B.C.P.-D.C.L.S. Agar

M219

Intended Use:

Recommended for isolation of *Salmonella* and *Shigella* species.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Tryptone	5.000
Yeast extract	3.000
HM peptone B #	3.000
Lactose	7.500
Sucrose	7.500
Sodium citrate	10.000
Sodium chloride	5.000
Sodium thiosulphate	5.000
Sodium deoxycholate	2.500
Bromocresol purple	0.020
Agar	14.000
Final pH (at 25°C)	7.2±0.2

Equivalent to Beef extract

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 67.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE or OVERHEAT. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Salmonella and *Shigella* are gram-negative, facultatively anaerobic, non-sporulating rods in the family *Enterobacteriaceae*. They are widely distributed in animals affecting mainly the stomach and the intestines. *Shigella* is the causative agent of bacterial diarrhoea and the faecal-oral route usually transmits the disease. Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta (6). Arizona group was originally named *Salmonella* Arizonae. It has been found mainly in reptiles and birds and occasionally in human patients with diarrhoea or septicemia. These organisms are difficult to differentiate biochemically from *Escherichia coli*, one of the most commonly recovered bacteria in clinical laboratory.

B.C.P-D.C.L.S Agar (Bromo Cresol Purple - Deoxycholate - Citrate - Lactose-Sucrose Agar) is the modification of the original formulation of Leifson (7), which was later, modified by Hajna and Damon (3). It allows easy isolation of *Salmonella, Shigella* and Arizona organisms from a mixed culture by differentiating between lactose-negative, sucrose-positive coliforms. It also inhibits all gram-positive bacteria and most of the *Proteus* species along with some strains of *S. dysentriae*(8).

Larger amount of the material can be inoculated into an enrichment medium followed by inoculation onto an agar plate, thereby, facilitating the isolation of *Salmonella,* when present only in small numbers. On incubation, *Salmonella* multiply rapidly, while E.coli and most other bacteria are inhibited. After enrichment, the enriched culture is plated onto a differential agar medium. B.C.P.-D.C.L.S. is a useful modification of D.C.A. (Deoxycholate Citrate Agar) that contains both lactose and sucrose (3). Some coliforms ferment sucrose more readily than lactose. Sucrose fermenting and lactose non-fermenting strains, e.g. some strains of *Proteus* and *E.coli,* form colonies distinguishable from the pale colonies of *Salmonella* and *Shigella,* which do not ferment sucrose, on this medium. Hence the number of false positive cultures requiring biochemical testing is reduced and the efficiency of isolation of *Salmonella* and *Shigella* is increased.

Peptone, Tryptone, yeast extract and HM peptone B in the medium provide nitrogen, vitamins and minerals necessary to support bacterial growth. Lactose and sucrose are the fermentable carbohydrates and therefore inclusion of these two sugars permits the formation of yellow colonies by organisms that ferment lactose, sucrose or both. Sodium thiosulphate is the indicator of H_2S production. Sodium citrate and sodium deoxycholate inhibit all gram-positive bacteria and coliforms but allow the gram-negative bacilli to grow. Sodium chloride provides essential ions. Bromo cresol purple is the pH indicator.

B.C.P.-D.C.L.S. Medium is unsuitable for the isolation of *Yersinia* species, which are sucrose positive. Non-selective media should be inoculated along with this media.
The medium can be directly inoculated with the test specimens. Alternatively, the sample can be enriched in GN Broth, Hajna (M242), Tetrathionate Broth (M032), or Selenite Broth (M052), and subsequently isolated on B.C.P.-D.C.L.S. Agar. A less inhibitory medium should be run in parallel to B.C.P.-D.C.L.S.

Type of specimen
Food and dairy samples; Water samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,9,10).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :
Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Light yellow to beige homogeneous free flowing powder

Gelling
Firm,comparable with 1.4% Agar gel.

Colour and Clarity of prepared medium
Purple coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 6.75% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	good-luxuriant	>=50%	colourless, may show faint bluish coloured colonies
Salmonella Enteritidis *ATCC 13076* (00030*)	50-100	good-luxuriant	>=50%	colourless, may show faint bluish coloured colonies
Shigella dysenteriae ATCC 13313	50-100	good	>=50%	colourless, may show faint bluish coloured colonies

Shigella flexneri ATCC 12022 (00126*)	50-100	good-luxuriant	>=50%	colourless, may show faint bluish coloured colonies
Shigella sonnei ATCC 25931	50-100	good-luxuriant	>=50%	colourless, may show faint bluish coloured colonies
Escherichia coli ATCC 25922 (00013*)	50-100	none-poor	<=10%	yellow
Proteus mirabilis ATCC 25933	50-100	none-poor	<=10%	colourless
Proteus vulgaris ATCC 13315	50-100	none-poor	<=10%	colourless

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Hajna A. A. and Damon S. R., 1956, Appl. Microbiol., 4, 341
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015)Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippincott Company.
7. Leifson E., 1935, J. Pathol. Bacteriol. 40, 581
8. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
9. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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