



Brilliant Green Phenol Red Lactose Agar

M1693

Brilliant Green Phenol Red Lactose Agar is used for selective isolation of *Salmonella* species from water samples.

Composition**

Ingredients	Gms / Litre
Meat extract	5.000
Peptone,enzymatic digest of animal tissue	5.000
Disodium hydrogen phosphate	1.000
Sodium dihydrogen phosphate	0.600
Lactose	10.000
Sucrose	10.000
Phenol red	0.090
Brilliant green	0.005
Agar	15.000
Final pH (at 25°C)	7.0±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.69 grams in 1000 ml distilled water. Heat with occasional agitation and bring just to the boil to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

This medium is formulated according to Edel and Kampelmacher (1, 2) for selective isolation of *Salmonella* species from water and is recommended by ISO specifications ISO 6340; 1995 / IS15187; 2002 (3). Solid selective media are used after the liquid enrichment steps for detection and isolation of *Salmonella* species. In order to increase the probability of detecting *Salmonella* organisms, at least two different medias are inoculated from selective enrichment cultures. This includes Brilliant green phenol red lactose agar (M1693), XLD agar (M031/M031I) or Bismuth sulphite agar (M027). The occurrence of typical colonies of *Salmonella* species on selective agar media is not sufficient evidence for the presence of *Salmonella* species. Therefore it is necessary to subculture presumptive *Salmonella* colonies on different media for biochemical and serological confirmation (3).

Water samples collected should be analysed within 24 hours. For water samples exceeding 10 ml in volume, add the sample to the same volume of buffered peptone water (M614/M614S) (double strength) or filter through a sterile membrane filter and place in 50 ml buffered peptone water (single strength). 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. Incubate at 36 ±20C for 16 -20 hours.

For further enrichment in selective media transfer 0.1 ml of preenrichment 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 ± 0.50C for 18-24 h. The larger volume of inoculum might increase the probability of detecting *Salmonella* organisms. In certain situations, the use of selenite cystine medium (M025) in addition to malachite green / magnesium chloride medium is recommended. Place a loopful of enrichment medium onto Brilliant Green Phenol Red Lactose Agar (M1693) and XLD agar (M031/M031I). Bismuth Sulphite Agar (M027) can be used an optional medium. Place in incubator at 36 +/- 20C for 24 h -48 h for Bismuth Sulphite Agar. For confirmation take all (or atleast five of) the distinct typical *Salmonella* colonies from each positive agar medium). Colonies on Brilliant Green Phenol Red Lactose Agar (M1693) which are red or slightly pink-white and opaque with red surroundings. Colonies on XLD which are colourless (but appear red) usually with black centre/ Black colonies on Bismuth Sulphite Agar usually surrounded by metallic sheen.

Plate out the selected colonies onto the surface of predried nutrient agar plates (M561A) in manner which will allow well isolated colonies to develop. Place these plates in an incubator at $36 \pm 20C$ for 18 h to 24 h. 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.

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

Greenish brown coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.66% w/v aqueous solution at 25°C. pH : 7.0 ± 0.1

pH

6.90-7.10

Cultural Response

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

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Cultural Response <i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	$\geq 50\%$	red or slightly pink-white and opaque with red surroundings
<i>Salmonella Enteritidis</i> ATCC 50-100 13076		luxuriant	$\geq 50\%$	red or slightly pink-white and opaque with red surroundings
<i>Escherichia coli</i> ATCC 25922	50-100	none-poor	$\leq 10\%$	

Storage and Shelf Life

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

Reference

- 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

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