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Colorex™ Orientation Agar
CHRP0016

Intended Use

Colorex™ Orientation agar is a non-selective chromogenic medium, manufactured using CHROMagar™ technology, used for the differentiation and enumeration of urinary tract pathogens based upon colony color and morphology.

Summary

Urinary Tract Infections (UTI) are one of the leading types of bacterial infections (1), with gram-negative bacteria and *Enterococcus* spp. being problematic for hospitalized patients (2). Urine samples sent to clinical microbiology laboratories are numerous, and contribute greatly to the daily workload. Traditional culture methods are constrictive in that their ability to differentiate gram-negative organisms relies solely on the determination of lactose utilization. Rapidly identifying the causative organism can aid clinicians in selecting the appropriate antibiotic therapy (2). This is important, as resistance to oral antibiotics used to treat UTI's is increasing (1). CHROMagar™ Orientation Agar can aid in the identification of organisms in mixed cultures from clinical samples, without the restraints of traditional culture methods (2-5). CHROMagar™ Orientation Agar has been shown to reduce workload in the clinical laboratories (3,4,5) and is easy to use (4, 5). This allows for clinical laboratories to collect information from one media, compared to the standard two-plate method for urine cultures, which can result in cost savings and identification of mixed cultures that may have been missed with traditional methods (5).

Principles

Dr. Alain Rambach first invented a chromogenic culture medium in 1979 using enzymatic chromogenic substrates. Colorex™ Orientation Agar uses these substrates to differentiate mixed cultures from clinical urine samples, with minimal confirmatory testing. Colorex™ Orientation contains specially selected peptones and yeast extracts as the nutrient source, and enzymatic chromogenic substrates (chromagens) to differentiate and detect certain groups of organisms. Bacterial enzymes act on the chromagens to yield colonies of unique and differential color. The enzymes transform the colorless and soluble chromagen into a color, located at the site of the colony, making differentiation clear and easy.

Formula

Composition	g/L
Agar	15.0
Peptone and Yeast Extract	17.0
Chromogenic Mix	1.0

Precautions

IVD For professional use only. Only trained and qualified personnel should use this product. This medium is meant to grow potentially pathogenic organisms, and universal biohazard precautions should be taken. Sterilize any biohazard waste prior to disposal. Do not use plates if they show any signs of microbial contamination. Do not use plates if they show evidence of deterioration, such as: drying, cracking, or discoloration. Do not use plates that are expired.

Storage

Plates should be stored in the dark prior to use in original packaging. Store at 2-8°C upon receipt. Exposure to light may affect the performance of the media. Plates may be used until the expiration date stated on the packaging and plate.

Specimen Collection

This medium is used for enumerating and differentiating bacteria in urine samples only. Use aseptic technique when collecting urine samples. Samples should be streaked on to the medium no more than 2 hours after collection, or must be refrigerated for no more than 24 hours to prevent overgrowth of urinary pathogens.

Procedure

Allow the plate to warm temperature prior to inoculation. Use calibrated loops or other validated techniques to collect a sample of undiluted urine. Use correct laboratory technique when loading the loop, and inoculate by streaking down the middle of the plate, and perform additional spreading of the sample. For enumeration, use a 0.01mL or 0.001mL loop. Incubate plates inverted in aerobic conditions at 37°C for 18-24 hours. Avoid exposure to light during incubation.

Results

After appropriate incubation, the plates should show isolated colonies where the inoculum was spread. Use the Table 1 for presumptive identification and as a guideline for additional biochemical or immunological confirmatory tests. For enumeration, if a 0.01mL loop was used, each colony is representative of 100 CFU/mL; if a 0.001mL loop was used, each colony is representative of 1000 CFU/mL of urine.

Microorganism	**Typical colony appearance**
Gram (-)	
E. coli	Dark pink to reddish
Klebsiella, Enterobacter, Citrobacter, Serratia	Metallic blue (+/- reddish halo)
Proteus, Morganella, Providencia	Brown halo
Proteus vulgaris	Blue with brown halo
Pseudomonas	Translucent (+/- natural pigmentation cream to green)
Acinetobacter	Cream
Stenotrophomonas	Colorless
Gram (+)	
Enterococcus	Turquoise blue
S. aureus	Golden, opaque, small
S. epidermidis	Cream, pinpoint colonies
S. saprophyticus	Pink, opaque, small
S. agalactiae	Light blue
Yeasts	
Candida albicans	Cream, pinpoint colonies

Table 1- Colony appearance for presumptive identification.

Confirmatory Tests

Confirm the results with additional biochemical or immunological tests. Table 2 lists suggested biochemical tests. Gram stain and microscopy can be used to confirm results. While performing confirmatory tests, do not apply the detection reagent directly onto the colonies on Colorex™ Orientation Agar. Use filter paper with growth from respective colonies. Follow all instructions accompanying confirmatory tests.

Organism	**Colony Size**	**Colony Color**	**Confirmatory Test**
E. coli	Medium- large	Dark to light pink, transparent colonies with or without halos	Indole Test E. coli is indole (+).
Proteus, Morganella, Providencia	Small- Large	Pale to beige colonies with brown halos	TDA Test (with $FeCl_2$ Test) for confirmation of *Proteus*
Enterococcus	Small	Turquoise blue	PYR Test

			Enterococcus is PYR (+)
Pseudomonas	Small-Large	Translucent with possible natural pigmentation, cream to green	Oxidase Test *Pseudomonas* is Oxidase (+)

Table 2- Confirmatory Tests

Quality Control:

Plate appearance: colorless to very light amber, clear.
pH: 7.0 ± 0.2

Inoculate the media with the strains listed below in Table 3. Streak for isolation in order to show colony morphology. Incubate the plates inverted at 35-37°C aerobically for 18-24 hours.

Organism	Typical Colony Appearance/ Growth Results
ATCC# 29212 *Enterococcus faecalis*	Turquoise Blue
ATCC# 25922 *Escherichia coli*	Reddish
ATCC# 25923 *Staphylococcus aureus*	Golden, opaque, small
ATCC# 13883 *Klebsiella pneumoniae*	Metallic blue
ATCC# 15305 *Staphylococcus saprophyticus*	Pink
ATCC# 12228 *Staphylococcus epidermidis*	Colorless to Cream, pinpoint

Table 3- Quality Control strains

Limitations of the Procedure:

The use of this media for other specimens than urine has not be documented.
This medium is non-selective, and will support the growth of other UTI pathogens. These pathogens may not react with the chromogenic substrates in the medium, and further confirmatory test must be used for differentiation.
All presumptive identifications based on colony color and morphology require confirmatory tests.
Occasionally, organisms may display a color similar to the typical colony appearance of another strain, and must be distinguished with confirmatory tests.
O-nitrophenyl-β-D-galactopyranoside negative *E. coli* colonies may appear colorless (2, 4).
Colorblind technicians using this medium may have difficulty interpreting the results.
For indole tests on dark pink to rose colonies, use dimethylaminocinnamaldehyde (DMACA) indole reagent. The color of the colonies may interfere with the interpretation of the results if Kovac's Indole Reagent is used.
This medium will not support the growth of fastidious organisms.

References

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5. Holly A. D'Souza, M. C. (2004). Practical Bench Comparison of BBL CHROMagar Orientation and Stnadard Two-Plate Media for Urine Cultures. *Journal of Clinical MicroBiology*, 60-64.

Colorex™ Orientation Agar is manufactured using CHROMagar™ powder.