Wilkins Chalgren Medium

Cat. 1503

For susceptibility testing as well as for the isolation and culture of anaerobic bacteria in general from clinical samples

Practical information

Aplications	Categories
Selective isolation	Clostridium
Selective isolation	Anaerobes

Industry: Clinical

Principles and uses

Wilkins Chalgren Medium was designed for use in the determination of minimum inhibitory concentrations (MIC) of antibiotics for anaerobic bacteria by the agar dilution method. It is also recommended for the isolation of anaerobic organisms from clinical specimens. It has the same performance in Petri dishes as in tubes.

It has the advantage over other media in that it does not need the addition of blood to obtain the satisfactory growth of clinically important anaerobic bacteria.

Tryptone and Bacteriological peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group and other growing factors to cultivate Bacteroides melaninogenicus and Peptostreptococcus anaerobius. Dextrose is the fermentable carbohydrate providing carbon and energy. L-Arginine provides amino acids for the growth of Eubacterium lentum. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium pyruvate acts as an energy source for asaccharolytic cocci such as Veillonella and to catalyze and degrade traces of hydrogen peroxide which affects the metabolism of anaerobes. Haemin and vitamin K1 are growth factors. Haemin is essential for the growth of Bacteroides species. Bacteriological agar is the solidifying agent.

Formula in g/L

Dextrose	1	Bacteriological agar	15
Bacteriological peptone	10	Hemin	0,005
L-Arginine	1	Sodium chloride	5
Sodium pyruvate	1	Tryptone	10
Vitamin K1	0,0005	Yeast extract	5

Preparation

Suspend 48 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. Cool to 45-50 ºC, mix well and dispense into plates.

Instructions for use

For antibiotic susceptibility testing:
- Dispense the medium in sterile Petri dishes.
- Adjust the density of the microorganism suspension to 0,5 Mac Farland. A denser inoculum will produce smaller zones of inhibition and a smaller inoculum will give rise to the opposite effect.
- The suspension should be used between 15 and 60 minutes from its preparation.
- Dip a sterile cotton swab in the suspension.
- To avoid over-inoculation of Gram-negative bacteria, remove the excess liquid by pressing and turning the swab against the inside of the tube.
- For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.
- Apply the strip before 15 minutes have passed since the inoculation.
- Incubate at a temperature of 35±2 ºC for 24-48 hours in an anaerobic atmosphere.
- The results are taken from the edge where complete inhibition is observed with the plate about 30 cm from the eye. That point will determine the CMI.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Amber	7,1±0,2

Microbiological test

Incubation conditions: (35±2 ºC / 24-48 h / anaerobic atmosphere).

Microrganisms	Specification
Clostridium perfringens ATCC 13124	Good growth
Bacteroides fragilis ATCC 25285	Good growth
Bacteroides melaninogenicus ATCC 25611	Good growth

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Wilkins T.D. and Chalgren S. (1976) Antimicrob. Agents. Chemother., 10. 926-928.
Sutter V.L., Barry A.L., Wilkins T.D. and Zabransky R.J. (1 979) and Microb. Agents Chemother, 16. 495-502. Brown W.J. and Waatti P.E. (1980) Antimicrob. Agents Chemother., 17. 629-635.