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 Albicans ID and CPSID2 (Biomérieux). 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 Microbilological Laboratory at Beilinson Medical Center.

Aim of te 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 (Tryptic soy 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 4-6°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 parallely on the three agars by using a calibrated sphere / 10 µl loop and incubated aerobically at 35±2°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 parallely from reference media.

III. Microorganisms Identification and Confirmation

Enterobacteriaceae isolates were identified by the following biochemical reactions : mobility, indole production, ONPG hydrolysis, glucose fermentation Whit or without CO_2 production, hydrogen sulfide production, urea hydrolysis, lysine and ornithine 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-pyrrolindonyl-beta-naphthylamide substrate by PYRase (PYR), 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 USED FOR THE EVALUATION

Test Microorganisms (According to NCCLS)	Reference media: Tryptic Soy Agar + 5% deb. sheep blood Lot No. 9816, 9993, 0308, 0738	CHROMagar Orientation Lot No. 0231, 0415, 0563, 0732, 0872, 0876, 3671	
	Colony Count from estimated dilution - Dilution 10^2 cfu/ml	Morphology	Colony Count Dilution 10^2 cfu/ml
	Mean of 4 batches ± SD		Mean of 7 batches ± SD
Kleb. pneumonia ATCC 13883	210 ± 77	Mucoid, metallic blue	230 ± 99
Ps. aeruginosa ATCC 27853	138 ± 15	Transparent, yellow to green serrated edges, diffused	128 ± 11
P. mirabilis ATCC 4630	110 ± 34	Clear beige on beige background	128 ± 43
S. aureus ATCC 25923	129 ± 69	Opaque, white	132 ± 68
Streptococcus faecalis ATCC 19433	168 ± 69	Dry, Turquoise	174 ± 75
E. coli ATCC 25922	174 ± 98	Small, pink-red	166 ± 47

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

Organisms	Morphology Description (18 - 24h incubation)
E. Coli	Small, pink-red
Kleb. pneumoniae	Mucoid*, metallic blue
Citrobacter freundii	Metallic blue**
Enterobacter spp.	Metallic blue
Proteus mirabilis	Clear diffusable beige on beige background
Morganella	Clear diffusable beige on beige background
Pseudomonas aeruginosa	Transparent, yellow serrated edges, diffused ***
Acinetobacter	Nontransparent, entire edges, white
Enterococcus spp.	Dry, turquoise
Streptococci Group B	Small translucent. Diffused light blue within agar
Streptococci Group C	Small translucent. Diffused light blue within agar
Staphylococci spp.	Opaque, white
Candida spp.	Creamy, wet convex

* Slight pink halo around periphery after 24-36 hours
** Strong purple-pink halo (diffused) after 24-36 hours
*** Green after 24-36 hours

TABLE III - TOTAL NUMBER OF THE DIFFERENT MICROORGANISMS IN THE 190 POSITIVE RESULTS

Organism	Numbers of Isolates on :		
	CHROMagar Orientation	Blood Agar	MacConkey Agar
E. Coli	87	85	87
Enterococci	33	33*	-
Proteus spp	13	13	13
Morganella	4	4	4
Kleb. pneumoniae	16	16	14
Enterobacter spp.	5	5	2
Ps. aeruginosa	20**	20**	20**
Citrobacter spp.	3	2	3
Acinetobacter	4	4	4
Staphylococci spp.	11	11	-
Streptococci Group B	5	5	-
Streptococci Group C	1	1	-
Candida	7	7	-

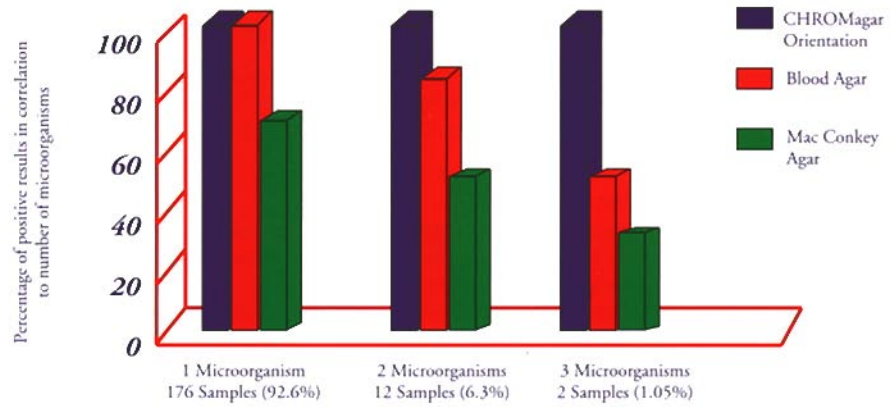
* In 8 out of the 33 isolates, additional tests were necessary to confirm the identification
** In 1 out of the 20 samples, only a few colonies were detected

TABLE IV - POSITIVE RESULTS FROM 176 PURE CULTURE OUT OF THE 900 URINE SAMPLES ASSAYED

Organism	Numbers of Isolates on :		
	CHROMagar Orientation	Blood Agar	MacConkey Agar
E. Coli	73	73	73
Enterococci	28	28*	-
Proteus spp	8	8	8
Morganella	2	2	2
Kleb. pneumoniae	15	15	13
Enterobacter spp.	2	2	2
Ps. aeruginosa	20**	20**	20**
Citrobacter spp.	2	2	2
Acinetobacter	4	4	4
Staphylococci spp.	9	9	-
Streptococci Group B	5	5	-
Streptococci Group C	1	1	-
Candida	7	7	-

* In 5 out of the 28 isolates, additional tests were necessary to confirm the identification
** In 1 out of the 20 samples, only a few colonies were detected

Figure 1: Distribution of the positive results and ability of the different media to detect the microorganisms



RESULTS:

The quality control assay results of the CHROMagar Orientation Media are given in Table I. Out of the 900 urine samples assayed, 190 were found positive : 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 II. 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) (Tables III-IV)
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.