



PRODUCT SPECIFICATION SHEET

Deoxycholate Agar (DM076)

Intended Use

Deoxycholate Agar (DM076) is a differential medium recommended for the enumeration of coliforms in dairy products. It may also be used for the isolation of Gram-negative enteric pathogens from rectal swabs, faeces and other pathological specimens.

Product Summary and Explanation

Deoxycholate Agar was first formulated by Leifson for the improved isolation of intestinal pathogens and the enumeration of intestinal pathogens in milk and water.⁽¹⁾ Deoxycholate Agar was an improvement over other media because chemicals, citrates and sodium deoxycholate worked well as inhibitors. This media is mainly inhibitory only on Gram positive bacteria, but has originally been used for the detection of *Salmonella* and *Shigella* from human fecal specimens.^(2, 6) The selectivity of medium permits the use of fairly heavy inocula without danger of overgrowth of the *Shigella* and *Salmonella* by other micro-flora. Today, the medium is used alternatively to MacConkey Agar as a universal differential isolation medium for all enteric bacilli.^(3, 4, 5) It is recommended that for routine examination of stool and urine specimens, particularly for the detection of *Shigella* and *Salmonella*, Deoxycholate Agar be used in conjunction with other media such as MacConkey Agar, Bismuth sulphite Agar, etc, as these organisms produce colorless colonies on this medium.

Principles of the Procedure

The Deoxycholate and Citrate salts inhibit the development of the Gram positive organisms. Peptic digest of animal tissue provides nitrogen, vitamins, minerals and amino acids essential for growth. Lactose is the fermentable carbohydrate providing carbon and energy. Lactose also helps in differentiating enteric bacilli as lactose fermenters which degrades the lactose causing acidification of the medium and under the pH indicator or neutral red forms red or pink colonies; while lactose non-fermenters produce colorless colonies. Coliform bacteria if present form pink colonies on this medium. These colonies usually are also surrounded by a turbid zone of precipitated deoxycholic acid formed due to acidification of the medium. Sodium deoxycholate combines with neutral red in an acidic environment, causing the dye to go out of the solution with the subsequent precipitation of deoxycholate. Citrate and iron (Fe) combination has a strong hydrolyzing effect on agar when the medium is heated, producing a soft and unelastic agar.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	10.00
Lactose	10.00
Sodium deoxycholate	1.00
Sodium chloride	5.00
Dipotassium phosphate	2.00
Ferric citrate	1.00
Sodium citrate	1.00
Neutral red	0.03
Agar	15.00
Final pH: 7.3 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	





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Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. DO NOT AUTOCLAVE OR REMELT. This medium is heat sensitive. Avoid excessive or prolonged heating during reconstitution.
4. If autoclaved the agar becomes soft and almost impossible to streak. Surface colonies of non-lactose fermenters often absorb a little color (pinkish) from the medium and organisms may be mistaken for coliforms.

Directions

1. Suspend 45.03 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely, with frequent agitation to avoid charring.
3. DO NOT AUTOCLAVE. Avoid overheating.
4. Mix well and pour into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Reddish orange coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 4.5% solution	pH 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Color of colony
1.	<i>Staphylococcus aureus</i> ATCC 25923	>=10 ³	inhibited	0%	--
2.	<i>Salmonella Typhi</i> ATCC 6539	50-100	good-luxuriant	>=50%	colorless or opaque with or without black centre
3.	<i>Enterococcus faecalis</i> ATCC 29212	>=10 ³	inhibited	0%	--
4.	<i>Escherichia coli</i> ATCC 25922	50-100	good	>=40- 50%	pink with bile precipitate
5.	<i>Salmonella enteritidis</i> ATCC 13076	50-100	good-luxuriant	>=50%	colorless
6.	<i>Salmonella typhimurium</i> ATCC 14028	50-100	good-luxuriant	>=50%	colorless
7.	<i>Shigella flexneri</i> ATCC 12022	50-100	good	>=40- 50%	colorless

The organisms listed are the minimum that should be used for quality control testing.





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Escherichia coli ATCC 25922- Pink colonies with bile precipitate
Salmonella Typhimurium ATCC 14028- Colorless colonies

Test Procedure

For Enumeration of Coliforms

1. Prepare specimen dilutions of 1/10, 1/100, 1/1000 in 1/4-Strength Ringers solution use this Inoculums within 15 min.
Pour plates : pipette 1 ml of each dilution into Petri dishes. Add 10-20 ml of Deoxycholate Agar, cooled at 45°C, and mix thoroughly
Spread plates: spread 1ml of each dilution over the surface of the solidified medium in a Petri dish.
Overlay thin layer of uninoculated Deoxycholate Agar over the surface of a gelled pour-plate, it assists subsequent counting.
2. Incubate at 35 ± 37°C for 18-24hours.

For Isolation of *Enterobacteriaceae*

1. For isolation of organisms of *Enterobacteriaceae* it is advisable to use Deoxycholate Agar in conjunction with other plating media.
2. Inoculate lightly a Deoxycholate Agar plate with faeces, rectal swab, or enrichment culture.
3. Incubate at 35-37°C for 18-24 hours.

Results

1. Select plates containing 10-300 colonies. Results are expressed as colonies per product tested.
2. Differentiation of enteric bacilli is based on fermentation of lactose.
3. Lactose fermenters produce acid and, in the presence of Neutral Red, form pink to red colonies, whereas Non-lactose fermenters form colorless colonies.
4. Non-lactose fermenters which are not of enteric origin are generally inhibited by the sodium desoxycholate in the medium.





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Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. This medium supports growth of organisms of the family *Enterobacteriaceae* and a variety of other Gram negative rods, such as *Pseudomonas* and *Aeromonas*.
2. The medium does not differentiate between *Proteus* and other lactose negative Gram negative rods such as *Salmonella* or *Shigella*.
3. Colonies of *Proteus* and *Salmonella* will not be black on this medium.
4. Although certain diagnostic tests may be performed directly on this medium, for complete identification biochemical and, if indicated, immunological testing is necessary.
5. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Deoxycholate Agar.

Product Code : DM076

Available Pack sizes : 100gm / 500gm

References

1. Leifson, E. 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. *J. Pathol.* 40:581-599.
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3. Farmer III, J. J., and M. T. Kelly. 1991. *Enterobacteriaceae*. In: A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C. 360-383.
4. MacFaddin, J. F. 1985. *Media for isolation-cultivation-identification-maintenance of medical bacteria*, vol. 1, p. 269-275.
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6. Speck M. L., (Ed.). 1984. *Compendium of Methods for the Microbiological Examination of Foods*, 2nd ed., APHA, Washington, D.C.

Further Information

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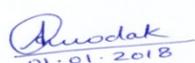
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