



## Sakazakii DHL Agar

M1619

Sakazakii DHL Agar (Deoxycholate Hydrogen sulphide Lactose Agar) is used for the detection and isolation of pathogenic *Enterobacteriaceae* from all types of specimens.

### Composition\*\*

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Meat peptone	10.000
Meat extract	3.000
Lactose	10.000
Sucrose	10.000
L-Cysteine hydrochloride. H <sub>2</sub> O	0.200
Sodium citrate	1.000
Sodium deoxycholate	1.500
Sodium thiosulphate	2.000
Ammonium iron (III) citrate	1.000
Neutral red	0.030
Agar	15.000
Final pH ( at 25°C)	7.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 63.71 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

*Enterobacteriaceae* have wide distribution. Many members form the normal gut and intestinal microflora in humans and animals. They are also found on plants and in soils and water (1). Some species occupy very limited ecological niches. They are a major component of the normal intestinal flora of humans but are relatively uncommon at other body sites. They account for nearly 50% of septicemia cases, more than 70% of urinary tract infections and a significant percentage of intestinal infections (2).

Sakazakii DHL Agar is modified Deoxycholate Agar (M065) as described by Sakazakii et al (3, 4). Sakazakii DHL Agar stands for Sakazakii Deoxycholate-Hydrogen sulphide-Lactose Agar. The medium is selective for the identification and isolation of *Enterobacteriaceae* due to inclusions of sodium deoxycholate. Sodium deoxycholate inhibits gram-positive bacteria and also prevents swarming growth of *Proteus* species. However due to the low concentration of sodium deoxycholate coupled with the nutritionally rich media, fastidious strains of *Salmonella* and *Shigella* are able to grow on this medium. Sulphur is released from thiosulphate or other sulphur-containing compounds in the form of sulphide. The H<sub>2</sub>S thus produced is detected by ferric ammonium citrate to form insoluble heavy metal sulphides that appear as a black precipitate (5). *Proteus*, *Morganella*, *Rettgerella* and *Providencia* colonies are surrounded by dark brown zones due to phenylalanine deamination. Phenylalanine is sourced from peptone that forms an iron complex with the ferric ions. The high concentration of sucrose in the medium permits the recovery of sucrose positive and lactose negative members of *Enterobacteriaceae*.

### Quality Control

#### Appearance

Light yellow to light pink homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel.

#### Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates

### Reaction

Reaction of 6.37% w/v aqueous solution at 25°C pH : 7.2±0.2

### pH

7.00-7.40

### Cultural Response

M1619: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	H <sub>2</sub> S production
<b>Cultural Response</b>					
<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	≥50%	red with bile precipitate	negative
<i>Klebsiella pneumoniae</i> ATCC 10031	50-100	good-luxuriant	≥50%	pink	negative
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	≥50%	colourless	positive
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	good-luxuriant	≥50%	colourless	positive
<i>Proteus vulgaris</i> ATCC 13315	50-100	fair-good	30-40%	pink with brownish zone	negative
<i>Proteus mirabilis</i> ATCC 25933	50-100	good-luxuriant	≥50%	colourless with brownish zone	variable
<i>Shigella flexneri</i> ATCC 12022	50-100	fair-good	30-40%	colourless	negative
<i>Staphylococcus aureus</i> ATCC 25923	≥10 <sup>3</sup>	inhibited	0%	-	-
<i>Enterococcus faecalis</i> ATCC 29212	50-100	none-poor	≤10%	colourless	negative
<i>Bacillus cereus</i> ATCC 10876	≥10 <sup>3</sup>	inhibited	0%	-	-

### Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium below 2-8°C. Use before expiry date on the label.

### Reference

- Krieg N. R. and Holt J. G., (Eds.), 1984, Bergeys Manual of Systematic Bacteriology Vol. I, P-408-516. Williams and Wilkins Co. Baltimore.
- Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
- Sakazaki R., Namioka S., Osada A., a. Yamada C. A., 1960, Japan. J. Ex. Med., 30; 13-22.
- Sakazaki R., Tamura K., Prescott L. M., Benzic Z., Sanyal S. C., a. Sinha, R., 1971, Indian J. Med. Res., 59; 1025-1034.
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Revision : 1 / 2011



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