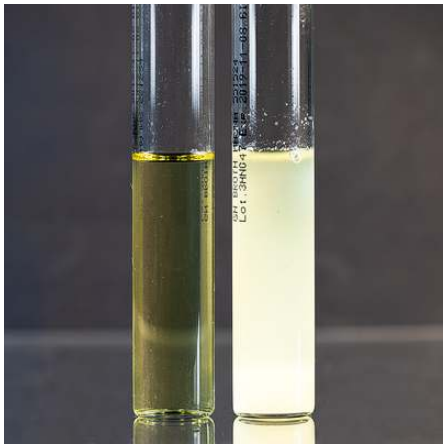
INSTRUCTIONS FOR USE

GN BROTH HAJNA

Ready-to-use tubes



GN Broth Hajna; from left: un-inoculated tube, growth of *Shigella flexneri*

1 - INTENDED USE

In vitro diagnostic device. Selective enrichment medium for the isolation and cultivation of Gram-negative enteric pathogenic bacteria (*Salmonella* and *Shigella*) from clinical samples and other materials.

2 - COMPOSITION -TYPICAL FORMULA *

Tryptose	20.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	4.0 g
Potassium dihydrogen phosphate	1.5 g
Sodium citrate	5.0 g
Sodium deoxycholate	0.5 g
Mannitol	2.0 g
Dextrose	1.0 g
Purified water	1000 mL

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

GN (Gram Negative) Broth is prepared according to the formulation devised by Hajna in 1955.[1] The medium is used for the enrichment of Gram-negative enteric pathogenic bacteria (*Salmonella* and *Shigella*), in samples of clinical, industrial and environmental origin.[2]
Carbohydrates are balanced with an excess of mannitol over glucose to favour growth of mannitol-fermenting *Salmonella* and *Shigella* over *Proteus* and *Pseudomonas* during the first 6 hours of incubation.[2] The phosphate buffers prevent over-acidification of medium by acidic metabolic production. The selective compounds are sodium citrate and sodium deoxycholate: the medium inhibits all Gram-positive bacteria, particularly enterococci, normal intestinal flora, the coliforms during the first 6 hours of incubation, aerobic and anaerobic spore-forming bacilli.
Croft and Miller[3] and Taylor and Schelhart[4] reported that the enrichment of stool cultures, compared to direct inoculation of plates, increases the sensitivity of isolation of *Salmonella* and *Shigella*, as these infections may be caused by low numbers of bacteria. In another study, Taylor and Schelhart[5] showed that GN Broth was superior to selenite enrichment media for the isolation of *Shigella*. GN Broth is also recommended for use in the microbiological examination of foods[6] and water[7].
For *Shigella* isolation from faecal specimens, the enrichment in GN Broth is advised, followed by subculture on two different selective media: XLD Agar and a second less selective medium (e.g. Mac Conkey Agar).[8]

4 - PHYSICAL CHARACTERISTICS

Medium appearance	pale yellow, limpid
Final pH at 20-25°C	7.0 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
GN Broth Hajna	Ready-to-use tubes	551524	20 x 10 mL glass tubes, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

GN Broth Hajna may be inoculated with human clinical specimens such as faeces or rectal swab. Collect specimens before antimicrobial therapy where possible. Good laboratory practices for collection, transport and storage of the specimens should be applied. For food and environmental samples refer to the quoted references.[6,7]

8 - TEST PROCEDURE

For stool testing, inoculate the tubes with 1 g of faeces or 1 mL of faecal suspension obtained by suspending 1 g of faeces in 1 mL of saline. Rectal swabs can be inserted directly into the broth.
Incubate the tube with loosened caps at 35 ± 2°C for 6-8 hours, but if microbial growth is observed already at the 6th hour, subculture to selective and differential plating media such as Mac Conkey Agar, XLD Agar, Hektoen Enteric Agar. Subculture again after 18-24 hours of incubation.
Consult appropriate references for information about processing and inoculation of other clinical specimens[8,9] or food samples[6,7].

10 - READING AND INTERPRETATION

After incubation, the growth of organisms is indicated by turbidity of the broth. Subculture by streaking a loopful of broth on selective enteric plating media. The plating media should be chosen as a combination of greater and lesser inhibitory selective agars.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, E-mail: export@biolifeitaliana.it;
Web: www.biolifeitaliana.it

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.[10]

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S.Typhimurium ATCC 14028	33-37°/ 18-24H / A	good growth after subculture on Mac Conkey Agar
S.flexneri ATCC 12022	33-37°/ 18-24H / A	good growth after subculture on Mac Conkey Agar
E.coli ATCC 25922	33-37°/ 6-8 H / A	partial or complete inhibition after subculture on Mac Conkey Agar

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready-to-use tubes of GN Broth Hajna and of the raw material used for the ready-to-use tube production (dehydrated GN Broth Hajna REF 401524) is tested for productivity and selectivity by comparing the results with a previously approved Reference Batch.

Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 35-37°C for 18-24 hours, and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: S.Typhimurium ATCC 14028, S.Enteritidis NCTC 5188, *S.flexneri* ATCC 12022, *S.sonnei* ATCC 9290, *S.boydii* ATCC 9207. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.

Selectivity is evaluated with dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of non-target organisms in test tubes, incubating at 35-37°C for 18-24 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Selectivity is tested with the following non-target strains: *E.coli* ATCC 25922, *P.vulgaris* ATCC 9484 (6-8 hours of incubation), *E.faecalis* ATCC 19433 e *S.aureus* ATCC 25923 (18-24 hours of incubation). The selectivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≥1.

13 - LIMITATIONS OF THE METHOD

- Since heavy growth of some saprophytes (non-pathogens) may exhibit growth on extended incubation, 6-8 hours is the recommended time period for initial subculturing.[2]
- GN Broth Hajna is not the optimal growth medium for *Shigella dysenteriae*.
- Enteric pathogens isolation techniques should include a variety of enrichment broths and isolation media.
- After the enrichment in GN Broth Hajna, even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that these products do not contain any transmissible pathogen. Therefore, it is recommended that the ready-to-use tubes be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each tube is for single use only; do not transfer or subdivide the tube content in other containers.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes of GN Broth Hajna are subject to terminal sterilization by autoclaving.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

14 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store tubes in their original pack at 2-8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. After opening the box, the tubes can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g. microbial contamination, atypical colour, precipitate).

15 – REFERENCES

1. Hajna AA. A new enrichment broth medium for gram-negative organisms of the intestinal group. Public Health Lab 1955; 13:83-89.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. Croft CC, Miller MJ. Isolation of *Shigella* from rectal swabs with Hajna “GN” broth. Am J Clin Pathol 1956; 26:411-417.
4. Taylor WI, Schelhart D. Isolation of shigellae. V. Comparison of enrichment broths with stools. App Microbiol 1968; 16:1383-1386
5. Taylor WI, Schelhart D. Isolation of shigellae. IV. Comparison of plating media with stools. Amer J Clin Pathol 1967; 48:356-362.

6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Bonadonna L, Ottaviani M. Rapporti ISTISAN 07/5 Metodi analitici di riferimento per le acque destinate al consumo umano ai sensi del DL.vo 31/2001. Metodi microbiologici. ISS, 2007.
8. Strockbine NA, Bopp CA, Fields PI, Kaper JB, Nataro JP. *Escherichia, Shigella* and *Salmonella*. *In* Jorgensen JH, Pfaller MA et al. editors. Manual of clinical microbiology,11th ed. Washington,DC: American Society for Microbiology; 2015
9. Buchan BW et al. *Escherichia*, *Shigella* and *Salmonella*. In Carrol KC, Pfaller MA et al. editors. Manual of clinical microbiology,12th ed. Washington, DC: American Society for Microbiology; 2019.
10. CLSI (formerly NCCLS) Quality Control of Commercially Prepared Culture Media. Approved Standard, 3rd edition. M22 A3 vol. 24 n° 19, 2004

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Do not reuse	Recyclable pack This side up
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Updated layout and content in compliance with IVDR 2017/746	2021/04
Instructions for Use (IFU) - Revision 2	Removal of obsolete classification	2023/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, E-mail: export@biolifeitaliana.it;
Web: www.biolifeitaliana.it