Wilkins Chalgren Anaerobic Agar Base

M832

Intended Use:

Used for the selective isolation and cultivation of anaerobic bacteria and also for susceptibility testing of anaerobes by the agar dilution method.

Composition**

Ingredients	g / L
Tryptone	10.000
Peptone	10.000
Yeast extract	5.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
L-Arginine	1.000
Sodium pyruvate	1.000
Hemin	0.005
Menadione	0.0005
Agar	10.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 43.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C before adding antibiotics to be tested. Mix gently and pour into sterile Petri plates. For cultivation of anaerobes, aseptically add the rehydrated contents of 2 vials each of Anaero Supplement (FD001) or G.N. Anaero Supplement (FD002) as desired into sterile molten medium before pouring into sterile Petri plates.

Principle And Interpretation

Anaerobic bacteria are widespread in soil, marshes, lake and river sediments, oceans, sewage, food and animals. In humans, anaerobic bacteria normally are prevalent in the oral cavity around the teeth, in the gastrointestinal tract, in the orifices of the genitourinary tract and on the skin. Anaerobic infections in humans and various animals can involve virtually any organ under immunocompromised conditions (1). Also, anaerobic infections are often associated with tissue necrosis and abscess formation, leading to impaired delivery of antimicrobial agents in blood to the actual site of infection. This explains why anaerobic infections are often aggressively managed with debridement, aspiration and/or surgical removal of infected tissue. Because of the technical and interpretive difficulties associated with anaerobic susceptibility testing, presentation of definitive recommendations is difficult (2).

Wilkins Chalgren Anaerobic Agar Base, formulated by Wilkins and Chalgren (3), along with Brucella Agar Base is the preferred medium for agar dilution tests with anaerobes. This medium is also recommended for testing anaerobic bacteria (4,5,6). Wilkins Chalgren Agar need to be appropriately supplemented to support the growth of certain anaerobic bacteria. Hemin and Menadione (Vitamin K3) enhances the growth of *Bacteroides* species and *Prevotella melaninogenica*, respectively and many other species of gram-negative anaerobic rods (5,7). The medium can also be supplemented with defibrinated or lysed blood for the growth of fastidious anaerobic bacteria (8). Tryptone and peptone serve as sources of essential nutrients including carbon and nitrogen. Yeast extract provides vitamins and other growth factors like purines and pyrimidines that are essential for the growth of *P. melaninogenica*. Arginine serves as an amino acid source while pyruvate serves as an energy source. The medium can be made selective for non-sporing anaerobic bacteria and gram-negative anaerobic bacteria by addition of Anaero Supplement (FD001) and G.N. Anaero Supplement (FD002) respectively.

Type of specimen

Clinical- stool, abscess

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Proper anaerobic conditions must be maintained for optimal recovery of organisms

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 4.3% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed with added Anaero Supplement (FD001) or G.N. Anaero Supplement (FD002) under anaerobic condition, after an incubation at 35-37°C of 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Bacteroides fragilis ATCC 25285	50-100	luxuriant	>=50%
Clostridium perfringens ATCC 12924	50-100	luxuriant	>=50%
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%
Prevotella melaninogenicus ATCC 15930	50-100	luxuriant	>=50%

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

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