savyonDIAGNOSTICS

CHROMAGENIC UTI AGAR
(Prepared 90mm plates - Pkg. of 10)
Catalogue No.: BP-015/BD-010

ISOLATION AND COUNTING OF URINARY PATHOGENS

DIRECT IDENTIFICATION OF *Escherichia coli,* Proteus, and group D streptococci

I. CLINICAL RELEVANCE

Chromagenic UTI Agar is an agar medium in a Petri dish that allows:

- Isolation and counting of all urinary tract organisms using a standard bacteriological loop plating method.
- Direct identification via demonstration of enzyme activities of the bacteria most often responsible for urinary tract infections, namely *Escherichia coli*, Proteus, and group D streptococci.
- Orientation for the diagnosis of the other urinary pathogens, in particular K. E. S. group enterobacterial (Klebsiella, Enterobacter, Serratia).

II. COMPOSITION

Chromagenic UTI Agar is a non-selective agar medium composed of:

- A rich nutrient base containing three peptones, which sustains growth of all urinary tract pathogens.
- two chromogenic substrates for detection of the bacterial enzymes ß-glucuronidase and ß-glucosidase.
- and tryptophan for detection of tryptophanase activity (indole production) and of tryptophan deaminase activity (TDA).

III. PRINCIPLE

- *E. coli* can be identified based on demonstration of the activity of two enzymes, ß-glucuronidase and tryptophanase; ß-glucuronidase cleaves the first chromogenic substrate contained in the medium, causing the colonies to turn purple.
- Proteus are characterized by tryptophan deaminase activity *Proteus mirabilis* is indole-negative.
- Group D streptococci produce a ß-glucosidase (esculinase) which cleaves the second chromogenic substrate contained in the medium causing the colonies to turn blue.

REVELATION OF THE VARIOUS ENZYME ACTIVITIES

- **Spontaneous**

For ß-glucuronidase and ß-glucosidase: change in color of the colonies after incubation at 37°C for 18 to 24 hours:

	Positive reaction	**Negative reaction**
ß-glucuronidase	Pink to purple or clear with a pink to purple centre	White
ß-glucosidase	blue to greenish-blue	or colorless

- **Spontaneous or after addition of a reagent**

	Positive reaction	**Negative reaction**
TDA	Spontaneously orange-brown color of the colony with or without a brown color of the agar. If the change in color is slight, deposit one drop of iron perchloride on an isolated colony: a brownish-green color develops within a few seconds.	White or colorless colony

- **After addition of a reagent**

To detect tryptophanase (indole production) deposit one drop of Kovac's reagent on in isolated colony, directly onto the agar (see section VI. Procedure).

	Positive reaction	**Negative reaction**
Indole	The reagent turns pink within no more than 15 seconds	The reagent is still colorless after 15 seconds

IV. PRESENTATION

Savyon Diagnostics Ltd.
3 Habosem St.
7761003 Ashdod, Israel

Tel.: 972.8.8562920
Fax: 972.8.8523176
info@savyondiagnostics.com

Boxes containing twenty 90-mm Petri dishes.

This product is intended for in vitro diagnostic use only.

V. REAGENTS AND ADDITIONAL MATERIAL

Kovacs reagent: 10-ml bottle

VI. COLLECTION OF URINE SAMPLES

Urine sampling methods impact significantly on the results of cytologic and bacteriologic examinations of urine; collection of urine samples at first voiding in the morning is recommended. To avoid contamination by saprophytic bacteria samples must be collected as follows

- **In males:** clean the urinary meatus and glans penis carefully using Dakin's solution
- **In females**: catheterization is not necessary; the external genitalia should be cleaned carefully using Dakin s solution insertion of a vaginal tampon is recommended especially during menstruation

In males and females, discard the first part of the stream and clean-catch a midstream sample in a sterile container.

- **In infants:** use a sterile urine bag.

Test the urine within 1 hr of sampling; if this is impossible the specimen should be stored at + 4°C to prevent bacterial growth

VII. PROCEDURE

Plating

- Use a standard 10 µl bacteriological loop.
- Hold the loop vertically and submerge it in the urine.
- Deliver the urine onto the agar by streaking the loop once along one radius of the dish (Figure 1).
- Starting at the top of the deposit, without reloading the loop streak at closely spaced intervals over the entire surface of the agar, perpendicularly to the initial streak (Figure 2).

Incubation

Incubate the plated dish in a 37°C oven for 18 to 24 hours.

Reading - interpretation

Counting

After incubation for 18 to 24 hrs at 37°C, compare the density of the colonies in the upper half of the disk to the diagram below:

Number of organisms per ml urine 10^3 10^4 10^5 10^6 10^7

Counts of $\leq 10^4$ usually indicate contamination but should be interpreted in the light of the urinary leukocyte count and of clinical manifestations. Counts of 10^5 organisms or more usually indicate infection provided the urine sample was collected properly.

Identification: identify the organism based on the color of the colonies.

PINK TO PURPLE OR CLEAR WITH A PINK TO PURPLE CENTER

ß-glucuronidase activity suggesting presence of *E. coli,* to be confirmed by testing for indole production:

- indole production indicates on *E. coli*:

- absence of indole production indicates a need for identification using conventional methods.

Detection of indole production

- For cultures containing only one kind of organism, deposit one drop of Kovac's reagent on a well-isolated suspect-colony,
  directly onto the agar:
  -if the reagent turns pink within no more than 15 seconds. the organism is indole positive.
  -if the reagent is still colorless after 15 seconds the organism is indole negative
- For cultures containing more than one kind of organism test for indole using the technique described in paragraph 3 of the "Comments" section.
-

Savyon Diagnostics Ltd.
3 Habosem St.
7761003 Ashdod, Israel

Tel.: 972.8.8562920
Fax: 972.8.8523176
info@savyondiagnostics.com

BLUE or GREENISH-BLUE

ß-glucosidase activity: examine the culture under a microscope.

- COCCI, with small colonies (0.5 to 1.0 mm in diameter) and a bright, frank blue color are group B streptococci. If any of these characteristics is lacking, identify the organism using conventional methods.
- BACILLI, with large colonies (2.0 to 3.0 mm in diameter) and a blue or greenish-blue color are probably KES group

ORANGE-BROWN (with or without a change in agar color to brown)

Tryptophan deaminase activity (TDA +) indicating an organism of the Proteus-Providencia-Morganella group.
If the change in color is slight, revelation of the enzyme can be enhanced by depositing a drop of iron perchlorite onto a colony: the reagent turns brown-green within a few seconds. Test for indole production:

- for cultures containing only one kind of organism, deposit one drop of Kovac's reagent directly onto a brown colony:

- if the color of the reagent does not change within 15 seconds, there is no indole production indicating a *Proteus mirabilis*.
- if the reagent turns pink within 15 seconds there is indole production indicating an indole+ Proteus, a Providencia, or a Morganella. Precise identification should be performed using a conventional method.

- For cultures containing more than one kind of organism, test for indole using the method described in paragraph 3 of the "Comments" section.

WHITE OR COLORLESS

Identification using a conventional method.

Savyon Diagnostics Ltd.
3 Habosem St.
7761003 Ashdod, Israel

Tel.: 972.8.8562920
Fax: 972.8.8523176
info@savyondiagnostics.com