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CHROMOSTREAK Urine Culture Device (Cat.No.BD-915)

POSITIONING & STRATEGY

CHROMOSTREAK Urine Culture Device (UCD) Cat.No.BD-915 is your superior device designed to be one step ahead of all urine culturing devices. DIPSTREAK (CHROMOSTREAK)®UCD (Cat.No.BD-915) is the advanced format of the previous model (Cat.No.BD-912) providing increase in the work surfaces of agars at 60 %. The advanced model of CHROMOSTREAK UCD (Cat.No.BD-915) emphasizes all advantages of a product.
The unique dip-tip technique differentiates CHROMOSTREAK UCD from all other commercial dipslides enabling dilution streaking similar to culture. CHROMOSTREAK UCD combines the sensitivity & specificity of the traditional Petri dish culture media, with the convenience of dipslide, which together with special unique features makes it the leading device for UTI testing! The simple and rapid protocol together with the see-through view results in a unique transport packaging position CHROMOSTREAK UCD as a premium high quality price product, providing easy to use friendly standardized culture process from the bedside or doctor's office up to the clinical microbiological laboratory.

INTENDED USE

CHROMOSTREAK UCD is a convenient semi-quantitative screening culture device for inoculating and transporting urine samples as well as for detecting, enumerating and identifying specific bacteria in urine.[1, 2] A unique streaking mechanism permits the isolation of single colonies even when the original bacterial population of the sample was as high as 10^7 organisms per milliliter. The device is intended for use in physician's office laboratories and clinical laboratories as an aid in the diagnosis of urinary tract infection (UTI).

SUMMARY AND EXPLANATION

CHROMOSTREAK UCD comprises a plastic paddle with two types of agar attached back-to-back, housed in a closed transparent plastic tube. A ring with elongated prongs is attached to the end of the paddle so that there are prongs on each side of the slide. The ends of the prongs are dipped into the urine sample. Upon re-insertion into the plastic tube, the prongs are prevented from moving and the agar surfaces are inoculated with the urine sample as the paddle passes over the prongs. The result is a series of streaks of decreasing bacterial concentration, which permits isolation of single colonies even when the original bacterial population of the sample was as high as 10^7 organisms per milliliter. Current routine methods for bacteriological examination of urine are the classic Petri dish culture method [5] and the dipslide techniques.[6] CHROMOSTREAK UCD combines the advantages of both techniques, enabling bacterial enumeration and isolation following a simple, user-friendly procedure.
A selective medium, giving an excellent differentiation between coliforms and non-lactose fermenters with inhibition of Gram-positive cocci.[4] Most urinary tract pathogens grow on the medium, whereas most contaminants are inhibited.

SPECIMEN COLLECTION

Cleanse the genital area and collect a midstream urine specimen in a clean container. Inoculate the urine as soon as possible following collection. If storage of the urine specimen is necessary, maintain the specimen at 4°C in a closed sterile container. Storage time should not exceed two hours.

PRINCIPLES OF THE PROCEDURE

The ends of the prongs are dipped into the urine specimen and take up a standard volume of urine (approximately 6µl). Upon re-insertion into the plastic tube, the prongs are prevented from moving and both agar surfaces are simultaneously inoculated with the urine sample by a streaking dilution as the paddle passes over the prongs. As a consequence, the number of bacteria deposited on the media is in direct proportion to the number of bacteria present in the specimen.[10] Following incubation, the number of bacterial colonies is compared with the Colony Density Chart to determine the colony forming units per ml (CFU/ml) of bacteria in the urine sample.

KIT CONTENTS

CHROMOSTREAK UCD contains two types of media: chromogenic agar (Uri*Select* agar) and MacConkey agar.
URI*Select* is a non-selective chromogenic agar medium composed of a rich nutrient base for isolation and counting of all urinary tract organisms, direct identification via demonstration of enzyme activities of the bacteria most often responsible for urinary tract infections, namely *Escherichia coli, Proteus*, and enterococci and orientation for the diagnosis of the other urinary pathogens, in particular K.E.S. group Enterobacteria (*Klebsiella, Enterobacter,Serratia and Citrobacter* species).
MACCONKEY agar is a selective and differential medium giving an excellent differentiation between coliforms and non-lactose fermenters with inhibition of Gram-positive cocci.[9] Most urinary tract pathogens grow on the medium, whereas most contaminants are inhibited. The concentration of bile salts in this medium is relatively low in comparison with other enteric plating medium; therefore, selectivity for gram-negative bacteria is not as great as in some other formulations. Crystal violet inhibits gram-positive microorganisms, especially enterococci and staphylococci. Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral

red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment lactose.

MATERIAL REQUIRED BUT NOT PROVIDED

Incubator (37 ± 1°C)

Incubation Stand

PRECAUTIONS

1. For In Vitro Diagnostic Use.
2. Use aseptic technique and established laboratory procedure in handling and disposing of infectious material.
3. Dispose of used CHROMOSTREAK UCD and tubes by burning, autoclaving or immersing in a suitable disinfectant overnight.
4. Incubation in a CO_2 - enriched atmosphere will cause inhibition of staphylococcal growth.[11]

STORAGE

1. Store CHROMOSTREAK UCD at 15-25°C for 4 months. Protect from direct light to ensure product stability through the expiration date. Expiration date is indicated on the cap of device and on the label of package.

EXPIRATION DATE

1. The expiration date applies to the product in its intact container when stored as directed.
2. Do not use CHROMOSTREAK UCD exhibiting any of the following characteristics: discoloration, dehydration, wrinkling or shrinkage of an agar surface; microbial growth before inoculation; or an atypical cultural response in Quality Control procedures.

PROCEDURE

1. Stand the CHROMOSTREAK UCD tube firmly on a table. Unscrew the cap. Pull the paddle out. Do not touch any part but the cap (Fig. 1).
2. Hold the paddle vertically and dip the white prongs into the sample up to about half of their length (see below (Fig. 2).
3. Center the prongs over the opening of the tube (Fig. 3).
4. Press the paddle into the tube in a quick, vertical and continuous motion and close the cap (Fig. 4).

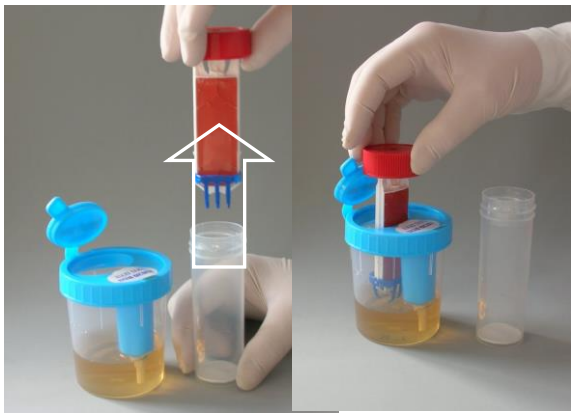


Fig. 1

Fig. 2

Fig. 3

Fig. 4

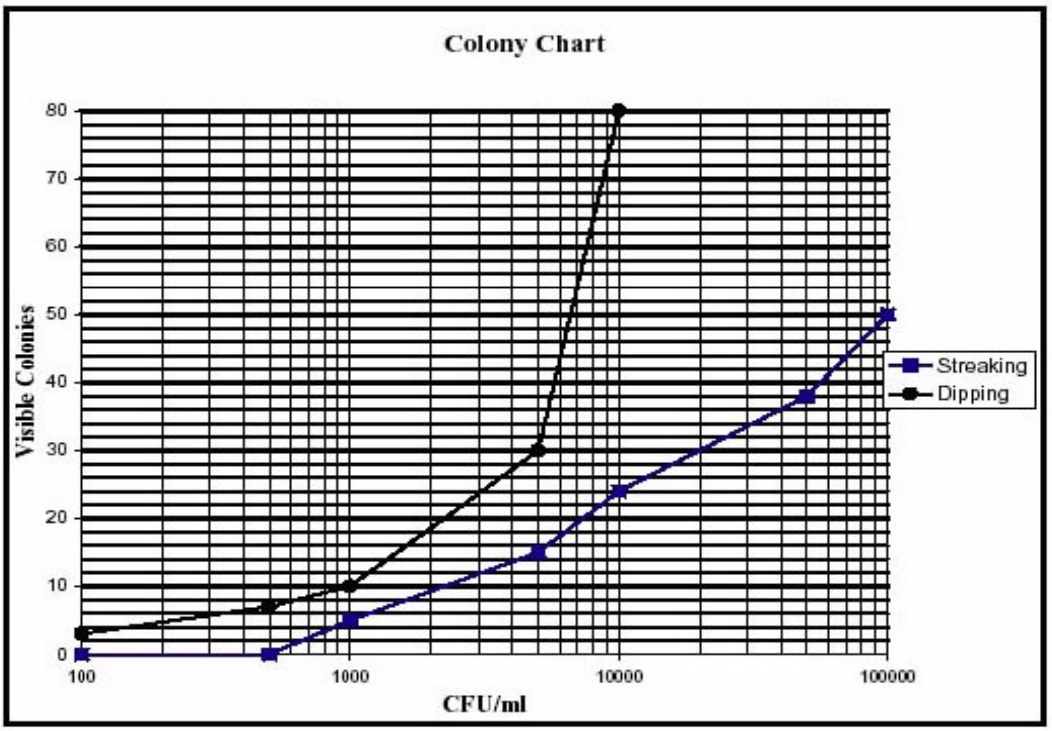
5. Label the inoculated CHROMOSTREAK UCD with patient ID. Transport to laboratory for incubation and examination.
6. Incubate the entire container in a vertical position at 35-37°C for 18-24 hours using aerobic conditions. Before incubation, loosen cap one-half turn. **DO NOT INCUBATE IN CO_2!**
7. Determine the number of colony forming units by comparison to the Colony Density Chart.

RESULTS

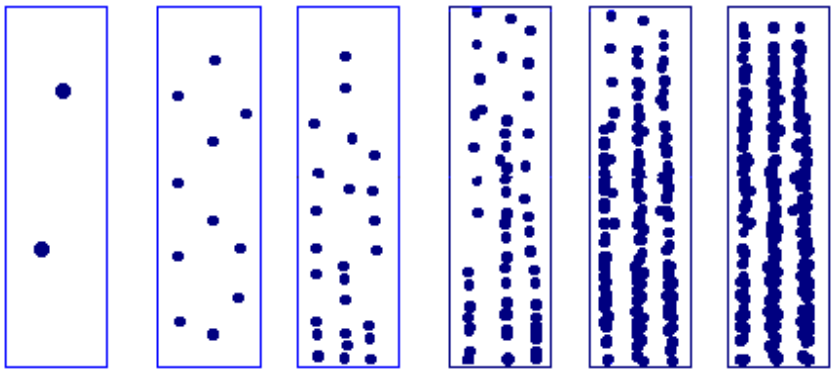
Following incubation, examine CHROMOSTREAK UCD for bacterial growth, which may be evidenced by visible colonies on the Chromogenic **URI*Select*** agar surface. Colony growth on the CHROMOSTREAK UCD is in discrete lines with isolated colonies from samples with as many as 10^7 organisms per milliliter. The same sample yields an undifferentiated pattern of confluent growth on an old-style dipslide. Since each colony results from growth of a single bacterial cell, and since the sampler takes up a standard volume of urine, the number of

colonies indicates the "colony count" of the specimen, the approximate number of bacteria per ml (CFU/ml) of urine. If microbial growth is present, match the colony density on the agar surface with the printed illustration it most closely resembles on the Colony Density Chart (See below).

TABLE 1: The relationship between bacterial concentration (CFU/ml) and the approximate colony count of the Colony Density Chart.



COLONY DENSITY CHART



Colonies	**<10**	**10-24**	**25-50**		**>50**	
CFU/ml	**10^3**	**10^4**	**10^5**	**10^6**	**10^7**	**10^8**

INTERPRETATION OF RESULTS
BACTERIAL COUNT

As a rule, for clean catch urine cultures, tests yielding $\geq 10^5$ CFU/ml are regarded as positive (significant level of organisms), $\leq 10^4$ CFU/ml as negative, and between 10^4 and 10^5 CFU/ml as borderline, which calls for a repeat assessment. Symptomatic patients having colony counts less than 10^5 CFU/ml require evaluation based on clinical information. The bacterial growth may consist of very small or very large colonies. It is important to remember that only the number of colonies, and not their size, should be considered when comparing CHROMOSTREAK UCD to the Colony Density Chart. Mixed bacterial growth, which means that different types of colonies are present, is usually caused by contamination of the specimen.

Many factors, such as use of antimicrobial therapy, time of urine incubation in the bladder (e.g., first voided urine), and proper specimen collection, may influence the colony count obtained. In all cases, the physician must be the final judge of the proper interpretation of CHROMOSTREAK UCD test results.

COLONY MORPHOLOGY & PRINCIPLE OF IDENTIFICATION WITH URI*Select* 3 AGAR

Presumptive identification is based on typical morphology and colony color. Chromogenic URI*Select* agar enables identification of bacteria by distinct color differences among different types of organisms.

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

REVELATION OF THE VARIOUS ENZYME ACTIVITIES:

1. Spontaneous

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

Positive reaction of β-galactosidase activity – pink color of colonies. β-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.

Positive reaction of β- glucosidase activity – turquoise blue color of colonies: 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 Enterococcus species or 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 organisms (Klebsiella, Enterobacter, Serratia or Citrobacter species) and should be identified using a conventional method.

Negative reaction - No coloration.

2. Spontaneous or after addition of a reagent:

Tryptophan deaminase activity (TDA) indicating an organism of the Proteus-Providencia-Morganella group.

Positive reaction of TDA - spontaneously, orange-brown color of the colony, with or without a change on agar color to brown. If the change in color is slight, revelation of the enzyme can be enhanced by depositing a drop of iron perchlorite ($FeCl_3$) 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.

Negative reaction - No coloration on an isolated colony.

3. Detection of indole production:

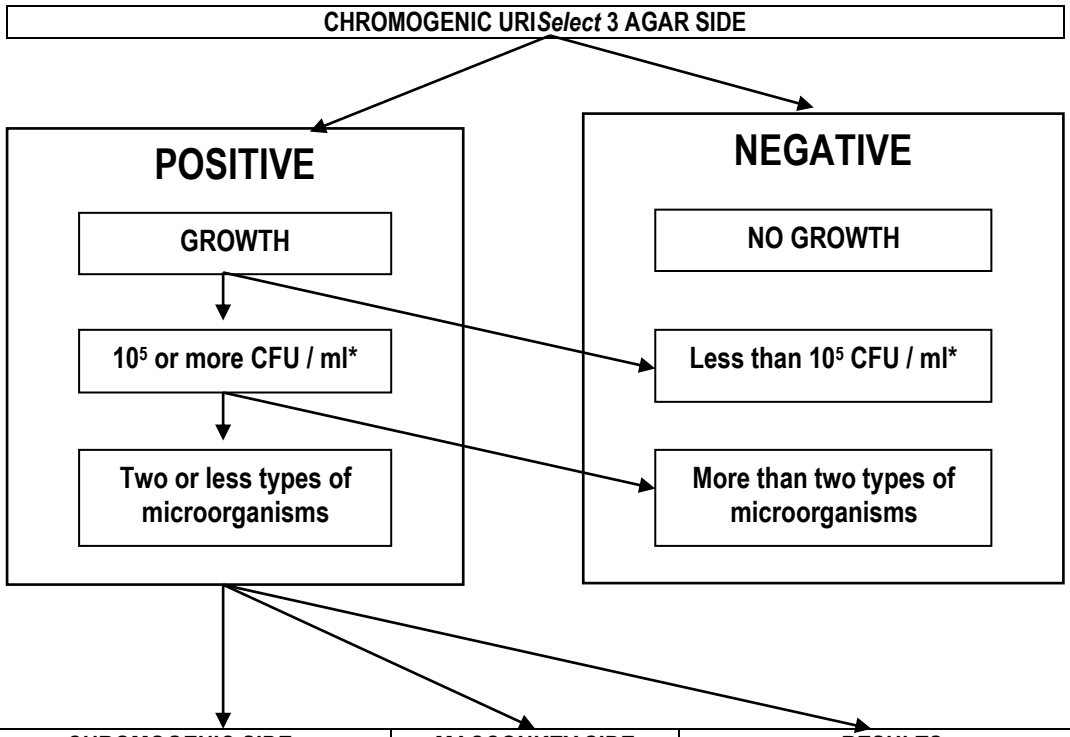
For cultures containing only one kind of organism, deposit one drop of Kovac's reagent on a well-isolated suspect-colony, directly onto the agar:

- 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.

TABLE 2: The relationship between bacterial enzyme activities and the colony morphology on chromogenic URI*Select* 3 agar

Organism	Colonies morphology		Enzyme activities			
	Size (Ø mm)	Color	β-glucuronidase	ß-glucosidase	Indole	TDA
E. coli	2.0 or 3.0	Pink	+	-	+	-
KES group organisms	2.0 or 3.0	Turquoise blue or blue purple	-	+	+/-	-
Proteus mirabilis	2.0	Orange brown	-	-	-	+
Proteus vulgaris, Morganella, Providencia	2.0	Orange brown	-	-	+	+
Enterococcus faecalis	0.5 – 1.0	Bright, frank, blue	-	+	-	-
Group B Streptococcus	0.5 – 1.0	Bright, frank, blue	-	+	-	-
Staphylococcus	1.0 or 2.0	White	-	-	-	-

TABLE 3: Interpretation of CHROMOSTREAK UCD results



CHROMOGENIC SIDE	MACCONKEY SIDE	RESULTS
Pink colonies. Confirm with a positive reaction of the colony with the Kovac's Reagent (the reagent turns pink within 15 sec.).	**Pink-red** colonies	***E.coli***
Turquoise blue or blue purple large colonies	**Pink-red** colonies	***Klebsiella, Enterobacter, Serratia and Citrobacter* species**
Orange brown color of the colony, with or without a **brown color** of the agar	Translucent colonies	***Proteus, Providencia and Morganella* species**
Spreading and flat **yellowish-green** colonies with metallic sheen	Clear colonies	***Pseudomonas* species**
Small or extremely small (0.5 to 1.0 mm in diameter) **bright, frank, blue** colonies	**No Growth**	***Enterococcus or group B Streptococcus*** *species*
White colonies	**No Growth**	***Staphylococcus* species**

*Each individual laboratory should determine its own cutoff point

LIMITATIONS OF THE PROCEDURE

1. CHROMOSTREAK UCD is a presumptive screening test. If the physician concludes that it is clinically indicated, full biochemical identification of the causative agent(s) and antimicrobial susceptibility testing should be performed.
2. CHROMOSTREAK UCD can detect bacteria concentrations as low as 1000 CFU/ml of urine. The Colony Density Chart allows the reporting of colony counts to the nearest power of 10. When used as directed, an overall correlation of 95% is obtained when CHROMOSTREAK UCD colony count results are compared to conventional agar plating methods.
3. If bacterial growth is mixed, repeat the test since this is most likely due to contamination.
4. Infants and certain patients may have true infection even if the bacterial count is less than 10^5 CFU/ml. Final interpretation of such results should be evaluated based on clinical information. Protocols for individual laboratory results must be established based on close cooperation between the medical and laboratory staff. The guidelines are based on the principle that four factors (number of isolates, density of isolates, type of specimen, and clinical information) must be considered to assess the significance of an isolate. Clinical information may alter the physician's final interpretation of culture results.
5. If bacterial content in a urine specimen is above 10^7 CFU/ml, no single colony can be isolated because

of confluent growth, even in the "isolation track". A standard quantitative urine culture should be performed.

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