BD™ DCLS Agar, Modified / Hektoen Enteric Agar (Biplate)

INTENDED USE

BD DCLS Agar, Modified / Hektoen Enteric Agar (Biplate) is used for the isolation of *Salmonella* and *Shigella* from human fecal specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

DCLS Agar, Modified is a modification of the Desoxycholate Citrate Agar media described by Leifson.[1] Coliform organisms capable of fermenting lactose or sucrose are generally inhibited. Gram positive bacteria are suppressed. While studying enteric pathogens on Endo medium, Holt-Harris and Teague used lactose and sucrose in the development of a nutrient agar containing methylene blue and eosin. Some coliforms ferment sucrose more readily than lactose.[2] The addition of sucrose (saccharose) allows nonpathogenic sucrose-fermenting organisms to produce rose-red (pink) colonies that are easily recognized, reducing the number of false positive reactions.

There exist several modifications of the original formula of DCLS Agar.[3,4] The formula of this medium contains more lactose and sucrose and less citrate and, therefore, supports growth of *Shigella* slightly better than regular DCLS Agar. Additionally, it allows the detection of *Yersinia enterocolitica.*

In DCLS Agar, Modified, meat extract and peptone provide nitrogen, and yeast extract provides vitamins. Lactose and saccharose (sucrose) are fermentable carbohydrates that are used by many non-pathogenic Gram negative rods, such as *Escherichia coli*, but not by *Salmonella* and *Shigella*. Sodium citrate, sodium thiosulfate and sodium desoxycholate are selective agents. Neutral red is the pH indicator.

Hektoen Enteric Agar (HEA) was developed in 1967 by King and Metzger of the Hektoen Institute in order to improve the isolation of *Shigella* and *Salmonella* organisms when compared with other media frequently utilized at that time.[5] This medium is considered to be moderately selective, and is particularly useful in the isolation of *Shigella* species. The present formulation differs from that of the original in that sodium desoxycholate has been eliminated and the concentration of bile salts reduced. Additionally, the peptone concentration has been increased in order to offset the inhibitory effects of the bile salts.[3]

Bile salts render the medium selective, inhibiting Gram positive organisms and reducing growth of some gram-negative bacteria other than *Salmonella* and *Shigella*. Lactose, sucrose and salicin are included for optimal differentiation by the color of the colonies and of the medium adjacent to the colonies. *Salmonella* and *Shigella* do not ferment these carbon compounds and thus do not cause a color change of the pH indicator system, while organisms fermenting one or several of these compounds to acids, e.g. *E. coli*, cause a color change to yellow, orange, or salmon. Ferric ammonium citrate and sodium thiosulfate in the medium enable the detection of hydrogen sulfide production by *Salmonella*. The pH indicator system consists of acid fuchsin and bromthymol blue. This formulation is recommended as one of several plating media for the culture of *Enterobacteriaceae* from stool specimens.[6-8]

BD DCLS Agar, Modified / Hektoen Enteric Agar (Biplate) is a combination of two selective differential media for the isolation of *Salmonella* and *Shigella* from human feces.

REAGENTS

BD DCLS Agar, Modified / Hektoen Enteric Agar (Biplate)

Formulas* Per Liter Purified Water

DCLS Agar, Modified		Hektoen Enteric Agar	
Meat Peptone (Pancreatic)	5.0 g	Peptic Digest of Animal Tissue	12.0 g
Yeast Extract	2.5	Yeast Extract	3.0
Meat Extract	2.5	Bile Salts	9.0
Lactose	10.0	Lactose	12.0
Sucrose	10.0	Sucrose	12.0
Ferric Ammonium Citrate	1.0	Salicin	2.0
Sodium Desoxycholate	2.5	Sodium Chloride	5.0
Sodium Thiosulfate	5.0	Sodium Thiosulfate	5.0
Sodium Citrate	1.0	Ferric Ammonium Citrate	1.5
Neutral Red	0.02	Bromthymol Blue	0.064
Agar	10.0	Acid Fuchsin	0.1
pH 7.5 +/- 0.2		Agar	13.5
		pH 7.6 +/- 0.2	

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.
Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.
Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate plates at 35 ± 2°C in an aerobic atmosphere. Occasionally, *Shigella* species may require a 42 to 48 hours incubation.
Examine plates after 18 to 24 and after 42 to 48 hours for amount of growth, colony size, pigmentation and selectivity.

Strains	DCLS Agar, Modified	Hektoen Enteric Agar
Escherichia coli ATCC™ 25922	No growth to fair growth; rose-red (pink) colonies, precipitates may surround the colonies	Inhibition partial to complete; yellow-orange colonies, precipitates may surround the colonies, salmon to orange halos
Enterococcus faecalis ATCC 29212	Inhibition complete	Inhibition partial to complete; tiny yellow colonies, salmon to orange halos
Proteus mirabilis ATCC 12453	Orange-red colonies; growth fair to good	Blue-green to blue with black centers
Salmonella Abony DSM 4224	Orange red to yellow colonies; growth good to excellent	Growth good to excellent; colonies green to blue-green with black center
Salmonella Typhi-murium ATCC 14028	Orange red to yellow colonies; growth good to excellent	Growth good to excellent; colonies green to blue-green with black center
Shigella flexneri ATCC 12022	Orange red to yellow colonies; growth fair to excellent	Growth fair to excellent; colonies light green
Uninoculated	Orange-red, slightly opalescent	Green, nearly transparent

PROCEDURE

Materials Provided
BD DCLS Agar, Modified / Hektoen Enteric Agar (90 mm **Stacker™** biplates). Microbiologically controlled.

Materials Not Provided
Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types
This medium is used for stool specimens from patients suspected to have a bacterial enteric infection and for similar materials e.g., rectal swabs (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).[7,8]

Test Procedure
Streak the specimen as soon as possible after it is received in the laboratory on both media of this biplate. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edges; then streak for isolation from the inoculated areas.
A less selective medium such as **BD MacConkey II Agar** and selective liquid enrichment media, such as Selenite F Broth should also be inoculated to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. For a complete discussion on the isolation and identification of enteric pathogens from clinical specimens, refer to the procedures described in appropriate references.[6-8]
BD DCLS Agar, Modified / Hektoen Enteric Agar (Biplate) may be used as media for subculturing from Selenite F Broth.
Incubate plates, protected from light, at 35 ± 2°C for 18 to 24 h. If negative, reincubate for an additional 18 to 24 hours.

Results
Typical growth appearance on **BD DCLS Agar, Modified / Hektoen Enteric Agar (Biplate)** is as follows:

Organisms	DCLS Agar, Modified	Hektoen Enteric Agar
E. coli	Large, flat, pink to red with a zone of bile precipitation	Large, yellow to salmon color; some strains may be inhibited
Enterobacter/Klebsiella	Large, mucoid, pink	Large, yellow to salmon color
Proteus	Colorless to red	Variable, blue-green to blue or salmon, most strains with black center
Salmonella	Colorless to pale pink	Blue-green to blue; most strains black center
Shigella	Colorless to pale pink	Green and moist, raised
Pseudomonas	Colorless to brown or green	Irregular, green to brown
Gram positives	No growth	No growth to slight growth

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
DCLS Agar, Modified / Hektoen Enteric Agar (Biplate) is used for the isolation of *Salmonella* and *Shigella* from human stool specimens and rectal swabs.[3,6-9]
If the number of pathogens in a specimen is low, they may be missed on highly selective media. It is recommended to include a medium with a lower degree of selectivity, e.g., **BD MacConkey II Agar** and/or a liquid selective enrichment.[6-8]
Proteus mirabilis colonies may resemble *Salmonella* on these media.
Certain *Shigella* strains may need a 42 to 48 h incubation.
Although certain diagnostic tests may be performed directly on these media, biochemical and, if indicated, immunological testing using pure cultures is necessary for complete identification.
Colonies suspected of being *Salmonella* or *Shigella* must be confirmed and identified serologically. [6]
Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on these media.

REFERENCES

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PACKAGING/AVAILABILITY

BD DCLS Agar, Modified / Hektoen Enteric Agar (Biplate)
Cat. No. 254553 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

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