PRODUCT SPECIFICATION SHEET

Lysine Iron Agar (DM137)

Intended Use
Lysine Iron Agar (DM137) is used for the differentiation of enteric organisms especially Salmonella serotype arizonae, based on their ability to decarboxylate or deaminate lysine and to form hydrogen sulphide.

Product Summary and Explanation
Edwards and Fife[1] developed the medium to detect lactose-fermenting salmonellae which will produce pink colonies on lactose-containing media like Deoxycholate Citrate Agar and Brilliant Green Agar. Lysine Iron Agar is a differential medium which detects Salmonellae (including lactose fermenting S. arizona) by lysine decarboxylase activity and H_2S production. Because S. arizona ferments lactose so rapidly, the author found that the expected H_2S production on triple sugar was suppressed. Since S. arizona strains are found occasionally in outbreaks of food borne infection, it is important to be able to detect them. The only recognized groups of Enterobacteriaceae which regularly decarboxylate lysine rapidly and which produce large amounts of hydrogen sulphide, are the Salmonellae.[2,3] Lysine Iron Agar is therefore a sensitive medium for the detection of lactose-fermenting and non-lactose-fermenting salmonellae.

Principles of the Procedure
Dextrose is a source of fermentable carbohydrate. Peptic digest of animal tissue and yeast extract provide essential nutrients. Ferric ammonium citrate and sodium thiosulphate are indicators of H_2S formation. Cultures that produce hydrogen sulphide cause blackening of the medium due to ferrous sulphide production. Lysine decarboxylation causes an alkaline reaction (purple color) to give the amine cadaverine and organisms which do not decarboxylate lysine, produce acid butt (yellow color). Organisms that deaminate lysine, form a - ketocarboxylic acid, which reacts with iron salt near the surface of the medium under the influence of oxygen to form reddish-brown compound. The medium is stabbed to the base of the butt and streaked on slant.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	5.00
Yeast extract	3.00
Dextrose	1.00
L-Lysine	10.00
Ferric ammonium citrate	0.50
Sodium thiosulphate	0.04
Bromocresol purple	0.02
Agar	15.00
Final pH:(at 25°C) 6.7 ± 0.2	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 34.56 grams of the medium in one liter of distilled water.
2. Heat if necessary, to dissolve the medium completely. Distribute into tubes as desired.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool the tubes in slanted position to form slants with deep butts.

Quality Control Specifications

Dehydrated Appearance	Light yellow to greyish yellow colored, homogeneous, free flowing powder
Prepared Medium	Purple coloured, clear to slightly opalescent gel forms in tubes as slants
Reaction of 3.45% w/v aqueous solution	pH 6.7 ± 0.2 at 25°C

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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	Butt	Slant	H_2S
1.	*Citrobacter freundii ATCC 8090*	50-100	Luxuriant	Acidic reaction, Yellowing of the medium	Alkaline Reaction, purple or no colour change	positive reaction, blackening of medium
2.	*Escherichia coli ATCC 25922*	50-100	Luxuriant	Alkaline Reaction, purple or no colour change	Alkaline Reaction, purple or no color Change	Negative reaction
3.	*Proteus mirabilis ATCC 25933*	50-100	Luxuriant	Acidic reaction, Yellowing of the medium	deep red,lysine deamination	Positive reaction, blackening of medium
4.	*Salmonella arizonae ATCC 13314*	50-100	Luxuriant	Alkaline Reaction, purple or no colour change	Alkaline Reaction, purple or no colour change	Positive reaction, blackening of medium
5.	*Salmonella enteritidis ATCC 13076*	50-100	Luxuriant	Alkaline Reaction, purple or no colour change	Alkaline Reaction, purple or no colour change	Positive reaction, blackening of medium
6.	*Salmonella typhimurium ATCC 14028*	50-100	Luxuriant	Alkaline reaction, purple or no colour change	Alkaline Reaction, purple or no colour change	Positive reaction, blackening of medium
7.	*Shigella flexneri ATCC 12022*	50-100	Luxuriant	Acidic reaction, Yellowing of The medium	Alkaline Reaction, purple or no colour change	Positive reaction, blackening of medium

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

Lysine Iron Agar (DM137)
1. Control
2. Escherichia coli ATCC25922
3. *Citrobacter freundii ATCC 8090*
4. *Proteus mirabilis ATCC 25933*
5. *Salmonella Typhimurium ATCC 14028*

Test Procedure
1. Using a straight needle, prick the centre of a well-isolated colony from a fresh, pure culture.
2. Inoculate by stabbing to the base of the butt and streaking the slant.
3. Cap the tube loosely to ensure aerobic conditions.
4. Incubate at 35°C for 18-48 hours.
5. Examine at 18-24 and 40-48 hours for growth and colour changes in the butt and the slant of the medium and for blackening at the apex of the slant.

Results
1. A positive lysine decarboxylase reaction is purple (alkaline0 butt, purple slant. A negative reaction is yellow (acid) butt, purple, (alkaline) slant.

2. A positive lysine deaminase reaction is a red slant. A negative reaction is a purple slant. (Proteus spp. And Providencia spp. Produce a red slant over a yellow [acid] butt.)
3. A positive hydrogen sulfide reaction is blackened medium at the apex of the slant.

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. Salmonella paratyphi A, unlike other salmonella spp., does not produce lysine decarboxilase resulting in an alkaline slant and an acid butt.
2. H_2S-producing Proteus spp. Do not blacken the medium[4,5] It is suggested that Lysine Iron Agar be used in conjunction with Triple Sugar Iron Agar or other media to confirm differentiation.
3. The reaction of Morganella morganii may be variable after 23 hour incubation and may require longer incubation[5].

Packaging

Product Name : Lysine Iron Agar
Product Code : DM137
Available Pack sizes : 100gm / 500gm

References

1. Edwards P. R. Fife Mary A. (1961) Appl. Microbiol. 9. 478-480.
2. Moeller V.(1954) Acta. Pathol. Microbiol. Scand. 355. 259-277.
3. Ewing W.H., Davis B. R. and Edwards P. R. (1960) Pub. Hlth Labs. 18. 77-83.
4. MacFaddin, J. F. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1 Willams and Wilkins, Baltimore,MD
5. Finegold, S M. and W. J. Martin. 1982. Bailey and Scott's diagnostic microbiology, 6th ed. The CV Mosby Company, St. Louis, MO.

Further Information

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