GC Agar Base

Cat. 1106

For the cultivation and selective isolation of fastidious microorganisms, especially Neisseria gonorrhoeae and Haemophilus spp.

Practical information

Aplications	Categories
Selective isolation	Neisseria
Selective isolation	Streptococcus
Selective isolation	Haemophilus

Industry: Clinical

CE
IVD

Principles and uses

GC Agar Base is used with various additives for the isolation and cultivation of pathogenic microorganisms such as Neisseria gonorrhoeae, Haemophilus influenzae and N. meningitidis. GC Agar Base is employed with the addition of hemoglobin and supplements for the preparation of Chocolate Agar and Thayer-Martin Medium.

The Chocolate Agar can be supplemented with the following supplements:
- VCN Supplement (Cat. 6013). Turns de medium into Thayer-Martin Medium.
- VCAT Supplement (Cat. 6014). For the selective isolation of Neisseria.
- VCNT Supplement (Cat. 6026). Also used for the isolation of Neisseria.
- LCAT Supplement (Cat. 6012). For the isolation of pathogenic Neisseria.

The addition of hemoglobin in Chocolate Agar 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.

In the base medium, peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Corn starch absorbs any toxic metabolites produced. Dipotassium and monopotossaium phosphates act as buffer systems. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological Agar is the solidifying agent.

Thayer and Martin improved the selectivity of the GC agar by incorporating antibiotics such as colistin, vancomycin or nystatin, in order to grow fastidious microorganisms that require different growth factors. 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. On Thayer-Martin Medium the typical colonies of N. gonorrhoeae 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.

Formula in g/L

Bacteriological agar	10	Dipotassium phosphate	4
Maize starch	1	Monopotassium phosphate	1
Peptone mixture	15	Sodium chloride	5

Typical formula g/L * Adjusted and/or supplemented as required to meet performance criteria.

Preparation

Suspend 18 grams of the medium in 250 ml of distilled water to make a double-strength base. Mix well and allow to stand for 5 minutes. Heat with frequent agitation and boil for one minute. Sterilize in autoclave at 121 ºC for 15 minutes.

Also, autoclave 250 ml of a 2% hemoglobin solution, elaborated by gradually adding water to 5 grams of dry hemoglobin to obtain a uniform suspension,

before exposing it to the autoclave's heat.

Cool both flasks to 50 °C and aseptically add the haemoglobin solution to GC Agar Base and mix gently. Aseptically add Polyenrichment Supplement (Cat. 6011) for 250 ml of the medium + 250 ml of sterile 2% hemoglobin solution. Mix carefully to avoid bubble formation. This completed medium is the general purpose Chocolate Agar. Pour into plates or tubes with screw caps. Allow tubes to solidify with a long slant.

Instructions for use

For clinical diagnosis, the type of sample is secretions of the respiratory tract.
- Use standard procedures to obtain isolated colonies from specimens.
- Since many pathogens require carbon dioxide on primary isolation, plates may be incubated in an atmosphere containing approximately 5-10% CO_2.
- Incubate at 35±2 °C for 40-48 hours.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber, slightly opalescent	7,2±0,2

Microbiological test

Incubation conditions: (35±2 ºC / 5-10 % CO_2 atmosphere / 40-48 h).

Microorganisms	Specification
Haemophilus influenzae ATCC 10211	Good growth
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.
Sept. 15. 1971. Venereal Disease Research Lab., C.D.C. Atlanta, Ga., USA.
Thayer, J. D. Martin J. E., 1966. Improved medium selective for the cultivation of N. gonorrhoeae and N. meningtidis. Public Health Rep. 81. 559-562.