IFU

Instructions for Use

GN BROTH

Cat. no. K01	GN Broth, 13x100mm Tube, 6ml	20 tubes/box
Cat. no. K39	GN Broth, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. R76BX	GN Broth, 13x80mm Tube, 4ml	100 tubes/box

INTENDED USE

Hardy Diagnostics GN (Gram-Negative) Broth is recommended for the selective enrichment of *Shigella* and *Salmonella* spp. in clinical and non-clinical specimens.

SUMMARY

GN Broth was originally developed by Hajna to improve the recovery of *Salmonella* and *Shigella* from clinical and non-clinical specimens. Hajna recommended specimens be enriched in GN Broth 1-6 hours prior to plating on a solid medium.(7) Hajna's formulation employed an increased amount of mannitol over dextrose. This formulation produced an accelerated growth of *Salmonella* and *Shigella* while limiting the growth of *Proteus* spp. and *Pseudomonas aeruginosa*. Inhibitory chemicals in the medium allow normal fecal flora to be maintained in a prolonged lag phase. The *Shigella* and *Salmonella* are less inhibited and enter a log or stimulated phase of growth during the first few hours of incubation.

Casein and meat peptones provide amino acids and other nitrogenous substances to support bacterial growth. Dextrose and mannitol supply the energy source. The pH of the medium is maintained by phosphate buffers and osmotic equilibrium is maintained by sodium chloride. Gram-positive microorganisms and early multiplication of coliforms are both inhibited by sodium citrate and sodium deoxycholate.

Direct inoculation of rectal swabs on plating media, compared to plating after 6-8 hours of incubation on GN Broth, was first reported by Craft and Miller.(9) Their results showed that more isolates of *Shigella* were obtained when using the GN Broth. Taylor and Schelhart reported that a greater frequency of isolation of *Salmonella* and *Shigella* was obtained when enrichment with GN Broth was used as opposed to direct plating.(8) Taylor and Harris compared various enrichment broths for their ability to support the growth of *Shigella* species.(10) They reported GN Broth more satisfactory than Silliker Broth, Selenite Broth or Tetrathionate Broth for propagation and recovery.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	10.0gm
Peptic Digest of Animal Tissue	10.0gm
Sodium Chloride	5.0gm

Sodium Citrate	5.0gm
Dipotassium Phosphate	4.0gm
Mannitol	2.0gm
Monopotassium Phosphate	1.5gm
Dextrose	1.0gm
Sodium Deoxycholate	0.5gm

Final pH 7.0 +/- 0.3 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1-4,6] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. Specimens should be delivered to the laboratory within 2-3 hours. Special attention is required for stools. They should be collected early in the course of the disease and need to be cultured within two hours after collection. Due to their delicate nature, *Shigella* species are best recovered by inoculating the media directly at the bedside. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation.

Method of use:

1. Place 1.0gm of feces or 1ml of liquid stool in tube. Swab specimens may be inserted directly into the broth.

2. Emulsify the specimen thoroughly.

3. Incubate aerobically for six to eight hours at 35ºC.

4. Place one to two drops of the incubated broth onto selective plate media, such as MacConkey or Hektoen Enteric Agar and streak for isolated colonies.

5. Incubate aerobically at 35ºC.

6. Examine for pathogens in 18-24 hours.

INTERPRETATION OF RESULTS

Culture analysis is made from the media to which the enriched specimen is subcultured. Consult listed references for the interpretation of growth and other identification tests to identify growth of organism in the medium to which subculture has been made.[1-6]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

GN Broth, due to its low concentration of deoxycholate, is partially inhibitory to *E. coli* and other coliforms. The coliforms will eventually begin to overgrow the pathogens. Subculturing within eight hours after initial inoculation is necessary for optimal recovery of pathogens.

GN Broth does not encourage the growth of *Shigella dysenteriae*.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Shigella sonnei ATCC® 9290	I	24hr	35°C	Aerobic	Growth upon subculture to MacConkey plate
Escherichia coli ATCC® 25922	I	24hr	35°C	Aerobic	Growth upon subculture to MacConkey plate
Salmonella enterica ATCC® 14028	I	24hr	35°C	Aerobic	Growth upon subculture to MacConkey plate

* Refer to the document "Inoculation Procedures for Media QC" for more information.

Note: GN Broth is inoculated with organism, incubated for 6-8 hours, then subcultured to MacConkey.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

GN Broth should appear clear to slightly hazy, amber in color; with no chips or debris, may contain precipitate.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

4. IIsenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

7. Hajna, A.A. 1955. *Public Health Lab*.; 13:83.

8. Taylor, W.I. and D. Schelhart. 1968. *Applied Microbiology*; 16:1383.

9. *American Journal of Clinical Pathology*; 26:411, 1956.

10. *American Journal of Clinical Pathology*; 44:426, 1965.

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