TM 2021 – BRILLIANT GREEN PHENOL RED LACTOSE AGAR

INTENDED USE

For selective isolation of *Salmonella* species from water samples.

PRODUCT SUMMARY AND EXPLANATION

This medium is formulated according to Edel and Kampelmacher for selective isolation of *Salmonella* species from water and is recommended by ISO specifications ISO 6340; 1995 / IS15187; 2002. 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, XLD agar or Bismuth sulphite agar. 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.

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 (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 or 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 in addition to malachite green / magnesium chloride medium is recommended. Place a loopful of enrichment medium onto Brilliant Green Phenol Red Lactose Agar and XLD agar. Bismuth Sulphite Agar 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 which are red or slightly pinkwhite 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 in manner which will allow well isolated colonies to develop. Place these plates in an incubator at 36 ± 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.

COMPOSITION

Ingredients	Gms / Ltr
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

PRINCIPLE

Meat extract and yeast extract supplies essential amino acids and long chains of peptides for enhanced growth. Phenol red serves as an acid base indicator giving yellow colour to lactose and or sucrose fermenting bacteria. This medium also contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive bacteria.

INSTRUCTION FOR USE

- Dissolve 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.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Light yellow to light pink homogeneous free flowing powder.
Appearance of prepared medium : Greenish brown coloured, clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 7.0±0.1

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
Salmonella Typhimurium	14028	50-100	Luxuriant	>=70%	Red or slightly pink-white and opaque with red surroundings	35-37°C	18-24 Hours
Salmonella Enteritidis	13076	50-100	Luxuriant	>=70%	Red or slightly pink-white and opaque with red surroundings	35-37°C	18-24 Hours
Escherichia coli	25922	50-100	None-poor	0-10%	-	35-37°C	18-24 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

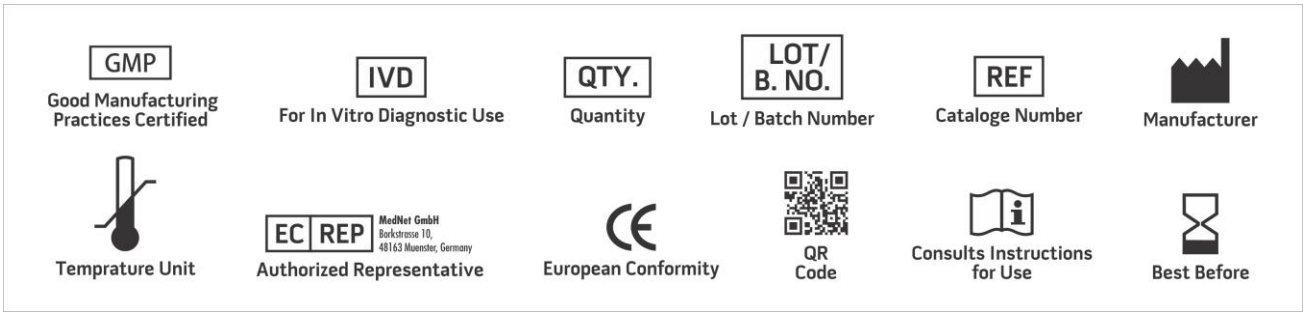
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

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



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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