

G-N Anaerobe Selective Supplement

PRODUCT INFORMATION

N001-5g - Nalidixic Acid, Powder, 5g

N001-25g - Nalidixic Acid, Powder, 25g

N001-100g - Nalidixic Acid, Powder, 100g

V001-250mg - Vancomycin HCl, Powder, 250mg

V001-1g - Vancomycin HCl, Powder, 1g

V001-5g - Vancomycin HCl, Powder, 5g

DESCRIPTION

Wilkins-Chalgren Anaerobe Agar with G-N Anaerobe Selective Supplement is for the selective isolation of Gram-negative anaerobes.

BACKGROUND

Menadione is a synthetic chemical compound sometimes used as a nutritional supplement because of its vitamin K activity.

Nalidixic acid is the first of the synthetic quinolone antibiotics. Nalidixic acid is effective against both gram-positive and gram-negative bacteria. In lower concentrations, it acts in a bacteriostatic manner; that is, it inhibits growth and reproduction. In higher concentrations, it is bactericidal, meaning that it kills bacteria instead of merely inhibiting their growth.

Vancomycin is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by Gram-positive bacteria.

Mechanism of action

Vancomycin acts by inhibiting proper cell wall synthesis in Gram-positive bacteria. Due to the different mechanism by which Gram-negative bacteria produce their cell walls and the various factors related to entering the outer membrane of Gram-negative organisms, vancomycin is not active against Gram-negative bacteria (except some non-gonococcal species of *Neisseria*).

APPLICATION IN WILKINS-

CHALGREN ANAEROBE AGAR

Recognising the need for a standard medium for antimicrobial susceptibility testing of anaerobic bacteria, Wilkins and Chalgren developed a new medium which would not require the addition of blood. Their formulation included yeast extract to supply vitamins and other growth factors such as purines and pyrimidines, that are necessary for good growth of *Peptostreptococcus anaerobius* and *Prevotella melaninogenica*. Arginine was added to ensure sufficient amino acid was available for the growth of *Eubacterium lentum*. Pyruvate was added as an energy source, for asaccharolytic cocci such as *Veillonella*. It also acts similarly to catalase and degrades traces of hydrogen peroxide, which may be produced by the action of molecular oxygen on medium constituents and interfere with the metabolism of anaerobes. Haemin was found to be essential for the growth of *Bacteroides* species and menadione for *Prevotella melaninogenica*.

Peptones derived from the single protein sources casein and gelatin, were used to improve standardisation of the medium. Wilkins and Chalgren considered that this medium consistently grew anaerobes as well or better than media such as Brucella Agar or Schaedler Anaerobe Agar. A collaborative study in ten laboratories showed that it could be used in an agar dilution method for susceptibility testing of anaerobic bacteria and recommended a procedure as a reference method.

The value of such a procedure was further confirmed by Brown and Waatti, who found that the incidence of resistance of anaerobic bacteria to frequently used antibiotics had increased. They considered it essential that diagnostic laboratories should have the capability of carrying out susceptibility tests on anaerobic bacteria.

Wilkins-Chalgren Anaerobe Agar is recommended for the isolation of anaerobic organisms from clinical specimens. It has been shown to function well both in Petri dishes and roll tubes. (B.S. Drasar, personal communication).

Wilkins-Chalgren Anaerobe Agar with N-S Anaerobe Selective Supplement is described as NAV Medium and is recommended for the isolation of Gram-negative anaerobes from clinical specimens.

NAV Medium is a modification of NAT Medium in

which 'Tween 80' and sodium pyruvate have been replaced by sodium succinate. Vancomycin has been added, thus making the medium totally selective for Gram-negative anaerobes.

G-N Anaerobe Supplement contains nalidixic acid and vancomycin as selective agents; haemin, menadione and sodium succinate as growth factors. Haemin was found to be essential for the growth of *Bacteroides* species and menadione for *Prevotella melaninogenica*. Some Gram-negative anaerobes require succinate as a source of energy.

The recovery of Gram-negative anaerobes on NAV Medium has been shown to be superior to that on media containing neomycin and kanamycin as selective agents.

In order to isolate the maximum non-sporing anaerobic bacteria from clinical specimens the following scheme must be followed.

Content concentrations

Typical Formula*	mg/litre
Wilkins-Chalgren Anaerobe Agar	
Tryptone	10
Gelatin peptone	10
Yeast extract	5
Glucose	1
Sodium chloride	5
L-Arginine	1
Sodium pyruvate	1
Menadione	0.0005
Haemin	0.005
Agar	10
Final pH 7.1 ± 0.2 @ 25°C	
G-N Anaerobe Selective Supplement	
Haemin	5
Menadione	0.5
Sodium succinate	2,500
Nalidixic acid	10
Vancomycin	2.5
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Wilkins-Chalgren Anaerobe Agar and G-N Anaerobe Selective Supplement

METHOD

Preparation

Suspend appropriate amount of Wilkins-Chalgren Anaerobe Agar in distilled water. Bring to the boil to dissolve completely and sterilise by autoclaving at 121°C

for 15 minutes. Cool to 50-55°C and aseptically add the contents of G-N Anaerobe Supplement rehydrated as directed, together with 25ml defibrinated blood. Mix gently and pour into sterile Petri dishes.

Protocol

1. Prepare supplies of Plate as described in the section marked Directions.
2. Inoculate the specimens on to plates. Best results are obtained if freshly prepared plates are used but plates may be stored at 4°C for up to 3 days.
3. Incubate the plates anaerobically at 35°C for 48 hours.
4. Examine the plates. If no growth has occurred then incubation should be continued up to 5 days before plates are discarded, as up to 20% of non-sporing anaerobes require prolonged incubation under unbroken anaerobic conditions.
5. Carry out confirmatory tests on the isolates and record the results.

Quality control

Positive control:

Bacteroides fragilis ATCC® 25285: Good growth; grey/white colonies

Fusobacterium necrophorum ATCC® 25286: Good growth; grey/white colonies

Negative control:

Escherichia coli ATCC® 25922: Inhibited

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