EVALUATION OF A NEW MEDIUM FOR ISOLATION, DIFFERENTIATION AND PRESumptive IDENTIFICATION OF MICROORGANISMS IN URINARY TRACT INFECTIONS

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INTRODUCTION

In the past few years several chromogenic media such as Alborian ID and CPHD2 (BioMerieux), CHROMagar Candida (CHROMagar), etc., have been commercialized allowing the direct identification of microorganisms on primary plates. CHROMagar Orientation is based on the same principles and proposes a simultaneous presumptive identification of gram negative and positive bacteria and yeasts on one single medium by means of distinct colony colors. The clinical evaluation of this medium was conducted at the Microbiological Laboratory at Bellinson Medical Center.

Aim of the Study

The aim of this study was to evaluate the sensitivity of the medium and its ability to differentiate urinary pathogens. Accuracy of antibiotic susceptibility testing according to standard methods by picking the isolates directly from CHROMagar Orientation agar was also tested.

MATERIALS AND METHODS

I. Study Population

900 urine samples from hospitalized patients were tested in this study.

II. Media and Bacteriological Procedures

1. Blood-Agar (Uprig. see broth No. 2 with 5% def. sheep blood)
2. MacConkey Agar
3. Mueller Hinton Agar
4. CHROMagar Orientation - Paris, France

CHROMagar Orientation was prepared by Hy-Labs - Rehovot, Israel according to manufacturers instructions. Plates were stored at 4°C protected from light, and were used within 10 weeks. Each lot of media tested for quality control with ATCC strains for growth promotion and performance.

The CHROMagar was evaluated in comparison to standard reference culture media (Blood Agar and MacConkey Agar plates). The urine samples were inoculated parallel on the three agar plates by using a calibrated sphere of 10 μl loop and incubated aerobically at 35°C overnight or 48 hours during the weekends. Accuracy of antibiotic susceptibility testing according to standard methods was done by picking isolates directly from CHROMagar Orientation to Mueller Hinton agar and compared to those performed parallel from reference media.

III. Microorganisms Identification and Confirmation

Enterobacteriaceae isolates were identified by the following biochemical reactions: motility, indole production, ONPG-hydrolysis, phage fermentation (citrate or without CO2 production), hydrogen sulphide production, urea hydrolysis, lysine decarboxylase and sodium citrate utilization. Other Gram negative microorganisms than Enterobacteriaceae were tested for gelatin, indole and oxidase reactions.

Streptococci identification was confirmed by haemolysis on blood agar, hydrolysis of L-lysine by alkaline phosphatase by PyRase (PIR), esculin hydrolysis and agglutination tests.

S. aureus was confirmed by coagulase reaction.

Candida isolates were subcultured to CHROMagar Candida, a medium allowing identification of C. albicans, C. tropicalis, and C. krusei by colony color.

TABLE I - QUALITY CONTROL OF CHROMAGAR ORIENTATION

<table>
<thead>
<tr>
<th>Test Microorganisms</th>
<th>Reference media*</th>
<th>CHROMagar Orientation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>Light brown, 19h. 20h.</td>
<td>Pink, 19h. 20h.</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>White, 19h. 20h.</td>
<td>White, 19h. 20h.</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>White, 19h. 20h.</td>
<td>White, 19h. 20h.</td>
</tr>
<tr>
<td>S. aureus</td>
<td>White, 19h. 20h.</td>
<td>White, 19h. 20h.</td>
</tr>
</tbody>
</table>

TABLE II - URINE PATHOGENS PRESUMPTIVELY IDENTIFIED ON CHROMAGAR ORIENTATION ACCORDING TO PIGMENT REACTION

<table>
<thead>
<tr>
<th>Organisms</th>
<th>18 - 24h Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klebsiella pneumoniae</td>
<td>Pink-red</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Pink-red</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>Pink-red</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Pink-red</td>
</tr>
<tr>
<td>E. coli</td>
<td>Pink-red</td>
</tr>
</tbody>
</table>

* Light pink halo around pigment after 24-48 hours
** Yellow pigment with pink halo after 24-48 hours
*** Green after 24-48 hours
The quality control assay results of the CHROMagar Orientation Media are given in Table 1. Out of the 790 urine samples assayed, 190 were found positive: 1 in 176 of them, only one microorganism was isolated; in 12 of the urine samples 2 microorganisms were isolated and in the two remaining samples, 3 microorganisms were detected. The description of the colony growth is given in Table 2. The distribution of the different urine pathogens in the positive samples are given in tables III an IV and in figure 1.

**DISCUSSION AND CONCLUSIONS**

1. Overnight incubation was enough for reading results on CHROMagar Orientation. A longer incubation of up to 72 hours only improved the results.
2. CHROMagar Orientation showed the same sensitivity as the combination of Blood Agar/MacConkey Agar for detecting urine pathogens.
3. CHROMagar Orientation has the ability of presumptively differentiating several microorganisms directly from primary plates (E. coli, Enterococci, Proteus) (Table III-I-V).
4. Differentiation of the different bacterial colonies was easier on CHROMagar Orientation than on the reference media.
5. This ability allowed performing susceptibility tests directly from the primary isolates on CHROMagar Orientation without need of subcultures in most of the cases.
6. CHROMagar Orientation was more sensitive than Blood Agar in detecting mixed flora. When one of the microorganisms was Proteus, confluent growth was observed on blood agar.
7. The results of susceptibility tests of microorganisms picked from CHROMagar Orientation showed excellent correlation with test results of microorganisms picked from reference media.