Product :
GC AGAR BASE ENRIQUECIDO

Specification

Solid medium base especially recommended for the isolation and culture of fastidious microorganisms.

Formula * in g/L

Special peptone..... 15.00
Starch..... 1.00
Sodium chloride..... 5.00
Dipotassium phosphate.....4.00
Potassium phosphate.....1.00
Dextrose.....1.50
Sodium bicarbonate..... 0.15
Yeast fractions..... 10.00
Agar.....12.00

Final pH 7,2 ±0,2 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 24,8 g of powder in 250 mL of distilled water and bring to a boil. Distribute into a 1 L flask and sterilize in the autoclave at 121°C for 15 minutes.
Dilute 5 g of haemoglobin powder in 250 mL of hot distilled water, constantly stirring, to obtain a homogeneous solution. Sterilize in the autoclave at 121°C for 15 minutes.
Cool both flasks to 50ºC and aseptically add the sterile haemoglobin solution to the medium base.To facilitate Neisseria detection, the addition of one vial of VCAT Selective Supplement (Art. No. 06-141LYO1) is recommended. Homogenize by rotation in order to avoid bubbles and pour into plates.

Description

The base can be used in the following applications:

Chocolate Agar
It is prepared with the Agar base and haemoglobin, without any inhibitor. Defibrinated blood can be used in the ratio of 1:10 if required i.e. 10 mL of blood per to 100 mL of prepared base, cooled to 45°C. Place the complete medium into a boiling bath for a few seconds, three consecutive times; the medium will become dark chocolate brown in colour. This medium enables the growth of very fastidious microorganisms, such as *Haemophilus influenzae*.

Thayer-Martin Agar
In 1966 Thayer and Martin described a medium that has been very effective in the isolation of Pathogens such as *Neisseria*. The medium is prepared with GC Agar base, haemoglobin and an inhibitor vial of VCNT Selective Supplement (Art. No. 06-142LYO1) that contains the antibiotics: vancomycin and colistin to inhibit the oxidase-positive contaminants; nystatin to prevent the growth of saprophytic fungi and trimethoprim to prevent *Proteus* overgrowth as demonstrated by Odegaard and Phillips in 1970. Recuperation of stressed cells is improved when a vial of GPS - Growth Promotion Supplement (Art. No. 06-144LYO1) is added.

Transgrow Agar
This medium has been demonstrated to be very effective for the storage, transport and culture of *Neisseria*.
The preparation of this medium is the same as that of Thayer-Martin Agar but it is distributed into hermetic screw-cap tubes solidified in the horizontal position.

Technique

When sampling is performed close to the laboratory, Thayer-Martin plates may be directly inoculated. If the sample has to be transported to the laboratory, Transgrow medium is preffered.

Gonococcal test:
In women, it is recommended that samples be taken from one of the following:
Where possible, from the cervix, after removing the cervical mucous.
If cervical culture is negative, rectal cultures may be carried out form samples taken form the rectum.
If a cervical sample is not suitable, e.g. in girls or after a hysterectomy, vaginal or urethral cultures may be performed with the corresponding samples.
In men, urethral culture from mucosal samples is recommended. Anal and pharynx cultures may also be appropriate.
For diagnosis of gonorrhoea in women, a growth of Gram negative cocci with a specific morphology is necessary, combined with a positive oxidase reaction. However, when diagnosing men, the demonstration of intracellular gonococci in the urethral exudations is sufficient. Culture for biochemical identification only needs to be performed when the first test is not possible. In special cases fermentations or reactions with fluorescent antibodies may be used to demonstrate the presence of Neisseria gonorreheae. Microscopic preparation from urethral exudates must be done very carefully in order to mantain the cellular morphology. Plate inoculation is performed by drawing a Z over the surface with the swab; ising a rolling motion. After this, the sample is dispersed with a loop. Incubate à 37°C±1, in a very moist, 10% CO2 enriched atmosphere.
N. gonorrheae and N. meningitidis produce colourless and translucent colonies.
Antibiotic incorporated in the medium with inhibitory supplement avoids the growth of almost all the non pathogenic microorganisms in the sample, including the saprophytic species of Neisseria. Thayer-Martin Medium inhibits also Mima polymorpha var oxidans, a microorganism that sometimes may be confused with Neisseria gonorrheae.
Transgrow tubes are inoculated by introducing the swab very carefully, squeezing it out against the walls and reaching the bottom of the tube. Extract the swab carefully to reduce the CO2 lost.

Transport:
If possible, the sample should be incubated à 37°C±1 for 12-16 hours before being transported to the laboratory. Transgrow Medium keeps neisseria alive for up to 48 hours, even à room temperature. In the laboratory, incubate the tubes or, if they have been already incubated, examine the growth.
Transgrow Medium, with 10% CO2 allows the growth of pathogenic neisseria and inhibits all the other contaminating microorganisms in the same way as Thayer-Martin medium.
Transgrow Medium tubes, if well closed, with a CO2 atmosphere and refrigerated, are usable for à least 3 months after preparation.

Necessary supplements

VCAT Selective Supplement (Art. No. 928220NL)
Vial Contents:
Necessary amount for 500 mL of complete medium.
Vancomycin..... 1,00 mg
Colistin sulfate..... 3,75 mg
Amphotericin B..... 0,50 mg
Trimethoprim..... 1,50 mg

Distilled water (Solvent)

Quality control

Incubation temperature: 37°C ±1,0 **Incubation time:** 24-48 h

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10^4-10^6 CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018 .

Microorganism	Growth	Remarks
Neisseria gonorrhoeae ATCC® 19424	Good	-
Neisseria meningitidis ATCC® 13090	Good	-

References

· ATLAS, R.M. & L.C. PARKS (1997) Handbook of microbiological media. CRC Press. BocaRaton .Fla. USA.
· ISO/TS 11133-1: 2009 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
· ISO/TS 11133-2: 2003 Corr. 2004 Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
· MacFADDIN, J. (1985) Media for isolation-cultivation-Identification-maintenance of medical bacteria. Vol. I. William & Wilkins. Baltimore.
· ODEGAARD, K. (1971) Trimethoprim for the prevention of overgrowth by swarming Proteus in the cultivation of gonococci. Acta. Path. Microbiol. Scand. Sect. (B) 79:545-548.
· THAYER, J. D. & J. E. MARTIN (1966). Improved medium selective for cultivation of Neisseria gonorrheae and N. meningitidis Pub. Health Rep. 81:559-562.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).