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Gram Negative Enrichment Broth

Recommended for selective enrichment of gram-negative enteric organisms.

REF: BS.1/DG01.100.0100	100 Gram	REF: BS.1/DG01.250.0250	250 Gram
REF: BS.1/DG01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

Gram Negative (GN) Broth was developed by Hajna (1) as a selective enrichment medium for the recovery of Salmonella and Shigella from both clinical and non-clinical specimens, including urine, blood clots, throat swabs, and swabs collected from eating and drinking utensils (1,2). The medium is also recommended by the APHA (3) for the microbiological examination of food samples. Hajna further reported the usefulness of this medium for enriching organisms from rectal swabs for 1–6 hours prior to inoculation onto solid culture media (2,4). In addition, Croft and Miller demonstrated that GN Broth enabled the recovery of a greater number of Shigella isolates from rectal swabs (9). Taylor and Schelhart also reported that GN Broth was superior to selenite enrichment media for the isolation of Shigella (10).

METHOD PRINCIPLE

The medium contains tryptose, which provides amino acids and other nitrogenous substances to support bacterial growth. The combination of sodium citrate and sodium deoxycholate inhibit gram-positive and some gram-negative bacteria such as coliforms. Phosphates serve as a buffering system. Sodium chloride maintains osmotic equilibrium. The higher concentration of mannitol over dextrose limits the growth of Proteus and enhances growth of mannitol fermenting Salmonella and Shigella Proteus, Pseudomonas and coliforms do not overgrow Salmonella and Shigella in GN Broth during the first 6 hours of incubation. This enrichment broth should be used in conjunction with selective and nonselective plating media to increase the probability of isolating pathogens (3, 5, 7). GN Broth, Hajna should be inoculated directly with the specimen. In case of stool specimens, approximately 1 gram should be used for inoculation. Appropriate references for processing of clinical and food samples should be followed (3, 5, 6, 8). After incubation of 6-8 hours and again after 24 hours, sub culturing on selective agar media should be carried out (7).

MEDIA COMPOSITION

Item	Formula in g/L
Tryptose	20
Dextrose	1
Mannitol	2
Sodium citrate	5
Sodium deoxycholate	0.5
Dipotassium phosphate	4
Monopotassium phosphate	1.5
Sodium chloride	5

pH 7.0 ± 0.2 at 25°C

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated people.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,..).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Handle specimens and inoculated culture bottles as though capable of transmitting infectious agents. All inoculated culture bottles, specimen collection needles, and blood drawing devices should be decontaminated according to country requirement for waste disposal.

  S56: dispose of this material and its container at hazardous or special waste collection point.

  S57: use appropriate container to avoid environmental contamination.

  S61: avoid release in environment.

For further information, refer to the **Gr**am Negative Enrichment Broth material safety data sheet.

MEDIA PREPARATION, STORAGE AND STABILITY

STORAGE AND STABILITY

BioScien Gram Negative Enrichment Broth should be stored between 10-30°C in a firmly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a 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 the stated expiry period.

PROCEDURE

Suspend 39 grams in 1000 ml distilled water. Heat if necessary to disssolve the medium completely Dispense in test tubes. Sterilize by autoclaving at 115°C (10 lbs pressure) for 15 minutes. AVOID EXCESSIVE HEATING.

Deterioration

BioScien Gram Negative Enrichment Broth is Cream to yellow homogeneous free flowing powder. Prepared medium is Light amber coloured, clear to slightly opalescent solution in tubes.

should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations

TYPE OF SPECIMEN

Water Samples
Environmental samples

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile cups
- Sterile tubes
- Autoclave
- Incubator

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact the technical support.

PERFORMANCE CHARACTERISTICS

The following organisms are used by us as part of the quality assurance of the product. The total inoculum challenge for each test organism per bottle is 10 to 50 colony forming units (CFU's).

Test Organisms	Growth
Escherichia coli ATCC 25922	Good
Enterococcus faecalis ATCC 19433	None-poor
Enterococcus faecalis ATCC 19433	Good
Pseudomonas aeruginosa ATCC 27853	Good
Salmonella Typhimurium ATCC 14028	Good
Shigella flexneri ATCC 12022	Good

REFERENCES

1. Hajna A. A., 1955, Publ. Health Lab., 13:59.
2. Hajna A. A., 1955, Publ. Health Lab., 13:83.
3. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
4. Hajna A. A., 1956, Air. Univ. Sch. Ar. Med., USAF, 56:39
5. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
6. Forbes B. A., Sahm A. S., and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.
7. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
8. Ewing, 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., New York, N.Y.
9. Croft C. C., Miller M. J., 1956, Am. J. Clin. Pathol., 26:411.
10.Taylor W.I., Schelhart D., 1968, Appl. Environ. Microbiol., 16:1383.

SYMBOLS IN PRODUCT LABELLING	
IVD For in-vitro diagnostic use	 Number of <n> test in the pack
LOT Batch Code/Lot number	 Caution
REF Catalogue Number	 Do not use if package is damaged
 Temperature Limitation	 Consult Instruction for use
 Expiration Date	
 Manufactured by	