PRODUCT SPECIFICATION SHEET

L-Arginine Dihydrolase Medium, Modified (DM1022)

Intended Use

L-Arginine dihydrolase medium, Modified (DM1022) is recommended for confirmation of *Enterobacter sakazakii (Cronobacter sakazakii)* from milk and milk products.

Product Summary and Explanation

Enterobacter sakazakii (Cronobacter sakazakii) is a gram negative, rod shaped, pathogenic bacterium. The bacterium is ubiquitous being isolated from a range of environments and foods, and the majority of *Cronobacter* cases are in the adult population. *Cronobacter sakazakii* is a bacterium that causes a rare but often fatal infection of the bloodstream and central nervous system. Its association with intrinsically or extrinsically contaminated powdered formula has attracted the main attention. Powdered infant formula is most likely contaminated after production, since the pasteurization process is normally adequate to kill these bacteria. However, if the powder is produced using dry blending process and not heated, the organisms can survive in the formula.

Decarboxylase Media was first introduced by Moeller for the detection of arginine dihydrolase and lysine and ornithine decarboxylase.[1-3] L-Arginine Dihydrolase Medium, Modified is one of the medium recommended for the confirmation of *Enterobacter sakazakii* from milk and milk products, in accordance with ISO specifications.[4] Bacteria can be differentiated on the basis of their decarboxylating activity towards the amino acid. Arginine is decarboxylated to putrescine by the bacteria producing arginine dihydrolase enzyme in this medium. The production of amine, putrescine elevates the pH of medium which is detected by the indicator, bromocresol purple which forms purple in alkaline condition. Colour change from purple to yellow and then back to purple is positive reaction.

Principles of the Procedure

L-Arginine dihydrolase medium, Modified contains yeast extract which provides the necessary nutrients for the growth of organisms. L-arginine stimulates the arginine dihydrolase synthesis. Glucose provides the carbon and energy source. Bromocresol purple is the pH indicator.

Formula / Liter

Ingredients	Gms / Liter
L-Arginine, monohydrochloride	5.00
Yeast extract	3.00
Glucose	1.00
Bromocresol purple	0.015
Final pH: 6.8 ± 0.2 at 25°C	
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 9.01 grams of the medium in one liter of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Distribute in 13 x 100mm tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Allow the tubes to cool in an upright position.

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Quality Control Specifications

Dehydrated Appearance	Light yellow to grey homogeneous free flowing powder
Prepared Medium	Purple coloured clear solution in tubes
Reaction of 0.9% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Not Applicable

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

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Arginine Dihydrolase
1.	*Enterobacter aerogenes ATCC 13048*	50 - 100	good-luxuriant	negative reaction, yellow colour
2.	*Klebsiella pneumoniae ATCC 13883*	50 - 100	good-luxuriant	negative reaction, yellow colour
3.	*Proteus vulgaris ATCC 13315*	50 - 100	good-luxuriant	negative reaction, yellow colour
4.	*Salmonella typhi ATCC 6539*	50 - 100	good-luxuriant	positive reaction, purple colour
5.	*Salmonella typhimurium ATCC 14028*	50 - 100	good-luxuriant	positive reaction, purple colour
6.	*Cronobacter sakazakii ATCC 12868*	50 - 100	good-luxuriant	positive reaction, purple colour

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

Test Procedure

1. The ISO specification mentions that the sample under test is enriched in Buffered Peptone Water (DM049).
2. This enriched medium is used for inoculation of Modified Lauryl Sulphate Tryptone Broth Base.
3. After incubation at 44°C for 24 hours, a loopful is streaked onto Enterobacter Sakazakii Agar, Modified. The presumptive blue-green coloured colonies obtained after incubation are confirmed by performing the biochemical tests.
4. L-Arginine Dihydrolase Medium, Modified is one of the biochemical medium used.
5. Inoculate the medium tubes with presumptive colonies of *Enterobacter sakazakii(Cronobacter sakazakii).*
6. Arginine reaction is strictly anaerobic; therefore the broth tubes must be overlayed with mineral oil.
7. Incubate at 35-37°C for 18-24 hours.
8. In differentiation of *Enterobacteriaceae*, control tubes without arginine must be used. If the tubes give purple reaction the test is considered as negative.

Results

Arginine Dihydrolase reaction is indicated as a colour change from purple to yellow and then back to purple is positive reaction.

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. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : L-Arginine dihydrolase medium, Modified
Product Code : DM1022
Available Pack sizes : 500gm

References
1. Moeller, 1954, Acta Pathol. Microbiol. Scand., 34:102.
2. Moeller, 1954, Acta Pathol. Microbiol. Scand., 34:259.
3. Moeller, 1955, Acta Pathol. Microbiol. Scand., 36:158.
4. International Organization for Standardization Draft ISO/TS 22964: 2006 (E).

Further Information
For further information please contact your local MICROMASTER Representative.

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DM1022PSS, QAD/FR/024,Rev.00