Anaerobic Agar

Cat. 1000

For the cultivation of anaerobes, especially Clostridium

Practical information

Aplications	Categories
Growth	Clostridium
Growth	Anaerobes

Industry: Water / Clinical / Food

CE
IVD

Principles and uses

Anaerobic Agar is used for cultivation of anaerobic microorganisms. Anaerobic bacteria are unable to use oxygen as a terminal electron acceptor.

Casein peptone and soy peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium thioglycollate and sodium formaldehyde sulfoxylate act as reducing agents generating a low oxidation reduction potential, thus securing good anaerobic conditions. Methylene blue acts as the redox indicator, the blue color indicating the presence of oxygen.

The testing procedures can be carried out using standard Petri dishes or Brewer's Anaerobic Agar Plates, both with the medium cooled to 45-50 ºC.

The seeding of the sample (clinical or food) can be performed by surface inoculation or by pour plate method. Normally the sample should never be heated to destroy the vegetative forms of the aerobe, as the anaerobic non-spore formers will also be destroyed. Nevertheless, sometimes it could be useful to heat the sample when spore formers such as Clostridium are sought, except C. perfringens, which rarel y forms spores. When heating i s indicated, warm the sample suspended in a liquid diluent (peptone water, buffering phosphate solution, etc.) to 70-80 ºC for 10 minutes.

The plates of Anaerobic Agar can also be incubated in a normal atmosphere covering the surface of the plates with a Brewerlid. When growth is observed, open the plate and pick the desired colonies. Incubate longer if necessary. If the medium has not been prepared fresh before use, it is necessary to heat and remelt to expel the dissolved oxygen.

Thioglycollate Medium (Cat. 1508) without Indicator is an excellent enrichment broth and, frequently using it previously, yields better results than direct seeding.

Formula in g/L

Bacteriological agar	15	Casein peptone	17,5
Dextrose	10	L-Cystine	0,4
Methylene blue	0,002	Sodium chloride	2,5
Sodium thioglicollate	2	Soy peptone	2,5
Sodium formaldehyde sulfoxylate	1		

Preparation

Suspend 51 grams of the 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. The medium can be incubated in an anaerobic jar or with Brewer lids for anaerobiosis.

Instructions for use

For clinical diagnosis, the type of sample is abscess, pus and blood.

Inoculation on the surface:
- Dispense the medium in Petri dishes, (previously cooled to 45-50 ºC), using 50-60 ml of the medium.
- Inoculate the surface of the medium by smears or scratches.
- Cover the inoculated plate with an anaerobic Brewer petri dish cover.
- Incubate aerobically at 35±2ºC for 18-48 hours.

- Reading and interpretation of the results.

Sowing in depth:
- Dispense 0,1 to 1 ml of inoculum into the plate and cover with 20-25 ml of medium. Spin to mix it and let it solidify.
- Incubate anaerobically at 35±2 ºC for 18-48 hours.
- Reading and interpretation of the results.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Clear beige	White with a blue tint	7,2±0,2

Microbiological test

Incubation conditions: (35±2 ºC/ 18-48 h).

Microrganisms	Specification
Clostridium sporogenes ATCC 11437	Good growth
Clostridium perfringens ATCC 12919	Good growth
Clostridium butyricum ATCC 9690	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Standard Methods for the Examination of Water and Wastewater 1 5th Ed. American Public Health Association, Inc, Washington, D.C.
1980. Andrew, W.H.C.D. Diggs, and C.R. Wilson, 1975.
Evaluation of medium for the rapid recovery of Escherichia coli from shellfish. App. Microbiol. 29: 130-131