Brilliant Green Phenol Red Lactose Agar (DM1319)

Intended Use

Brilliant Green Phenol Red Lactose Agar (DM1319) is recommended for selective isolation of *Salmonella* species from water samples.

Product Summary and Explanation

Edel and Kampelmacher[1,2] formulated Brilliant Green Phenol Red Lactose Agar for selective isolation of *Salmonella* species from water and is recommended by ISO specifications ISO 6340; 1995 / IS15187; 2002.[3] After the liquid enrichment steps solid selective media are used for detection and isolation of *Salmonella* species. In order to increase the probability of detecting *Salmonella* organisms, at least two different media are inoculated from selective enrichment cultures which include Brilliant green phenol red lactose agar (DM1319), XLD agar (DM297/DM297I) or Bismuth sulphite agar (DM039). It is necessary to subculture presumptive *Salmonella* colonies on different media for biochemical and serological confirmation as the occurrence of typical colonies of *Salmonella* species on selective agar media is not sufficient evidence for the presence of Salmonella species.[3] Water samples collected should be analysed within 24 hours.

Principles of the Procedure

Brilliant Green Phenol Red Lactose Agar contains meat extract and peptone, enzymatic digest of animal tissue which stimulates bacterial growth. Lactose and sucrose are carbohydrate sources. Phenol red is a pH indicator. Brilliant green inhibits gram-positive organisms and many gram-negative bacteria, except *Salmonella*. Phosphates provides buffering capability.

Formula / Liter

Ingredients	Gms / Liter
Meat extract	5.00
Peptone, enzymatic digest of animal tissue	5.00
Disodium hydrogen phosphate	1.00
Sodium dihydrogen phosphate	0.60
Lactose	10.00
Sucrose	10.00
Phenol red	0.09
Brilliant green	0.005
Agar	15.00
Final pH: 7.0 ± 0.1 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 **46.69** grams of the medium in one liter of distilled water.
2. Heat with occasional agitation and bring just to the boil to dissolve the medium completely.
3. DO NOT AUTOCLAVE. Cool to 50°C.
4. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Light yellow to light pink homogeneous free flowing powder
Prepared Medium	Greenish brown coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 4.66% Solution	pH : 7.0 ± 0.1 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	Growth	Recovery	Colour of Colony

		(CFU)			
1.	*Salmonella typhimurium ATCC 14028*	50-100	good-luxuriant	>=50%	red or slightly pink-white and opaque with red surroundings
2.	*Salmonella enteritidis ATCC 13076*	50-100	good-luxuriant	>=50%	red or slightly pink-white and opaque with red surroundings
3.	*Escherichia coli ATCC 25922*	50-100	none-poor	>=10%	--

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

Test Procedure

Pre-enrichmnet:

1. For water samples exceeding 10 ml in volume, add the sample to the same volume of buffered peptone water (DM049/DM049BS) (double strength) or filter through a sterile membrane filter and place in 50 ml buffered peptone water (single strength).
2. Filtering aids can be used when needed. For sample volumes of 10 ml or less, use a minimum of 50 ml of buffered peptone water (single strength) or atleast 10 times the volume of the sample.
3. Incubate at 36 ±2 °C for 16 -20 hours.

Enrichment:

1. For further enrichment in selective media transfer 0.1 ml of pre-enrichment culture to 10 ml or 1 ml to 100 ml of Malachite green/ magnesium chloride medium i.e Modified Rappaport Vassiliadis medium (M1658I) for water testing and incubate in a water bath at 42 ± 0.5°C for 18-24 h.
2. The larger volume of inoculum might increase the probability of detecting Salmonella organisms.
3. In certain situations, the use of selenite cystine medium (DM475) in addition to malachite green / magnesium chloride medium is recommended.
4. Place a loopful of enrichment medium onto Brilliant Green Phenol Red Lactose Agar (DM1319) and XLD agar (DM297/DM297I). Bismuth Sulphite Agar (DM039) can be used an optional medium.
5. Place in incubator at 36 +/- 2 °C for 24 h -48 h for Bismuth Sulphite Agar.

Comfirmation Test:

1. Take all (or atleast five of) the distinct typical Salmonella colonies from each positive agar medium).
2. Colonies on Brilliant Green Phenol Red Lactose Agar (DM1319) are red or slightly pink-white and opaque with red surroundings.
3. Colonies on XLD are colourless (but appear red) usually with black centre/ Black colonies on Bismuth Sulphite Agar usually surrounded by metallic sheen.
4. Plate out the selected colonies onto the surface of pre-dried nutrient agar plates (DM180) in order to obtain well isolated colonies. Place these plates in an incubator at 36 ± 2 °C for 18 h to 24 h.
5. Use single isolated colonies only. Basic biochemical reactions for confirmation of Salmonella species must be studied which includes Lactose (negative) and Glucose (positive) fermentation reaction, Hydrogen sulphide production (positive), Urea negative and Lysine decarboxylase (positive) reactions.Refer appropriate references for specific test procedures.

Results

Refer appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 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 : Brilliant Green Phenol Red Lactose Agar

Product Code : DM1319
Available Pack sizes : 500gm

References

1. Edel W. and Kampelmacher E.H., 1969, Bull. W.H.O., 41:297.
2. Edel W. and Kampelmacher E.H., 1969, Bull. W.H.O., 39:487.
3. Water Quality- Detection of Salmonella species, International Organization for Standardization, ISO 6340-1995/ IS 15187:2002

Further Information

For further information please contact your local MICROMASTER Representative.

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