N-S Anaerobe Selective Supplement

PRODUCT INFORMATION

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

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

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

DESCRIPTION

Wilkins-Chalgren Anaerobe Agar with N-S Anaerobe Selective Supplement is for the selective isolation of non-sporing anaerobes.

BACKGROUND

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

Sodium pyruvate is commonly added to cell culture media as an additional source of energy, but may also have protective effects against hydrogen peroxide.

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.

Mechanism of action

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 for non-sporing anaerobes is referred to in the published literature1 as NAT Medium and is recommended for the isolation of non- sporing anaerobes from clinical specimens.

The recovery of non-sporing anaerobes from clinical material may sometimes prove difficult in specimens containing mixtures of aerobic and anaerobic bacteria. A medium which contains nalidixic acid as the selective agent was described by Wren for isolating these organisms. It was shown to be virtually non-inhibitory to most non-sporing anaerobes whilst retaining good selectivity for these organisms when present in mixed cultures. The medium is particularly useful for the recovery of non-sporing Gram-positive anaerobes since the presence of `Tween 80' stimulates their growth.

Another advantage of this medium is the earlier colonial pigmentation of the *Prevotella melaninogenica* group due to the slow lysis of the blood by `Tween 80' during incubation. It is also a less inhibitory medium than

aminoglycoside- containing media for non-sporing anaerobes in general.

The N-S Anaerobe Supplement for non-sporing anaerobes contains nalidixic acid as the selective agent, together with haemin, menadione and sodium pyruvate as an additional energy source.

Haemin was found to be essential for the growth of *Bacteroides* species and menadione for *Bacteroides melaninogenicus*. Pyruvate, in addition to being an energy source, acts similarly to catalase and degrades traces of hydrogen peroxide which may be produced by the action of molecular oxygen on media constituents. Hydrogen peroxide is known to affect the metabolism of anaerobes.

Downes et al. showed that NAT Medium was superior to kanamycin agar (KA) and neomycin agar (NA) in the recovery of all non-clostridial anaerobes. The major superiority was in the recovery of anaerobic, Gram-positive cocci.

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	
N-S Anaerobe Selective Supplement	
Haemin	5
Menadione	0.5
Sodium pyruvate	1000
Nalidixic acid	10
* Adjusted as required to meet performance standards	

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

METHOD

Preparation

Suspend appreciate amount of Wilkins-Chalgren Anaerobe Agar in distilled water containing 0.5 ml `Tween 80'. 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 N-S Anaerobe Supplement rehydrated as directed, together with 25 ml of 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:

Prevotella loescheii ATCC® 15930: Good growth; grey/white colonies

Peptostreptcoccus anaerobius ATCC® 27337: Good growth; grey/white colonies

Negative control:

Escherichia coli ATCC® 25922: Inhibited

REFERENCES

1. Wilkins T. D. and Chalgren S. (1976) Antimicrob. Agents Chemother. 10. 926-928.

2. Rogosa M. (1964) J. Bacteriol. 87. 162-170.

3. Hoffman P. S., George H. A., Kreig N. R. and Smibert R. A. (1979) Can. J. Microbiol. 25. 8-16.

4. Quinto G. and Sebald M. (1964) Am. J. Med. Technol. 30. 381-384.

5. Gibbons R. J. and MacDonald J. B. (1960) J. Bacterio. 80. 164-170.

6. Sutter V. L., Barry A. L., Wilkins T. D. and Zabransky R. J. (1979) Anti-Microb. Agents Chemother. 16. 495-502.

7. Brown W. J. and Waatti P. E. (1980) Antimicrob. Agents Chemother. 17. 629-635.

8. Castel O., Grollier G., Agius G. et al (1990) Eur. J. Clin. Microbiol. Inf. Dis. 9. 667-671.

9. Drasar B.S. Personal communication.

10. Wren M. W. D. (1977) J. Med. Microbiol. 10. 195-201.

11. Holdeman L. V and Moore W. E. C. (1977) Anaerobe Lab. Manual

(4th edition).

12. Wren M. W. D. (1980) J. Clin. Pathol. 33. 61-65.

13. Gibbons R. J. and MacDonald J. B. (1960) J. Bacteriol. 80. 164-170.

14. Downes J., Stern L. and Andrew J. H. (1986) Pathology 18. 141-144.

15. Wren M. W. D. (1981) Personal Communication.

16. Lev M., Keudell K. C. and Milford A. F. (1971) J. Bact. 108. 175-178.