

ENGLISH

Copan GN Broth – Product Insert & How to Use Guide

INTENDED USE

GN Broth is an enrichment and selective broth for enteric Gram-Negative organisms, especially indicated for cultivation of salmonella and shigella.

SUMMARY AND PRINCIPLES

GN Broth (Hajna formulation) is an enrichment and selective broth 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.

PRODUCT DESCRIPTION

GN Broth is available in the following configuration:

Catalogue Number	Product Description	Pack Size
OC017N.A	GN BROTH IN BULK – US MARKET: 4 ml of GN Broth in a 12X80 mm polypropylene screw-cap tube with internal conical shape.	50 devices per inner box 6X50 devices per box

REAGENTS

GN Broth components (per liter)²:

Components name	Grams
Triptosis	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 (lot release time)

REQUIRED MATERIALS BUT NOT INCLUDED

Appropriate materials for the cultivation and isolation of bacteria. Refer to laboratory reference manuals for recommended protocols for culture and identification techniques.

STORAGE

This product is ready-to-use and no further preparation is necessary. The packaged and unopened device can be stored at 5 – 25 °C until used or until the expiration date³. Do not overheat. Do not incubate or freeze prior to use. Improper storage will result in loss of efficacy. Do not use after expiration date, which is clearly printed on the outer and inner label.

LIMITATIONS

- Performance testing with Copan GN Broth was conducted using laboratory ATCC strains spiked into the GN Broth tube and not using human specimens.
- Proper specimen (condition, timing, and volume) collection from the patient is extremely critical for successful isolation and identification of infectious organisms. For the swirling procedure and specific guidance regarding specimen collection procedures, consult published reference manuals. Specimens should be collected as soon as possible after the clinical onset of disease⁴.
- Microorganisms Gram negative other than *Salmonella spp.* or *Shigella spp.* may be supported or inhibited or partially inhibited.

WARNING AND PRECAUTIONS

- For In Vitro Diagnostic Use.
- For professional use only. Use the device in accordance with the Package Insert.
- Do not use if the tube is open, there is evidence of damage or leakage, deterioration or contamination (i.e. broth is turbid).
- Do not use if the expiration date has passed.
- Do not re-pack.
- Do not re-use the device.
- Use of this device in association with diagnostic kits and/or instrumentation should be validated prior to use.
- All specimens must be considered potentially infectious. Wear protective gloves and other protection commensurate with universal precautions when handling clinical specimens. Observe CDC Biosafety recommendations, or other appropriate alternative.

PROCEDURE

An adequate collection of samples from the patient is an extremely critical factor for the successful isolation and identification of infectious organisms. Consult the published reference manuals for specific instructions on the sample collection procedures⁴⁻¹¹.

The Copan Fecal Swab system with a regular size flocked swab can be used as the sampling system. Contact Copan or refer to the specific product instructions.

Direct specimen collection:

- Unscrew the cap of the GN Broth tube.
- Inoculate the sample in the open tube.
- Using the transfer device (loop or micropipette) transfer minimum 1 µl of amies liquid medium (i.e. eSwab) into the GN Broth. Using the swab, swirl for minimum 5 seconds or 5-10 times to release the sample into the GN tube and discard the transfer device. The transfer device can be left inside after breakage, if it has a breaking point suitable to tube size.
- Re-cap the tube of GN Broth and Vortex the tube for 5-10 seconds at 2000/2500 rpm in order to mix tube content.
- Incubate inoculated GN Broth tubes at 35 ± 2 °C.
- After 6-8 hours or 18-24 hours, inoculate 1 to 10 µl of GN Broth onto appropriate bacteriology culture plate medium.

NOTE: The stool specimens in dry containers must be processed within two hours after collection.

Using Collection system (i.e. Fecal Swab) for specimen:

- Take the tubes of GN Broth and unscrew the cap.
- Vortex the Fecal Swab specimen tube for 10 seconds.
- Unscrew the cap and transfer an amount of minimum 10 µl from the Fecal Swab to the GN tube or transfer directly the Flocked Swab of the Fecal Swab.
- NOTE: the Fecal Swab cap can be transfer with its swab directly to the GN Broth tube. The Fecal Swab cap can be used to close the GN tube; Re-cap the Fecal Swab tube with the GN's cap.
- Using a loop or a micropipette, plate minimum 1 µl of GN Broth onto appropriate bacteriology selective plate medium.
- Incubate inoculated GN Broth tubes at 35 ± 2 °C.
- After 6-8 hours or 18-24 hours, inoculate 1 to 10 µl of GN Broth onto appropriate bacteriology culture plate 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, after this time coliforms are no longer suppressed and may overgrow the target pathogens.

WASTE DISPOSAL

Waste disposal must be done according with national laws. Use the precautions for potential infectious material, when necessary.

QUALITY CONTROL PROCEDURE

The enrichment procedure described below is applied by manufacturer to release the lots:

- Prepare a 0.5 McFarland bacterial suspension in PBS from a fresh culture of microorganism in test.
- Prepare a 10⁻⁵ serial dilution from the 0.5 McFarland bacterial suspension.
- Inoculate the GN Broth with 400 µl of the dilution prepared.
- Recap all tubes.
- Vortex for 10 seconds at 2500 rpm.
- Plate immediately 100 µl of GN Broth inoculated (count at time zero).
- Incubate the GN Broth tube at 35 °C ± 2 °C for 18-24 hours.
- After incubation, check the turbidity and plate 100 µl of GN Broth onto appropriate culture plate medium.
- Incubate the plates at 35 °C ± 2 °C for 18-24 hours.
- Read the plate and record the results to release the lot.

The inhibition procedure described below is applied by manufacturer to release the lots:

- Prepare a 0.5 McFarland bacterial suspension in PBS from a fresh culture of microorganism in test.
- Prepare a 1,5x 10⁻¹ to 1,5x 10⁻² serial dilution from the 0.5 McFarland bacterial suspension.
- Inoculate the GN Broth with 400 µl of the dilution prepared.
- Recap all tubes.
- Vortex for 10 seconds at 2500 rpm.
- Plate immediately 100 µl of GN Broth inoculated (count at time zero).
- Incubate the GN Broth tube at 35 °C ± 2 °C for 6-8 hours.
- After incubation, check the turbidity and plate 100 µl of GN Broth onto appropriate culture plate medium.

- Incubate the plates at 35 °C \pm 2 °C for 18-24 hours.
- Incubate the GN Broth tube at 35 °C \pm 2 °C till the end of incubation (18-24 hours).
- After incubation, check the turbidity and plate again 100 μ l of GN Broth onto appropriate culture plate medium.
- Incubate the plates at 35 °C \pm 2 °C for 18-24 hours.
- Read the plate and record the results to release the lot.

STRAINS	GROWTH AT ZERO TIME (CFU)	GROWTH AT 6-8 H (CFU)	GROWTH AT 18-24 H (CFU)
<i>S. typhimurium</i> ATCC 14028	54	Confluent growth	Confluent growth
<i>S. sonnei</i> ATCC 9290	54	Confluent growth	Confluent growth
<i>S. aureus</i> ATCC 6538	Confluent growth	251	4

The QC procedure described below can be applied by End User, in compliance to CLSI M22-A3⁷:

- Prepare a 0.5 McFarland bacterial suspension in nonbacteriostatic saline, from a fresh culture of microorganism in test (growth or inhibition organisms).
- Inoculate the GN Broth with 10 μ l of undiluted 0.5McF.
- Recap the tube.
- Vortex for 10 seconds at 2500 rpm.
- Incubate the GN Broth tube at 35 °C-37 °C for 18-24 hours.
- After incubation, check the turbidity and streak for isolation of GN Broth onto appropriate culture plate medium.
- Incubate the plates at 35 °C-37 °C for 18-24 hours.

The GN Broth should be tested in aerobic condition at 35-37 °C with ATCC strains *S. typhimurium* ATCC 14028, *S. sonnei* ATCC 9290 and *E. coli* ATCC 25922 as reported in CLSI M22-A3. Selective media perform satisfactorily if the quality control organisms exhibits adequate growth, expected colony size, typical morphology, and inhibition of growth of certain organism⁷.










RESULTS

Results obtained will largely depend on proper and adequate sample collection, as well as timely transport and processing in the laboratory.

REFERENCES

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INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning
	Manufacturer		Temperature limit
	In vitro diagnostic medical device		Use-by date
Rx Only	For professional use		Consult instructions for use
	Do not re-use		Batch code (Lot)
	Catalogue number		Contains sufficient for <n> tests



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