 DOI: 10.18231/2394-5478 .2017.0023

# Evaluation of urichrom –II for isolation of uropathogens in a tertiary care hospital

**Meenakshi Aggarwal[1,*], Sonal Saxena[2]**

[1]Specialist, Dept. of Microbiology, Kalawati Saran Children Hospital, New Delhi, [2]Professor, Dept. of Microbiology, Lady Hardinge Medical College & Associated Hospitals, New Delhi

***Corresponding Author:**
Email: drmagl2007@gmail.com

## Abstract

Urinary tract infections constitute a large share of human systemic infections and are one of the commonest bacterial infections that lead patients to seek medical care. Positive culture results are a prerequisite for timely and effective treatment of UTIs. In resource limited settings, conventional media and biochemicals are used for isolation of pathogens and a positive urine culture sometimes may require more than 48 hours for identification. To overcome this time lag, chromogenic media have been developed which help in early presumptive identification of the uropathogens and timely initiation of therapy. We have evaluated one such media Urichrom–II™ (International Microbio, France) in comparison to in house conventional media for growth of common urinary pathogens.

**Keywords:** UTI Pathogens, Chromogenic Media.

## Introduction

Urinary tract infections constitute a large share of human systemic infections and are one of the commonest bacterial infections that lead patients to seek medical care.(1) Understandably, the urine culture laboratory of a hospital receives very high number of daily samples. Quality processing of such large sample numbers requires uninterrupted supply of media, biochemical tests and dedicated manpower.

Positive culture results are a prerequisite for timely and effective treatment of urinary tract infections. Conventionally, a positive urine culture requires more than 48 hours for identification and sensitivity testing. Automated systems though desirable, are usually not available in laboratories of resource limited settings. Blood agar (BA), MacConkey agar and Cysteine Lactose Electrolyte Deficient (CLED) medium are routinely recommended for processing of urine samples.(2) Cysteine Lactose Electrolyte Deficient Media has been found to be superior to MacConkey agar as it supports better growth of Gram positive cocci and yeasts and inhibits swarming of Proteus spp.(3)

A variety of chromogenic media have been developed in recent years which help in early presumptive identification of the uropathogens and timely initiation of therapy in high risk cases. By the inclusion of chromogenic enzyme substrates targeting microbial enzymes, such media are able to target pathogens with high specificity.(4) In this study an attempt has been made to evaluate URICHROM–II™ (International Microbio, France) in comparison to in house conventional media for growth of common urinary pathogens.

## Materials and Methods

**Clinical samples:** The study included 671 midstream and/or catheter catch urine samples obtained from both in and outpatients of Smt. Sucheta Kriplani and Kalawati Saran Children Hospitals which were found to have significant pyuria on direct microscopy.

**Conventional Culture media:** Culture plates of MacConkey agar and CLED agar were prepared in-house according to the manufacturer's recommendations, using commercially available dehydrated media (Hi Media Laboratories, Mumbai, India). They were dispensed into petridishes, checked for sterility and stored at 2-8°C till use.

**Urichrom II™:** This is an enzyme substrate medium available as pre-prepared media in bottles. The medium was liquefied and poured into plates according to the manufacturer's instructions. The prepared plates were stored at 2-8°C. Every fresh batch of medium was tested for its ability to support the growth of ATCC strains such as *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichiacoli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

**Inoculation of media and incubation:** All urine samples were inoculated simultaneously using standard loop for semiquantitative estimation on all three media. These plates were incubated aerobically at 37°C overnight and examined the next morning. A presumptive identification of the isolates was attempted based on the colony colour on the chromogenic media and compared to conventional biochemical results from the other media plates. The results are interpreted as per manufacturer's charts. *Escherichia coli* grew as distinct pink -burgundy colored colonies while the *KES group and Enterococcus* produced distinct blue colonies. Further *Proteus spp.* gave brown colonies, *Pseudomonas aeruginosa* showed light green colonies and Staphylococcus *aureus* grew as whitish colonies.

## Results

Growth characteristics were observed, recorded and compared for 671samples with significant pyuria on all three media. Out of total samples, in 506 (75.4%) samples, we encountered exact matching results in terms of growth and count of organisms. These results included either sterile cultures (184, 27.4%), single pathogenic species (187, 27.8%) or polymicrobial cultures (135, 20.1%). Table 1 shows the distribution of samples having similar/matching results.

**Table 1: Distribution of samples having similar/matching results in conventional and test media (n=671)**

| No. of samples showing matching results | 506 |
|---|---|
| Sterile culture | 184 |
| Single pathogenic Species | 187 |
| Polymicrobial Growth | 135 |

**Table 2: Break up of samples growing single pathogenic species where conventional and test media showed similar results**

| Single organism isolated | No. of samples |
|---|---|
| Escherichia coli | 93 |
| Klebsiella spp. | 13 |
| Pseudomonas spp. | 8 |
| Proteus spp | 5 |
| Staphylococcus aureus | 4 |
| Coagulase negative staphylococus | 6 |
| Enterococcus spp. | 27 |
| Candida spp. | 24 |
| Diptheroids/micrococci | 7 |
| Total | 187 |

**Table 3: Break up of polymicrobial urine cultures where conventional and test media showed similar results**

| Organisms isolated | No. of samples |
|---|---|
| Escherichia coli + Enterococcus spp. | 24 |
| Klebsiella spp. + Enterococcus spp. | 11 |
| Mixed bacterial species (>=3 similar types in both categories) | 74 |
| Escherichia coli + Coagulase negative staphylococus | 6 |
| Escherichia coli + Klebsiella spp. | 10 |
| Proteus spp. + Coagulase negative staphylococus | 6 |
| Acinetobacter spp.+ Coagulase negative staphylococus | 4 |
| Polymicrobial Matching Total | 135 |

In 165 samples (24.5%), the growth results varied between both the groups. Out of these 165 samples, in 116 samples (17.2%) better growth was seen on Urichrom II. Table 4 shows the distribution of samples showing different results on the test media and conventional media.

**Table 4: Break up of samples showing different results**

| | | | |
|---|---|---|---|
| **Better growth on UrichromII** | | | **116** |
| | Yeast isolated only on Urichrom II | | 41 |
| | Polymicrobial/mixed growth-Additional organism (N=54) | Gram Negative bacteria | 19 |
| | | Enterococuus | 26 |
| | | CONS | 09 |
| | Mixed Growth on Urichrom against no growth on conventional media | | 16 |
| | Escherichia coli isolated only on Urichrom | | 5 |
| **Better growth on conventional media** | | | **14** |

| | | |
|---|---|---|
| | Yeast isolated only on conventional media | 5 |
| | More count of pathogenic Gram Negative bacteria | 4 |
| | Escherichia coli isolated only on conventional media | 3 |
| **β-glucuronidase negative E.coli strains** | | **35** |
| Total | | 165 |

Striking results were found in the case of yeasts where in 41(6%) samples grew significant number of yeasts on the chomogenic media while both MacConkey and CLED gave sterile cultures. In 54 (8%) samples showing polymicrobial/mixed growth, there was an additional organism growth on the chromogenic media making the interpretation of mixed bacterial species more confirmatory.

In case of Escherichia coli, some special strains were encountered. Usually Escherichia coli are expected to be β-glucuronidase positive. Thirty Five (5.2%) strains of Escherichia coli were detected to be β-glucuronidase negative. They presented as lactose fermenting(26)/late lactose fermenting(5)/non lactose fermenting(4)on MacConkey and CLED but grew as white colonies on Urichrome–II™. These strains were identified as Escherichia coli on basis of biochemical tests. Also ten isolates of Escherichia coli were β glucuronidase positive but grew as non-lactose fermenting colonies on MacConkey and CLED.

For *Enterococcus* species, Urichrome II gave excellent results. Distinctive blue colour of *Enterococcus* spp. was immensely helpful in differentiation and we were able to detect Enterococcus as an additional organism in 26 samples with polymicrobial growth. Four samples were positive for *Staphylococcus aureus.* Growth was seen on all three media.

We found fourteen cases (2%) wherein there was more growth on conventional media as compared to Urichrom-II™ (Table 4).

## Discussion

Blood agar, MacConkey and CLED Medium are the three most commonly used media for processing of urine samples.[2] In resource limited settings, use of blood agar for high number of urine samples has always been a debatable issue. MacConkey medium is very useful to identify lactose and non-lactose fermenters but is not suitable for isolation of gram positive cocci. Cysteine Lactose Electrolyte Deficient (CLED) medium which is now routinely recommended for processing of urine samples has been found to be superior to MacConkey agar as it supports better growth of Gram positive cocci and yeasts and inhibits swarming of Proteus spp.[3] A common feature for all these media is that identification tests need to be performed to further differentiate between the lactose fermenters like the *Escherichia coli* and the *KES* species and biochemical tests such as catalase and coagulase have to be performed for identifying gram positive cocci. This delays the reporting further by 18-24 hrs.

To simplify this and for reducing the burden of biochemical identification, manufacturers are developing media which allow direct differentiation of various bacteria from the primary plate itself. Over the last two decades, several chromogenic media have been made available commercially.[4] Most of these media specifically identify organisms of one genus e.g., BBL chromagar orientation and CPS ID2 agar for uropathogens, Chromagar for *S. aureus* and MRSA, Chromagarorientation with sodium azide or nalidixic acid and vancomycin for detection of enterococci and VRE, Chromagar for gram negative bacteria and enterococci.[5,6,7]

Chromogenic media are based on the principle that certain bacteria possess specific enzymes which can degrade the specific substrates incorporated in the media, thereby imparting visually distinctive color to the bacterial colonies. For the utilization of βD-Glucuronide, an enzyme β-Glucuronidase should be present in the organism. It was found that the enzyme β-Glucuronidase is present mostly in Escherichia coli only and the detection of this enzyme is considered as one of the confirmatory test in the identification of *Escherichia coli*. Similarly to utilize β-D-Galactoside, organism should have an enzyme β-Galactosidase. *Klebsiella, Enterobactor, Enterococcus* are found to be positive for this test. When these chromogenic substrates are incorporated in the agar medium, β-Glucuronidase enzyme positive organisms (*e.g. Escherichia coli*) form pink or burgundy colored colonies. β-Galactosidase positive organisms form blue-to-blue green color colony. *Proteus, Providencia, Morganella* group positive for tryptophan deaminase produces orange to brown colonies. The medium also allows direct biochemical testing on the plate itself for tests like catalase, oxidase, indole production and tryptophan deaminase activity, after looking for colony characters and Gram's staining.

Urichrom-II™ showed slightly better abilty to detect uropathogens as 17% (116) of our samples showed better growth on this chromogenic media. In our case, the chromogenic agar not only supported the growth of gram-negative uropathogens but also

effectively supported the growth of gram-positive and fungal uropathogens. Due to distinctive colours shown by the different organisms, there was significant ease in distinguishing mixed cultures and direct inoculation for antimicrobial sensitivity testing without the need for subcultures. This is in accordance with previous studies for uropthogens.[9,10,11]

To summarize, Urichrom II™ has been found to a competent media in the presumptive identification of common uropathogens like *E.coli, Klebsiella and Enterococci* in our setting. It has proved to be useful for detection of low number yeasts which could be pathogenic in some cases as compared to CLED and MacConkey Visual identification of bacteria alleviates the need for biochemical characterization to a certain extent thereby reducing the turnaround time for identification from 48 hrs. to 24 hours. Cost reduction from less use of biochemical media compensates for the higher price of chromogenic medium. Use of Urichrom-II™ as a primary plating media for urine samples can thus assist in early reporting and prompt treatment of urinary tract infections.

## References


1. Forbes, B.A. Sahm DF and Weissfeld AS. et al. 2007.Infections of the urinary tract. In: Bailey and Scott's Diagnostic Microbiology, 12th ed. C.V. Mosby Company, St. Louis, MO.
2. Baveja CP. Urinary Tract Infections. In: Textbook of Microbiology, 3rd edition. Arya publications, India;2009:564-7.
3. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A. Laboratory strategy in diagnosis of infective syndromes. In: Collee JG, Fraser AG, Marmion BP, Simmons AC, editors. MacConkeykie and McCartney Practical Medical Microbiology. Singapore: Churchill Livingstone; 1996:53-94.
4. Perry, J.D. and Freydière, A.M. (2007), The application of chromogenic media in clinical microbiology. Journal of Applied Microbiology, 103:2046–2055. doi: 10.1111/j.1365-2672.2007.03442.
5. Merlino J, Siarakas S, Veal D. The development of new chromogenic screening test for the phenotypic identification and differentiation of Enterococci with application in the detection of vancomycin resistance. In: *Medical Microbiology*. Proffered paper. 1998:5.6.
6. Merlino J, Lezoi M, Braudbury R, Veal D, Harbour C. A new chromogenic medium for identification and detection of *S.aureus* and MRSA. *J ClinMicrobiol*2000;38:990-994.
7. Hengstler KA, Hammann R, Fahr AM. Evaluation of BBL Chromagar orientation for detection and presumptive identification of urinary tract pathogens. *J ClinMicrobiol*1997;35:2773-2777.
8. Anuradha M, Madhavi Y. Urinary isolates with special reference to Urichrom II™. Int J Res Med Sci 2013;1:58-61.
9. Lakshmi V, Sateeshkumar T, Kulkarni G. Utility of Urichrom II™- a chromogenic medium for uropathogens. Indian J Med Microbiol 2004;22:153-8.
10. Samra Z, Heifetz M, Talmor J, Bain E, Bahar J. Evaluatio n of use of a new chromogenic agar in detection of urinary tract pathogens. J. Clin. Microbiol.1998.36:990–994.
11. Qaiser S, Zeeshan M, Jabeen K, Ahsan T, Zafar A. Comparison of chromogenic urinary tract infection medium with cysteine lactose electrolyte deficient media in a resource limited setting. J Pak Med Assoc. 2011 Jul;61(7):632-5.