and may thereby prevent outbreaks. ⁽¹⁾

For many years Blood, Cystine lactose electrolyte-deficient, and MacConkey agars have been used for the detection of urinary tract pathogens, as well as for the differentiation of a few of them. ⁽²⁾ These current detection methods are time-consuming, most typically consisting of 2 to 3 days of culture. ⁽¹⁾ Therefore any new method or medium with the ability to streamline urine culture processing in a meaningful way should be welcomed.

The aim of the microbiology laboratory is to reduce morbidity through accurate identification of pathogens with appropriate antimicrobial sensitivity testing in short turnaround time. ⁽³⁾

In the last few years several chromogenic media have been developed and commercialized, allowing for more specific direct differentiation of microorganisms on primary plates. The chromogenic agar offers simultaneous presumptive identification of gram-positive and gram-negative bacteria and yeasts on a single medium by means of distinct colony colours produced by reactions of genus- or species-specific enzymes with a suitable chromogenic substrate. ⁽²⁾

The present study was undertaken to validate the usefulness of HiCrome UTI agar as a primary urine culture medium for its rate of isolation and presumptive identification of uropathogens in comparison to CLED agar, Blood and MacConkey agar in a Pravara Tertiary Care Hospital, Loni, Ahmednagar.

Materials and Methods:

The protocol was approved by Institutional Ethical Committee, Pravara Medical College and Hospital, Loni and the study included 1500 midstream and/or catheter-catch urine samples from clinically suspected UTI patients of different age and sex groups attended either at the outpatient department or admitted in Pravara Tertiary Care Hospital, Loni, from March 2011 to August 2012. A detail medical history of the patient was taken and the data was recorded.

Preparation of media: HiCrome UTI agar, MacConkey agar, Blood agar and CLED agar media were obtained as a dehydrated powder from HiMedia laboratories (HiMedia Laboratories Pvt. Ltd. Mumbai-400086, India). All culture petri plates were prepared in laboratory by following manufacturer's instructions and recommendations.

Quality control: Each batch of medium used in this trial was tested for sterility, biochemicals and chromogenic reactions with American Type Culture Collection (ATCC) strains.

ATCC Control strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* 13883, and *Candida albicans* ATCC 10231 were used for quality control. ⁽²⁾

Urine culture was performed for samples that showed pus cells ≥ 5 /HPF on microscopy of a centrifuged deposit of urine (HiCrome 2). All the urine samples were inoculated aseptically on HiCrome UTI agar, CLED agar, Blood agar and MacConkey agar media with a calibrated loop and were incubated aerobically at 37°C for 18-20 hours.

Criteria for significant bacteriuria ⁽⁴⁾:

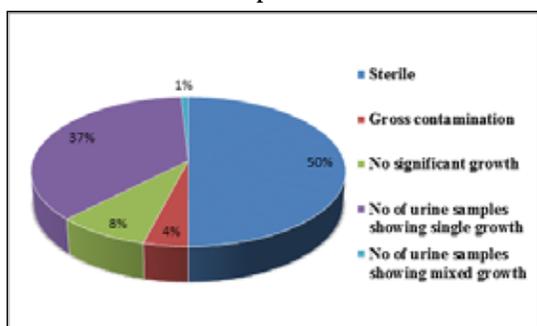
1. Presence of $>10^5$ cfu/ml of non-coliforms or $>10^2$ cfu/ml of coliforms in a symptomatic woman.
2. Presence of $>10^3$ cfu/ml of bacteria in a symptomatic man.
3. Growth of two different organisms from possible uropathogens at a concentration of 10^4 cfu/ml.

Presumptive Identification: Presumptive identification of bacterial growth was done on HiCrome UTI agar according to colony morphology and colour as depicted by the manufacturer. Colonies on the MAC agar and BA were also identified following colony characteristics against each of the uropathogens. The final identification of the isolates was done using standard identification protocol such as Gram's staining, motility test, catalase test, coagulase test, oxidase test and other relevant biochemical tests as appropriate for the isolates. ⁽⁶⁾

Results:

In the present study, a total of 1500 urine samples were processed and out of them, 552 urine samples showed significant growth of a single isolate and 13 urine samples showed mixed growth of two organisms. 50% samples were sterile and 4% samples showed gross contamination. No significant growth was observed in 125 of total urine samples (Figure 1).

Figure 1
Distribution of urine samples



Out of 578 urinary isolates, 363 were Gram negative bacilli and 171 were Gram positive cocci. *Candida spp.* was isolated from 44 urine samples.

Among all the bacterial isolates (n=578), *E.coli* were maximum in number (195), and out of 195, 185 *E.coli* showed single growth and 10 *E.coli* were in mixed culture. In the same way, *Klebsiella spp.* was found in total 81 samples, out of them 74 showed single growth and 7 were in mixed culture. *Staphylococcus aureus* and *Candida spp.* constitute 12.97% and 7.61% of the total urinary isolates, respectively (Table 1).

Table 1
Spectrum of organisms isolated from culture positive cases

Organism	Single	Mixed	Total No. (%)
<i>E. coli</i>	185	10	195 (33.74)
<i>Klebsiella spp</i>	74	7	81 (14.01)
<i>Staphylococcus aureus</i>	70	5	75 (12.97)
<i>Enterococcus spp.</i>	55	3	58 (10.04)
<i>Staphylococcus saprophyticus</i>	38	0	38 (6.58)
<i>Pseudomonas spp</i>	35	1	36 (6.23)
<i>Citrobacter spp</i>	23	0	23 (3.98)
<i>Acinetobacter spp</i>	15	0	15 (2.59)
<i>Proteus spp</i>	13	0	13 (2.25)
<i>Candida spp.</i>	44	0	44 (7.61)
Total no. of organisms	552	26	578 (100)

These urinary isolates were presumptively identified on HiCrome UTI agar, CLED agar, Blood and MacConkey agar. As shown in

Table 2, maximum number of isolates was presumptively identified on HiCrome UTI agar (88.06%) and there is no significant difference in the rate of presumptively identification from CLED agar and Blood agar and MacConkey agar in combination (62.97% and 61.24, respectively).

Table 2
Presumptive identification of organism from different primary culture plate

Organism presumptively identified	On HiCrome UTI agar (%)	On CLED agar (%)	On Blood agar and MacConkey agar (%)
<i>E. coli (n= 195)</i>	189 (96.92)	176 (90.26)	172 (88.21)
<i>Klebsiella spp (n= 81)</i>	81 (100)	81 (100)	80 (98.76)
<i>Staphylococcus aureus (n= 75)</i>	69 (92)	46 (61.33)	52 (69.33)
<i>Enterococcus spp. (n= 58)</i>	51 (87.93)	21 (36.20)	0 (0)
<i>Staphylococcus saprophyticus (n=38)</i>	29 (76.31)	0 (0)	0 (0)
<i>Pseudomonas spp (n=36)</i>	31 (86.11)	29 (80.5)	33 (91.66)
<i>Citrobacter spp (n= 23)</i>	12 (52.17)	0 (0)	0 (0)
<i>Acinetobacter spp (n= 15)</i>	3 (20)	0 (0)	0 (0)
<i>Proteus spp (n= 13)</i>	12 (92.30)	11 (84.61)	13 (100)
<i>Candida spp. (n= 44)</i>	32 (72.72)	0 (0)	4 (9.09)
Total (n= 578)	509 (88.06)	364 (62.97)	354 (61.24)

HiCrome UTI agar supported not only the growth of all uropathogens but mixed infections can also be diagnosed more easily. As shown in Table 3, presumptively identification of mixed growth of organisms on HiCrome UTI agar was 100%, but CLED agar, Blood agar and MacConkey agar in combination failed to presumptively identify 100% mixed growth of organisms.

Table 3
Presumptive identification of mixed growth on different primary culture plate

Mixed growth of Organisms presumptively identified	On HiCrome UTI agar (%)	On CLED agar (%)	On Blood agar and MacConkey agar (%)
<i>E. coli + Klebsiella spp (n=5)</i>	5 (100)	4 (80)	4 (80)
<i>E.coli + Staphylococcus aureus (n=3)</i>	3 (100)	1(33.33)	2(66.66)
<i>Klebsiella spp +Staphylococcus aureus (n=2)</i>	2 (100)	1 (50)	2 (100)
<i>E. coli + Enterococcus spp. (n=2)</i>	2 (100)	0 (0)	0 (0)
<i>Pseudomonas spp+ Enterococcus spp (n=1)</i>	1 (100)	0 (0)	0 (0)

Discussion:

In the present study, out of 1500 urine samples, 36.80% urine samples showed significant growth of a single isolate and 0.86% urine samples showed mixed growth of two organisms. 50% samples were sterile and 4% samples showed gross contamination. No significant growth was observed in approximately 9% of total urine samples (Figure 1).

Our results correlate with the study by R Parveen *et al* in which 31.67% samples showed single growth, 2% showed mixed growth and 66.33% samples yielded no growth. ⁽⁶⁾

Findings of the study done in Bangladesh which showed 38.5% single growth and 4% mixed growth ⁽⁷⁾ are also simi-

lar with our study. In contrast to the findings of the present study a higher rate was reported from UK (54.2% single growth and 21.6% mixed growth).⁽⁶⁾ This might be due to the fact that urine samples having pus cell > 200/cmm were included in that study.

A lower bacterial isolation rate also was reported from Israel (19.55% single growth and 1.66% mixed growth),⁽⁹⁾ India (20% single growth and 4% mixed growth)⁽¹⁰⁾ and California (24.5% single growth and 17.5% mixed growth).⁽¹¹⁾ Such lower isolation rate in their study were probably due to fact that all urine samples were cultured irrespective of pus cell count, while in the present study urine samples having pus cells \geq 5/HPF were included.

The rate of isolation and pattern of major uropathogens of the present study are in accordance with a few studies carried out on both chromogenic and conventional media. According to present study, *E.coli* was the predominant causative agent (33.74%), followed by *Klebsiella spp.* (14.01%). *Staphylococcus aureus* and *Candida spp.* constitute 12.97 % and 7.61% of the total urinary isolates, respectively (Table 1).

A study by Laila Akter stated, *E. coli* was the leading bacteria isolated from 59.30% samples followed by *Enterococcus spp.* 11.56%, *Klebsiella spp.* 11 (5.53%), *Pseudomonas spp.* 2.01% and *Proteus spp.* 1.51%.⁽⁴⁾

Similar results were found in a study from Hyderabad (India) and noted the most common organism was *Escherichia coli* (47.5%) followed by *Klebsiella spp.* (18.1%), *Enterococcus* (10.8%), *Pseudomonas* (4.7%) and *Proteus mirabilis* (1.4%).⁽¹²⁾ Higher isolation rate of *E.coli* was noted in the study by R Parveen *et al* i.e.64.49% followed by 11.21% *Klebsiella spp.*⁽⁶⁾

Presumptive identification of bacterial isolates in urine culture is time consuming and requires a great deal of experience when using traditional media like Blood agar, MacConkey agar and CLED agar. On the contrary, HiCrome UTI agar medium was found to be much superior over conventional media for its higher rate of isolation and uniform interpretation for identification of uropathogens. As many of the extra tests for bacterial identification associated with conventional culture methods were no longer required, chromogenic medium substantially reduced the laboratory workload with concomitant high bench throughput.⁽⁴⁾

In our study, on Hicrome UTI agar approximately 88% of the isolates were presumptively identified while CLED agar, BA and MA in combination could presumptively identify only 62.97% and 61.24% respectively (Table 2).

These results are somewhat similar with the results noted by R Perveen *et al.* According to this study 94.39% of isolates were presumptively identified by HiCrome UTI agar media, 74.77% by CLED agar media and 77.57% by Blood agar and MacConkey agar media in combination.⁽⁶⁾

On the other hand, HiCrome UTI agar presumptively identified 97.49% of isolates whereas MA and BA contributed in 67.34% and 36.68% presumptively identification of organisms, respectively, according to the study by Laila Akter in 2014.⁽⁴⁾ While, Saba Quiser *et al.* in 2011⁽¹³⁾ found both the media (chromogenic UTI medium and CLED agar) were comparable as far as isolation of bacteria was concerned.

Rafaat *et al*⁽¹⁴⁾ showed that the isolation and identification of isolates was found to be best on chromogenic UTI agar (98.9%), followed closely by US (97.7%) and least favorable by the conventional method (94.4%).

A study by Sharmin *et al.*⁽¹⁵⁾ also concluded that the chromogenic medium considerably reduced workload and minimized the use of conventional identification tests.

HiCrome UTI agar contains all essential nutrients to support the growth of possible uropathogens that is why all isolates were possible to be grown on to this medium. Slightly lower yielding rate on MAC agar can be explained by its limitations of not supporting all organisms involved in UTI like *Staph. saprophyticus* and *Enterococcus spp.*, because it is a selective medium for members of Enterobacteriaceae.⁽⁴⁾

As far as the presumptive identification of bacterial species is concerned, significantly high percentage of bacterial species were possible to be identified on HiCrome UTI agar by matching with standard colours as opposed to conventional culture system. This high rate of identification could be correlated with the ease of identification technique by seeing the distinct and perceivable colony colour produced by each of the bacterial species on chromogenic agar medium.

On the HiCrome UTI agar colonies of *E. coli* appear as purple to magenta because of α -galactosidase production thus allows its definite identification without need for further biochemical tests.

The present study revealed that, 96.92% *E.coli* were presumptively identified on Hicrome UTI agar whereas 90.26% and 88.21% *E.coli* were presumptively identified on CLED agar and in combination of BA and MA, respectively.

This results are consistent with the study by R Parveen which stated 94.20% *Esch.coli*, were identified in HiCrome UTI agar, whereas 79.71% were in CLED agar media and 82.61% in MacConkey & Blood agar media.⁽⁶⁾ Similarly another study in India reported 90% *Esch. coli* in chromogenic media (Uricrom II)⁽¹⁶⁾ whereas a study by Raafat *et al* noted sensitivity of Chromogenic UTI agar for *E. coli* was 95%.⁽¹⁴⁾

The chromogenic media also added the advantage on identification of a few non-lactose variety of *E. coli*, which might be the reason of decreased rate of identification on MA agar.⁽⁴⁾

Strains that produce α -glucosidase, such as *Enterococci* and the *Klebsiella-Enterobacter-Serratia* group form blue colonies result from hydrolysis of glucoside, a chromogenic substrate incorporated in the medium. Similarly, tryptophan is also present in the medium to detect members of the *Proteus* group, which generates a diffuse brown coloration as a result of tryptophan deaminase production.⁽¹⁷⁾

The present study also reveals that Hicrome UTI agar and CLED agar contributed 100% presumptively identification of *Klebsiella spp.* whereas BA could identify 98.76% of *Klebsiella* isolates. These results are very similar with the results in a study by Laila Akter which stated 100% *Klebsiella* isolates were presumptively identified on Hicrome UTI agar.⁽⁴⁾

Raafat *et al* found the sensitivity of chromogenic UTI agar for the identification of KESC group was 75%⁽¹⁴⁾, while a study by BioMerieux in France⁽¹⁸⁾ found chromogenic UTI agar could identify 97.67% KESC group.

Enterococci produce Beta-glucosidase, that attacks Beta-glucoside chromogenic substrate, generate distinct small blue colour colonies.

In our study, approximately 88% of *Enterococcus spp.* were presumptively identified on Hicrome UTI agar while 36% on CLED agar and none of the *Enterococcus spp.* was presumptively identified on BA and MA in combination. Very less number of *Enterococcus spp.* was presumptively identified on CLED agar which was explained that on CLED medium the presence of *Enterococci* was frequently masked by larger colonies of Gram negative.

A study by Sharmin et al.⁽¹⁵⁾ in Bangladesh revealed that 100% of Enterococci were identified on chromogenic agar but only 95% on CLED agar. According to R Parveen 100% were identified on HiCrome UTI agar media but on CLED agar media only 33.33% *Enterococci* were identified.⁽⁶⁾

Coloured colonies also facilitated more accurate detection of mixed cultures which helped to diagnose contaminated specimens, leading to reduced time spent and unnecessary work up of clinically insignificant organisms.⁽¹⁴⁾

Out of 565 culture positive samples, 13 samples showed mixed growth. Among the mixed growth, 100% organisms were identified on HiCrome UTI Agar media due to distinct colour produced by the different organisms, whereas only 6 samples (mixed growth) were identified on CLED agar and 8 samples (mixed growth) were identified on BA and MA in combination.

Very similar results were found in the study by R Parveen and Laila Akter.^(6, 4) The rate of identification of mixed culture on the BA and MAC agar was very poor due to their limitations in differentiating the colonies. Improved detection of mixed cultures may help to identify contaminated specimens and therefore lead to a reduction in the prescription of unnecessary antibiotics.⁽⁴⁾

Conclusion:

The overall findings of this study suggests that though expensive, chromogenic media like HiCrome UTI Agar media, is an acceptable alternative to traditional media for the isolation of urinary pathogens. HiCrome UTI Agar may facilitate improved sensitivity of identification of some Gram positive cocci (e.g., enterococci) in mixed cultures with *Enterobacteriaceae* and may promote more uniform interpretation of urine culture plates by less experienced bench technologists. Chromogenic media may also promote more rapid identification of the etiological agent(s) of infection and may provide clinicians with relevant information regarding their choice of empirical antimicrobial therapy for patients.

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