

**GN Broth, Hajna****M242**

GN Broth, Hajna, is recommended for selective enrichment of gram-negative enteric organisms.

**Composition\*\***

Ingredients	Gms / Litre
Tryptose	20.000
Dextrose	1.000
Mannitol	2.000
Sodium citrate	5.000
Sodium deoxycholate	0.500
Dipotassium phosphate	4.000
Monopotassium phosphate	1.500
Sodium chloride	5.000
Final pH ( at 25°C)	7.0±0.2

\*\*Formula adjusted, standardized to suit performance parameters

**Directions**

Suspend 39 grams in 1000 ml distilled water. Dispense in test tubes. Sterilize by autoclaving at 115°C for 15 minutes.

AVOID EXCESSIVE HEATING.

**Principle And Interpretation**

Hajna (1) developed Gram Negative (GN) Broth as an enrichment medium for recovery of *Salmonella* and *Shigella* from clinical and non-clinical specimens such as urine, blood clots, throat swabs, swabs from eating and drinking utensils etc (1, 2). GN Broth, Hajna is also recommended by APHA (3) for the microbiological examination of foods. Croft and Miller isolated more strains of *Shigella* from rectal swabs using this medium (9). Taylor and Schelhart showed the superiority of GN Broth to selenite enrichment media for isolation of *Shigella* (10). Hajna (2, 4) also suggested the enrichment of organisms from rectal swabs in this medium 1-6 hours before plating on solid media.

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).

**Quality Control****Appearance**

Cream to yellow homogeneous free flowing powder

**Colour and Clarity of prepared medium**

Light amber coloured, clear to slightly opalescent solution in tubes.

**Reaction**

Reaction of 3.9% w/v aqueous solution at 25°C. pH : 7.0±0.2

**Cultural Response**

M242: Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Organism	Inoculum (CFU)	Growth in GN broth	Growth after 24 hours on MacConkey Agar	Colour of colony		
<i>Escherichia coli</i> ATCC 25922	50-100	good	good	pink-red with bile ppt		

<i>Enterococcus faecalis</i> ATCC 19433	50-100	none-poor	none-poor	pale pink-red		
<i>Proteus mirabilis</i> ATCC 25933	50-100	good	good	colourless		
<i>Pseudomonas aeruginosa</i> ATCC 27853	50-100	good	good	colourless		
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good	good	colourless		
<i>Shigella flexneri</i> ATCC 12022	50-100	good	good	colourless		

**Reference**

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**Storage and Shelf Life**

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.