LBM™
GN Broth

PI21A Rev.01 Date 2016.07

LBM™ GN Broth

Package insert and How to use guide

ENGLISH

Copan GN Broth (Hajna) – Product Insert & How to Use Guide

INTENDED USE
Copan GN broth is an enrichment and selective medium for enteric Gram Negative organisms, especially salmonelle and shigelle.

SUMMARY
GN broth Hajna is an enrichment and selective medium for enteric Gram negative bacilli that especially promotes the recovery of Salmonellae spp and Shigella spp. The increased amount of mannitol over dextrose promotes the growth of salmonella and shigella while slowing the growth of non fermenting mannitol species as Proteus or Pseudomonas. Sodium Citrate and Sodium Deoxycholate during the first 6-8 hours of incubation inhibits the growth of Gram positive bacteria and coliforms but after this time the coliforms are no longer suppressed and may overgrow the target pathogens. After 6-8 hours or after 18-24 hours of incubation, the broth is subcultured on appropriate selective agar plates.

REAGENTS
GN broth Hajna components (per liter) :

Components name	g/liter
Triptosio	20.0
Dextrose	1.0
Mannitol	2.0
Sodium chloride	5.0
Sodium Citrate	5.0
Sodium Deoxycholate	0.5
Dipotassium phosphate	4.0
Monopotassium phosphate	1.5
Distilled Water	1000 ml

pH 7,0 ± 0,2 at 25°C

PRECAUTIONS
1. For in vitro diagnostic use.
2. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified personnel.
3. Work under a biological safety cabinet or according to internal laboratory procedures and wear gloves
4. All specimens and materials used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel. Sterilize all biohazard waste including specimen, containers and media after their use.
5. Directions should be read and followed carefully.

STORAGE
This product is ready to use and no further preparation is necessary. The unopened product can be stored at 5 - 25°C until used or until the expiration date. Do not overheat. Do not incubate or freeze prior to use. Improper storage may result in loss of efficacy. Do not use after expiration date, which is clearly printed on the outer box and on every single tube.

PRODUCT DETERIORATION
Do not use **Copan GN broth** if:
1. The product shows visible marks of damage or contamination;
2. There is evidence of leakage;
3. The expiration date has passed;
4. There are other signs of deterioration.(i.e. medium is turbid).

MATERIALS SUPPLIED

Catalog No.	Product Descriptions	Pack Size	Suitable for WASP Automation
085CU.A	12X80 mm plastic tube, with black screw cap, filled with 4 ml of GN broth	6 boxes of 50 pcs each	YES

MATERIALS REQUIRED BUT NOT SUPPLIED
Appropriate materials for the cultivation and isolation of bacteria. Refer to laboratory reference manuals for recommended protocols for culture and identification techniques.

INSTRUCTIONS FOR USE

GN broth in bulk:

1. Label a tube of GN broth with the patient ID and unscrew the cap.
2. Inoculate the broth by transferring the specimen into the opened tube.

LBM™ GN Broth

FOR SPECIMEN COLLECTED AND TRANSPORTED TO LABORTATORY IN APPROPRIATE TRANSPORT MEDIUM (i.e. Copan ESwab or Fecal Swab):

3a. Transfer the swab directly from the transport medium to GN broth
OR
Transfer a minimum aliquot of 30 ul of the patient sample medium into the GN broth.

For STOOL SPECIMEN

3b. Use a sterile loop or a swab when samples are solids and a pipette or a sterile loop when samples are liquid to transfer the specimen into the broth tube. The best ratio between sample and medium is 1:10.

NOTE: Specimens should be collected early in the course of the disease and stool specimens give better results if transported to laboratory with an appropriate transport media (i.e. Copan ESwab or Fecal Swab) to maintain microorganisms viability (especially Shigella spp). The stool specimens in dry containers must be processed within two hours after collection.

4. Re-cap the tube of GN broth and Vortex the tube for 5-10 seconds at 2000/2500 rpm in order to mix tube contents
5. Incubate inoculated GN broth tubes at 35 ± 2 °C.
6. After 6-8 hours or 18-24 hours, inoculate 1 to 10 ul of GN broth onto appropriate bacteriology culture medium.

Incubate GN broth according to laboratory Standard Operating Procedures and taking under consideration that GN broth formulation inhibit growth of Gram positive bacteria and coliforms only up to 6-8 hours incubation, past this time coliforms are no longer suppressed and may overgrow the target pathogens.

LIMITATIONS

1. In the laboratory, wear latex gloves and other protection commensurate with universal precautions when handling clinical specimens.
2. Condition, timing, and volume of specimen collected for culture are significant variables in obtaining reliable culture results. Follow recommended guidelines for specimen collection.
3. Performance testing with Copan GN broth was conducted using laboratory strains spiked into the GN broth tube and not using human specimens.
4. Proper specimen collection from the patient is extremely critical for successful isolation and identification of infectious organisms. For specific guidance regarding specimen collection procedures, consult published reference manuals. Specimens should be collected as soon as possible after the clinical onset of disease. Highest bacterial titres are present during the acute illness.

WARNINGS

1. ② This product is for single use only; reuse may cause a risk of inaccurate results.
2. For professional use only.
3. Do not re-pack.
4. Not suitable for any other application than intended use.
5. The use of this product in association with any diagnostic assay or with any diagnostic instrumentation should be validated by the user before using.
6. Do not use if the product is visibly damaged
7. Do not ingest the medium.
8. Directions for use must be followed carefully. The manufacturer cannot be held responsible for any unauthorized or unqualified use of the product.
9. It must be assumed that all specimens contain infectious micro-organisms; therefore all specimens must be handled with appropriate precautions. After use, tubes must be disposed of according to laboratory regulations for infectious waste.
10. Copan GN broth is for in-vitro diagnostics use only and is in no way intended for a curative or prophylactic purposes.
11. Observe approved biohazard precautions and aseptic techniques. Product to be used only by adequately trained and qualified personnel.
12. Work under a biological safety cabinet and wear gloves.

WASTE DISPOSAL

Unused reagents may be considered as non hazardous waste and disposed of accordingly. See Material Safety Data Sheet for additional information. Dispose of used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products. It is the responsibility of each laboratory to handle wasteand effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

RESULTS

Results obtained depends on adequate specimen collection and timely transport and processing in the laboratory.

QUALITY CONTROL PROCEDURE PERFORMANCE TEST

Procedure for testing:

1. Starting from a fresh culture prepare 0.5 Mc Farland suspension of each tester organism (Salmonella enterica subsp enterica thyphimurium ATCC 14028 or Shigella sonnei ATCC 9290) in PBS.
2. From the 0.5 McF suspension prepare appropriate dilution in order to have from 1000 to 3000 CFU/30 ul of inoculum
3. Using a micropipette inoculate 30 ul of each prepared bacteria suspension (inoculum) into a tube of GN broth
4. vortex inoculated GN tube for 10 seconds at 2000-2500 rpm to mix .
5. plate 100 ul of inoculate GN broth onto XLD (or other selective appropriate agar plates) in order to have the colonies count at time zero.
6. Incubate the inoculated GN broth tube at 35± 2°C for 6-8 hours.
7. After 6-8 hours take-out from the incubator the GN tube and vortex for 10 seconds.
8. Plate 1 ul of the inoculated GN broth onto XLD (or other selective appropriate agar plates) and incubate the plates at 35± 2°C for 18-24 hours in order to have the colonies count at time point 6-8 hours.
9. Re-incubate the inoculated GN broth tube at 35± 2°C for 12-16 hours (18-24 hours time point vs the time zero)

10. After 12-16 hours (18-24 hours time point vs the time zero) take-out from the incubator the GN broth tube and vortex for 10 seconds at 2000-2500 rpm to mix
11. Plate 1 ul of the inoculated GN broth onto XLD agar plates (or other selective appropriate agar plates) and incubate the plates at 35± 2°C 18-24 hours in order to have the colonies count at time point 18-24 hours.
12. Reads the results Expected results are for the plates at time 6-8 hours and the plates at time 18-24 hours: growth

INHIBITION TEST

Procedure for testing:

1. Starting from a fresh culture prepare 0.5 Mc Farland suspension of each tester organism (S.aureus ATCC 6538) in PBS.
2. From the 0.5 McF suspension prepare appropriate dilution in order to have from 1,5x 10 5 to 1,5x 10 4 CFU/ml of inoculum
3. Using a micropipette inoculate 30 ul of each prepared bacteria suspension (inoculum) into a tube of GN broth
4. vortex inoculated GN tube for 10 seconds at 2000-2500 rpm to mix.
5. plate 100 ul of inoculate GN broth onto MS agar (or other selective appropriate agar plates) in order to have the colonies count at time zero.
6. Incubate the inoculated GN broth tube at 35± 2°C for 6-8 hours.
7. After 6-8 hours take-out from the incubator the GN tube and vortex for 10 seconds.
8. Plate 100 ul of the inoculated GN broth onto MS agar (or other selective appropriate agar plates) and incubate the plates at 35± 2°C for 18-24 hours in order to have the colonies count at time point 6-8 hours.
9. Re-incubate the inoculated GN broth tube at 35± 2°C for 12-16 hours (18-24 hours time point vs the time zero)
10. After 12-16 hours (18-24 hours time point vs the time zero) take-out from the incubator the GN broth tube and vortex for 10 seconds at 2000-2500 rpm to mix
11. Plate 100 ul of the inoculated GN broth onto MS agar plates (or other selective appropriate agar plates) and incubate the plates at 35± 2°C 18-24 hours in order to have the colonies count at time point 18-24 hours.
12. Reads the results. Expected results are for the plates at time 6-8 hours and the plates at time 18-24 hours : partial to total inhibition

PERFORMANCE & INHIBITION TEST RESULTS

STRAIN	ZERO TIME COUNT	CFU COUNT on XLD at time point 6-8 hours	CFU COUNT on XLD at time point 18-24 hours
Salmonella enterica subsp enterica thyphimurium ATCC 14028	33; 56; 74	Confluent growth	Confluent growth
Shigella sonnei ATCC 9290	31 ; 62; 70	Confluent growth	Confluent growth
STRAIN	**ZERO TIME COUNT**	**CFU COUNT on MS at time point 6-8 hours**	**CFU COUNT on MS at time point 18-24 hours**
S.aureus ATCC 6538	Confluent growth	27; 30;19	2; 5; 6

ITALIANO

Brodo Copan GN (Hajna) - Presentazione e guida all'uso del prodotto

USO PREVISTO
Il brodo Copan GN è un mezzo di arricchimento e selettivo per organismi enterici Gram negativi, in special modo salmonella e Shigella.

RIASSUNTO
Il brodo GN Hajna è un mezzo di arricchimento e selettivo per i bacilli enterici Gram negativi che promuove in special modo il recupero di Salmonella spp. e Shigella spp. La maggiore quantità di mannitolo rispetto al destrosio promuove la crescita di salmonella e shigella e rallenta la crescita di specie di mannitolo non fermentanti come Proteus o Pseudomonas. Il citrato di sodio e il sodio desossicolato inibiscono la crescita di batteri Gram positivi e coliformi durante le prime 6-8 ore di incubazione, ma dopo questo periodo i coliformi non sono più soppressi e potrebbero crescere più dei patogeni target. Dopo 6-8 ore o dopo 18-24 ore di incubazione, il brodo viene sottoposto a subcoltura su apposite piastre selettive di agar.

REAGENTI
Componenti del brodo GN Hajna (per litro):

Nomi delle componenti	g/litro
Triptosio	20,0
Destrosio	1,0
Mannitolo	2,0
Cloruro di sodio	5,0
Citrato di sodio	5,0
Sodio desossicolato	0,5
Fosfato dipotassico	4,0