GC Broth

Cat. 2075

For the selective isolation and cultivation of gonococci and Haemophilus species when used with hemoglobin and supplements.

Practical information

Aplications	Categories
Selective isolation	Neisseria
Selective isolation	Streptococcus
Selective isolation	Haemophilus

Industry: Clinical

Principles and uses

GC Broth is used to prepare the GC Agar Base with the adequate addition of agar. It is used with various additives for the isolation and cultivation of pathogenic microorganisms such us Neisseria gonorrhoeae, Haemophilus influenzae and N. meningitidis.

Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Corn starch absorbs any toxic metabolites produced. Dipotassium and monopotassium phosphates act as buffer systems. Sodium chloride supplies essential electrolytes for transport and osmotic balance.

GC Agar Base is employed with the addition of hemoglobin and supplements for the preparation of Chocolate Agar and Thayer-Martin Medium. Chocolate Agar is prepared with the addition of 2% hemoglobin. The addition of hemoglobin provides hemin (X factor), required by Haemophilus species and promotes the growth of Neisseria species.

A chemical enrichment composed of cofactors, vitamins and nicotinamide adenine dinucleotide (NAD) is also required for the growth of Haemophilus and Neisseria spp. If required, antimicrobial supplements are added as inhibitors for an improved selectivity of the medium.

Thayer-Martin Medium is recommended for the primary isolation of N. gonorrhoeae and N. meningitidis from specimens with mixed flora taken from throat, vagina, rectum and urethra samples. It is designed to reduce the overgrowth of gonococci and meningococci by contaminants, to suppress saprophytic Neisseria species growth and to encourage pathogenic Neisseria growth. The typical colonies of N. gonorrhoeae on Thayer-Martin Medium are white-gray, opaque, sometimes shiny, finely granular in appearance, variable in size (1-2 mm), round with entire or lobate edges and mucoid after 48 hours of incubation.

The specimen should be placed on the surface of the plate making sure that a heavy inoculum is contained in a relatively small area. Streaking out from this area will produce well-isolated colonies. Incubate in a humid atmosphere of 5-10% CO2 and at 35 ºC for 40-48 hours. For suspect isolated colonies, perform a Gram stain and oxidase test.

In carbohydrate studies using the CTA Medium (Cat. 1502) with selected 1% sugars, N. gonorrhoeae ferments only glucose with acid production but no gas production. N. meningitidis ferments both glucose and maltose with acid but no gas production. The carbohydrate tests are carried out incubating the medium for 1-4 days at 35 ºC, aerobically and without CO2.

The antimicrobial agents in selective formulas such as Thayer-Martin Medium inhibit some strains of N. gonorrhoeae, therefore it is wise to streak non-selective Chocolate Agar plates to culture these organisms.

Formula in g/L

Corn starch	1	Dipotassium phosphate	4
Monopotassium phosphate	1	Peptone mixture	15
Sodium chloride	5		

Preparation

Suspend 26 grams of medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes.

Add 10 g of agar to the broth before sterilize to prepare GC Agar Base. Also, autoclave 250 ml of a 2% haemoglobin solution elaborated by gradually adding water to 5 grams of dry haemoglobin to obtain a uniform suspension, before exposing it to the autoclave's heat. Cool both flasks to 50 ºC and aseptically add the haemoglobin solution to GC Agar Base and mix gently. Aseptically add Polyenrichment Supplement (Cat. 6011), 1 vial A reconstituted in 1 vial B, for 250 ml of the medium + 250 ml of sterile 2% hemoglobin solution. Mix carefully to avoid bubble formation. Pour into plates or tubes with screw caps. Allow tubes to solidify with a long slant.

The GC Agar Base can be also prepared adding the following supplements:

1. VCN Supplement (Cat. 6013). This supplement turns the medium into Thayer Martin Medium.
2. VCAT Supplement (Cat. 6014). This supplement is used for the isolation of Neisseria.
3. VCNT Supplement (Cat. 6026). This supplement is also used for the isolation of Neisseria.
4. LCAT Supplement (Cat. 6012). This supplement is used for the isolation of pathogen Neisseria. It must be added after adding lysed horse blood instead of haemoglobin and Polyenrichment Supplement.

Note: VCAT Supplement and VCNT Supplement contains different antibiotics (one each) and different concentrations of the same antibiotics (three) within both. The election must be made according to the selectivity required.

When VCN Supplement, VCAT Supplement and VCNT Supplement are added, Polyenrichment Supplement and 2% haemoglobin solution must be used.

Instructions for use

Refer to appropiate references and procedures for result.
- Incubate at a temperature of 35±2 ºC for 40-48 hours.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
Opalescent	Fine powder	Beige	Clear amber, slightly opalescent	7,2±0,2

Microbiological test

Incubation conditions: (35±2 ºC / 40-48 h).

Microorganisms	Specification
Neisseria meningitidis ATCC 13090	Good growth
Streptococcus pyogenes ATCC 19615	Good growth
Streptococcus pneumoniae ATCC 6305	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Bailey and Scott. Diagnostic Microbiology. Fifth Edition, 1978. The C.V. Mosby Company. St. Louis, USA. Preparation of Transgrow.
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