Dipstreak®

URINE CULTURE DEVICE

For transport, screening, isolation and identification of bacterial contamination in urine samples

Instruction Manual

(Catalog No. BD-915)

For professional in vitro diagnostic use only
Store at +2+25oC until expiry date indicated on the label.
Store away from direct sunlight.

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INTENDED USE

Dipstreak ® is a convenient device for transporting urine samples as well as for isolating, enumerating and identifying specific bacteria in urine. A unique streaking mechanism permits the isolation of single colonies even when the original bacterial population of the sample was as high as 10^8 organisms per milliliter. ***Dipstreak*** ® is intended for use in both physicians' office laboratories and clinical laboratories.

SUMMARY AND EXPLANATION

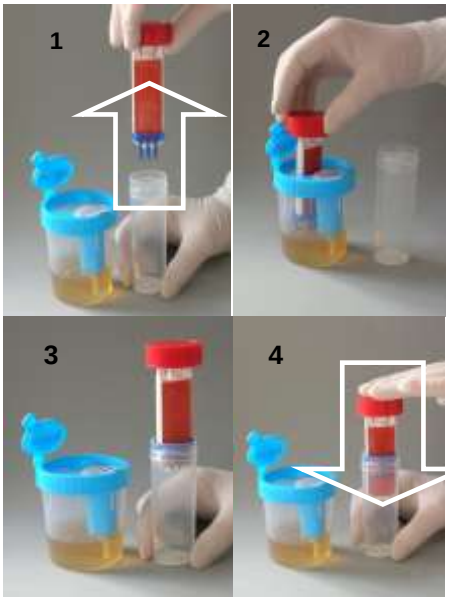
Dipstreak ® comprises a plastic paddle with two types of agar attached back-to-back, housed in a closed transparent plastic tube. A ring with elongated prongs is attached to the end of the paddle so that there are prongs on each side of the slide. The ends of the prongs are dipped into the urine sample. Upon re-insertion into the plastic tube, the prongs are prevented from moving and the agar surfaces are inoculated with bacteria 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 10^8 organisms per milliliter.

SPECIMEN COLLECTION

Cleanse the genital area and collect a midstream urine specimen in a sterile container. Inoculate the urine as soon as possible after collection.

PROCEDURE

1. Unscrew the ***DipStreak*** ® cap. Pull the paddle out. Do not touch any part but the cap.
2. Hold the paddle vertically and dip the white prongs into the sample up to about half of their length (see below).
3. Return the paddle to its container in a quick, vertical and continuous motion and tighten cap.
4. Transport the tube to laboratory for incubation and examination.
5. Before incubation loosen cap one-half turn.
6. Incubate the entire container in a vertical position at 35±2ºC for 18-24 hours.
7. Determine the number of colony forming units by comparison to the Colony Density Chart.



REF **BD-915: UriSelective agar/ MacConkey agar**

MacConkey Agar is a selective and differential medium for detection of coliform organisms and enteric pathogens. 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 micro-organisms 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.

UriSelective Agar (Savyon Diagnostics Ltd, Israel) is a non-selective agar medium composed of a rich nutrient base containing three peptones, which sustains growth of all urinary tract pathogens; 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).

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Use aseptic technique and established laboratory procedure in handling and disposing of infectious materials.
3. Dispose of used ***DipStreak*** ® and tubes by burning, autoclaving or immersing in a suitable disinfectant overnight.

STORAGE

Store at +2+25ºC until expiry date indicated on the label.
Store away from direct sunlight.

READING THE RESULTS

UriSelective Agar / MacConkey Agar

Remove the paddle from the tube. Observe the bacterial growth on both agars. Count and compare the number of bacteria growing on the UriSelective Agar to the chart or to the guidelines for enumeration of bacteria. ***DipStreak*** ® is capable of detecting bacteria in urine at concentrations as low as 1000 CFU/ml. The colony density chart allows the reporting of colony counts to the nearest power of 10. If bacterial content is above 10^7 CFU/ml, no single colony can be isolated because of confluent growth. A standard quantitative urine culture should be performed.

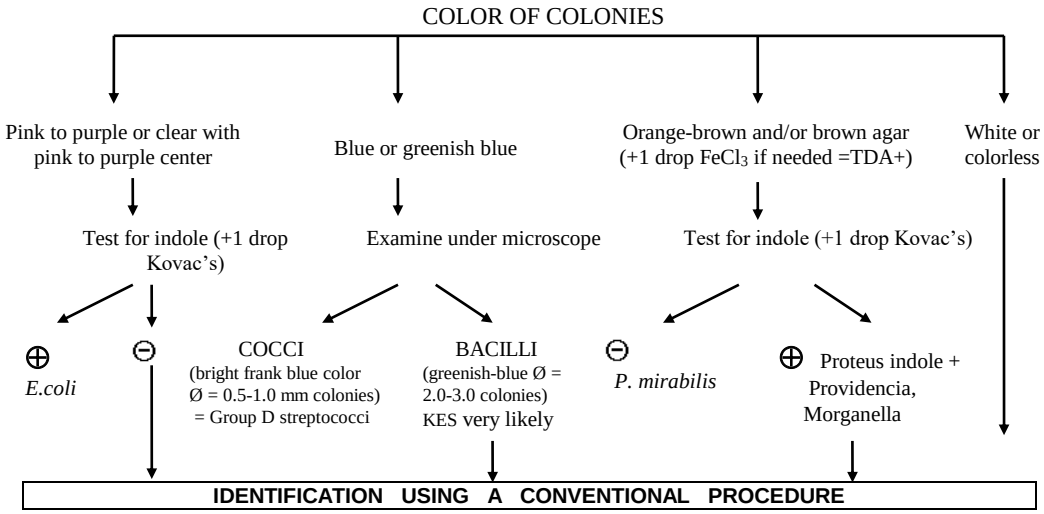
Interpretation of Results

In the past, the presence of $\geq 10^5$ CFU/ml was regarded as a positive result while $\leq 10^4$ CFU/ml was negative. From 10^4 to 10^5 CFU/ml were considered borderline cases, which called for repeat examination. Today, the trend is for specific medical departments such as Urology, Nephrology and Pediatrics to report down to 10^3 CFU/ml. Use the attached colony density chart for reporting results. If bacterial growth yields three or more different kinds of colonies, this is most likely due to contamination. The test should be repeated. Preliminary identification of the bacteria can be made based on type and color of colonies.For example:

Organism	UriSelective Agar	MacConkey Agar
Escherichia coli	Pink to purple or clear with a pink to purple center colonies	Pink to rose-red colonies (may be surrounded by a zone of precipitated bile)
Klebsiella	Blue to greenish-blue large mucoid colonies	Mucoid, pink large colonies
Citrobacter, Enterobacter, Serratia	Blue to greenish-blue large colonies	Pink colonies, less mucoid than Klebsiella
Pseudomonas aeruginosa	Spreading and flat yellowish-green colonies with metallic sheen	Irregular, colorless colonies
Proteus	Brown colonies with/ without brown color of the agar	Colorless colonies, swarming growth
Enterococci	Blue to greenish-blue small colonies	No growth to slight growth
Staphylococcus aureus	White or colorless colonies	No growth
Staphylococci coagulase-negative	White or colorless colonies	No growth

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

Interpretation of Results for UriSelective Agar (incubation 18-24 hr at 35±2ºc)



Bacteria		Colonies	Characteristics			
	Ø (mm)	Color	ß-glucuronidase	ß-glucosidase	Indole	TDA
E. coli	2.0 or 3.0	Purple	+	-	+	-
P. mirabilis	2.0	Orange-brown	-	-	-	+
E. faecalis	0.5 - 1.0	Bright frank blue	-	+	-	
P. stuartii	2.0	Orange-brown	-	+	+/-	-
S. aureus	1.0 or 2.0	White	-	-	-	-

USER QUALITY CONTROL

Quality control tests are performed on each lot of ***Dipstreak*** ® at the time of manufacture. Product users who wish to perform their own quality control may use the following procedure.

1. Prepare a suspension (10^4-10^5 CFU/ml) of each of the following organisms in culture-negative urine from a healthy individual. Confirm the exact organism concentration by inoculating 10 µl with a calibrated loop on reference plates of UriSelect3 and MacConkey agar.
2. Test according to the PROCEDURE.
3. If the device does not support the expected growth of organisms, it has deteriorated and should not be used.

TYPICAL CULTURAL RESPONSE (after 24 hours at 35±2ºC)

ORGANISM	ATCC	MacConkey Agar	UriSelective Agar
E. coli	25922	Pink colonies	Purple colonies
S. aureus	25923	Inhibited	White colonies
P. mirabilis	12453	Translucent, colorless colonies	Orange-brown colonies

Performance Characteristics

Savyon Diagnostics Ltd ***Dipstreak*** ® was compared to another commercially available urine culture device (the Diaslide®).

In Study 1 a total of 322 samples were obtained from a sick fund clinic and tested with both urine culture devices. From this group 188 samples yielded no bacterial growth on either device

In Study 2 a group of 200 samples, identified as positive by a catalase screening assay, were tested at a Sick Fund clinic with the *DipStreak* ® and Diaslide ® culture devices. From this group 47 samples yielded no bacterial growth on either device.

	No. of the following result by Diaslide ®					No. of the following result by Diaslide ®			
Study 1					**Study 2**				
Cutoff (CFU/ml) and result by *Dipstreak* ®	Positive	Borderline	Negative	Total	**Cutoff (CFU/ml) and result by *Dipstreak* ®**	Positive	Borderline	Negative	Total
10^4					**10^4**				
Positive	105	3	2	110	**Positive**	131	2	0	133
Borderline	1	3	7	11	**Borderline**	0	20	0	20
Negative	0	3	198	201	**Negative**	0	0	47	47
Total	106	9	207	**322**	**Total**	131	22	47	**200**
10^5					**10^5**				
Positive	90	4	2	96	**Positive**	98	0	2	100
Borderline	5	4	3	12	**Borderline**	0	33	0	33
Negative	0	1	213	214	**Negative**	0	0	67	67
Total	95	9	218	**322**	**Total**	98	33	69	**200**

Totals	**Study 1 + Study 2 No. of the following result by Diaslide ®**				**Summary of statistical data (borderlines excluded from sensitivity and specificity calculations)**			
Cutoff (CFU/ml) and result by *Dipstreak* ®	Positive	Borderline	Negative	Total	**Cutoff (CFU/ml) and result by *Dipstreak* ®**	**Sensitivity**	**Specifity**	**Total agreement**
10^4					**10^4**	236/238 99.2%	245/245 100%	503/522 96.4%
Positive	236	5	2	243				
Borderline	1	22	7	31				
Negative	0	3	245	248				
Total	237	34	254	**522**				
10^5					**10^5**	188/192 97.9%	280/280 100%	506/522 96.9%
Positive	188	4	4	196				
Borderline	5	37	3	45				
Negative	0	1	281	281				
Total	193	42	287	**522**				

Precision study: Five dilutions of known bacteria were tested with six different Dipstreak ® devices. The results listed in the following table show that the Dipstreak ® yields consistent, reproducible results at all dilutions for 1 x 103 to 1 x 10^7 CFU/ml.

Interpretation of results from colony chart

Organism	CFU/ml*	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
E. coli	1×10^3	1×10^3	1×10^2	1×10^3	1×10^3	1×10^3	1×10^2
	1×10^4	1×10^4	1×10^4	1×10^4	1×10^4	1×10^4	1×10^4
	1×10^5	5×10^5	1×10^5	5×10^5	1×10^5	5×10^5	1×10^5
	1×10^6	1×10^6	1×10^6	1×10^6	1×10^6	1×10^6	1×10^6
	1×10^7	1×10^7	1×10^7	1×10^7	1×10^7	1×10^7	1×10^7
Klebsiella pneumonia	1×10^3	1×10^3	1×10^3	1×10^3	1×10^2	1×10^3	1×10^2
	1×10^4	1×10^4	1×10^4	1×10^4	1×10^4	5×10^3	1×10^4
	1×10^5	1×10^5	1×10^5	1×10^5	1×10^5	1×10^5	1×10^5
	1×10^6	1×10^6	1×10^6	1×10^6	1×10^6	1×10^6	1×10^6
	1×10^7	1×10^8	1×10^7	1×10^7	1×10^7	1×10^7	1×10^7
Proteus vulgaris	1×10^3	5×10^3	1×10^3	1×10^3	1×10^3	1×10^3	1×10^3
	1×10^4	1×10^4	1×10^4	1×10^4	1×10^4	1×10^4	1×10^4
	1×10^5	1×10^5	1×10^5	1×10^5	1×10^5	1×10^5	1×10^5
	1×10^6	1×10^6	1×10^6	1×10^6	1×10^6	1×10^6	5×10^5
	1×10^7	1×10^7	1×10^7	1×10^7	1×10^7	1×10^7	5×10^7

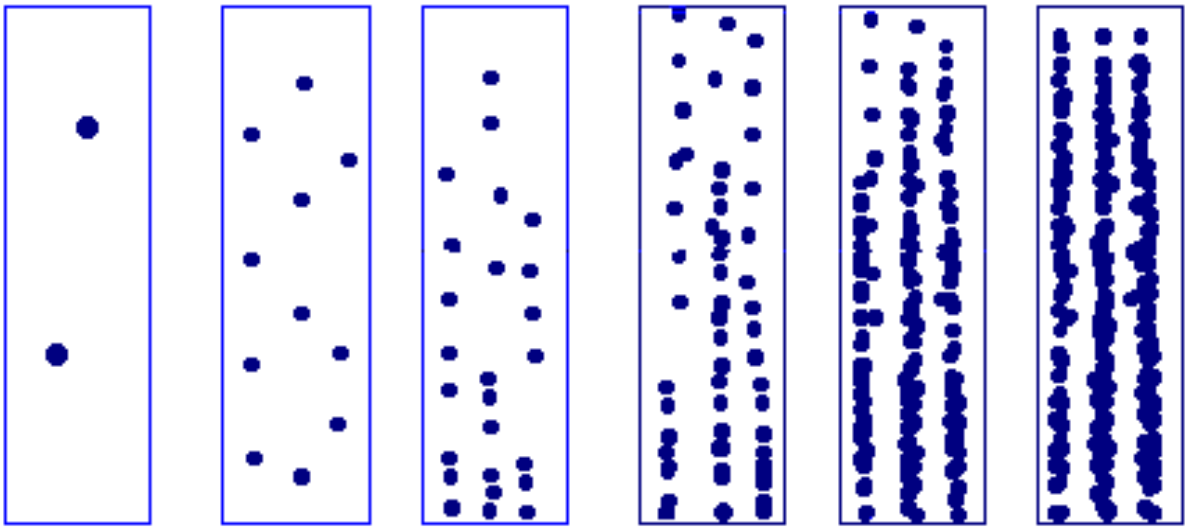
*as determined by culture on trypticase soy agar plates

References

[1] Balows, A. et al. eds. 1991. Manual of clinical microbiology, 5th ed. Amer. Soc. Microbiol., Washington DC.
[2] Clarridge, J.E. 1987. Laboratory diagnosis of urinary tract infections. Amer. Soc. Microbiol., Washington DC.
[3] Guttmann, D. 1967. Dip-slide: an aid to quantitative urine culture in general practice. Br. Med. J. 3:343-345.
[4] Blondeau, JM et al. 1995. Evaluation of the Cult Dip Plus dip slide method for urinary tract infection. J. Clin. Pathol. 48:710713.
[5] Difco Laboratories. 1984. Dehydrated culture media and reagents for microbiology, p.546-551. *In* Difco manual, 10th ed.

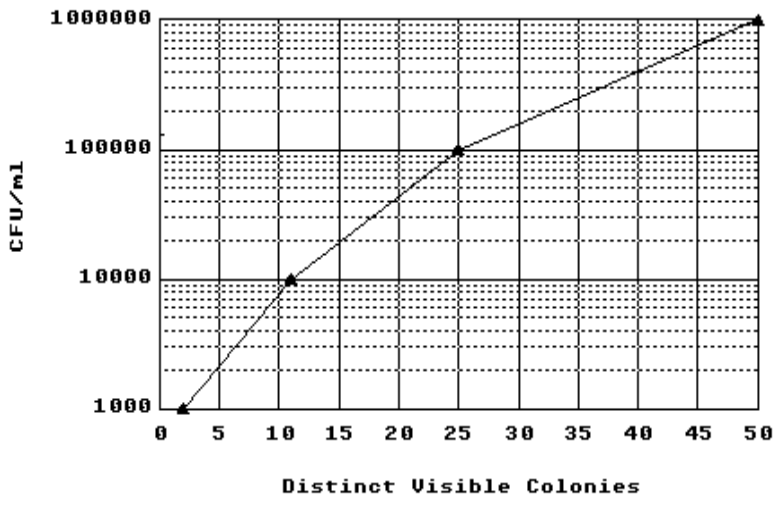
DipStreak ®

Colony Density Chart



Colonies	<10	11-24	25-50	>50		
CFU/ml	10^3-10^4	10^4-10^5	10^5-10^6	>10^6	10^7	10^8

Colony Forming Unit /ml, Read on UriSelective Agar



Colonies	CFU/ml
1-10	10^3-10^4
11-24	10^4-10^5
25-50	10^5-10^6
>50	>10^6

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EU REP

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Symbols for IVD components and Reagents			
	Manufacturer		Do not reuse
IVD	For *in vitro* diagnostic use only		Temperature limitation
REF	Catalogue Code		Use by
LOT	Lot Number		Consult instructions for use
n Σ	Contains Sufficient for <n> Tests	EU REP	EU Authorized Representative
STERILE A	Sterile by aseptic processing		

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