Gardnerella Vaginalis Selective Supplement

PRODUCT INFORMATION

G006-1g - Gentamicin Sulfate, Powder, 1g

G006-5g - Gentamicin Sulfate, Powder, 5g

G006-25g - Gentamicin Sulfate, Powder, 25g

G035-10mg - Gentamicin A Sulfate, EvoPure™, 10mg

G031-10mg - Gentamicin C1 Sulfate, EvoPure™, 10mg

G032-10mg - Gentamicin C1a Sulfate, EvoPure™, 10mg

G033-10mg - Gentamicin C2 Sulfate, EvoPure™, 10mg

G034-10mg - Gentamicin C2a Sulfate, EvoPure™, 10mg

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

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

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

A007-100mg - Amphotericin B, Powder, 100mg

A007-250mg - Amphotericin B, Powder, 250mg

A007-1g - Amphotericin B, Powder, 1g

A007-5g - Amphotericin B, Powder, 5g

DESCRIPTION

Columbia Blood Agar Base with Gardnerella Vaginalis Selective Supplement is a selective supplement for the isolation of *Gardenerella vaginalis*.

BACKGROUND

Gentamicin is an aminoglycoside antibiotic, used for many Gram-negative organisms However, gentamicin is not used for *Neisseria gonorrhoeae*, *Neisseria meningitidis* or *Legionella pneumophila*. It is synthesized by *Micromonospora*, a genus of Gram-positive bacteria widely present in the environment (water and soil). Gentamicin is one of the few heat-stable antibiotics that remain active even after autoclaving, which makes it particularly useful in the preparation of some microbiological growth media.

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.

Amphotericin B is a polyene antifungal drug. Two amphotericins, amphotericin A and amphotericin B are known, but only B is used clinically, because it is significantly more active in vivo.

Mechanism of action

As with other polyene antifungals, amphotericin B associates with ergosterol, the main component of fungal cell membranes, forming a transmembrane channel that leads to monovalent ion (K+, Na+, H+ and Cl−) leakage, which is the primary effect leading to fungal cell death.

APPLICATION IN COLUMBIA BLOOD AGAR BASE

Gardnerella Vaginalis Selective Supplement, is based on the formulation of Ison et al. and is recommended for the selective isolation of *G. vaginalis* from the vaginal discharge of patients with symptoms of Non-specific Vaginitis (NSV). The symptoms of this mild condition prior to the isolation of the aetiological agent(s) are:

1. The absence of recognised pathogens.

2. Foul smelling discharge.

3. pH greater than 4.5.

4. Release of `fish' odour on the addition of potassium hydroxide (10%) to the discharge.

5. The presence of `clue' cells in prepared wet mounts (these are epithelial cells with a characteristic stippled or granular appearance caused by Gram variable bacilli adhering to the cell surface).

Several media and techniques have been described for the isolation of *G. vaginalis*. Gardnerella Vaginalis Selective Medium can be used for the surface inoculation technique or the double layer technique.

With added human blood or rabbit blood, a betahaemolytic reaction is exhibited by *G. vaginalis*. This can be used as a preliminary diagnosis feature1. The addition

of `Tween 80' (0.02% v/v) to the medium containing human blood has been found to give enhanced beta-haemolytic zones.

G. vaginalis is a Gram variable, small, pleomorphic bacillus which forms 0.25-0.5 mm diameter colonies producing beta-haemolysis on medium containing human blood.

Content concentrations

Typical Formula*	mg/litre
Columbia Blood Agar Base	
Special peptone	23
Starch	1
Sodium chloride	5
Agar	10
Final pH 7.3 ± 0.2 @ 25°C	
Gardnerella Vaginalis Selective Supplement	
Gentamicin sulphate	4
Nalidixic acid	30
Amphotericin B	2
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Columbia Blood Agar Base Gardnerella Vaginalis Selective Supplement

METHOD

Preparation

Add appreciate amount of Columbia Blood Agar Base to distilled water. Boil to dissolve and sterilise by autoclaving at 121°C for 15 minutes. Aseptically add the contents to sterile Columbia Blood Agar Base cooled to approximately 50°C, and supplement with 50 ml of sterile human, rabbit or horse blood. Mix well and pour into sterile Petri dishes. For the double layer technique hold the medium in a water bath at 50°C.

Protocol

Surface Inoculation Method (Isolation):

1. Prepare the selective medium from Columbia Blood Agar Base, Gardnerella Vaginalis Selective Supplement and defibrinated Horse Blood, according to the preparation. To demonstrate the characteristic haemolysis substitute horse blood with human or rabbit blood when preparing the medium.

2. Using a swab inoculate the vaginal discharge the medium.

3. Incubate, at 35°C for 48 hours in an atmosphere containing 7% carbon dioxide.

4. Carry out confirmatory tests on all colonies from medium containing horse blood and on betahaemolytic colonies from medium containing human blood or rabbit blood.

Double Layer Method (Isolation and Presumptive identification):

1. Prepare two lots of selective medium from Columbia Blood Agar Base, Gardnerella Vaginalis Selective Supplement and sterile human blood according to the preparations.

2. Use one lot to prepare base medium plates and place the second lot in a water bath at 50°C.

3. Using the swab inoculate the vaginal discharge on to the surface of the prepared plates. Allow to dry at room temperature for half an hour.

4. Overlay with 5 ml of the selective medium at 50°C.

5. Allow the overlay medium to set.

6. Incubate at 35°C for 48 hours in an atmosphere containing 7% carbon dioxide.

7. Carry out confirmatory tests on isolates that show a beta-haemolytic zone. Use an inoculating wire to stab through the agar overlay to reach the colonies beneath.

The following tests have been compiled from the literature and personal communication.

Test or Substrate	Test Result	% Positive
Oxidase	Negative	0
Catalase	Negative	0
Haemolysis of:		
Human blood	Positive	967
Rabbit blood	Positive	96
Horse blood	Negative	some strains
Sheep blood	Negative	7
Hippurate hydrolysis	Positive	92
Starch hydrolysis	Positive	90
Metronidazole 50 µg	Susceptible	90
Trimethoprim 5 µg	Susceptible	100
Sulphonamide 1000 µg	Resistant	0

Quality control

Positive control:

Gardenerella vaginalis ATCC® 14018: Good growth; grey/white colonies

Negative control:

Proteus mirabilis ATCC® 29906: Inhibited

REFERENCES

1. Ison C. A., Dawson S. G., Hilton J., Csonka G. W. and Easmon C. S. F. (1982) J. Clin. Path. 35. 550-554.

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3. King E. A. (1964) `The Identification of Unusual Pathogenic Gram negative Bacteria' Center for Disease Control, Atlanta GA (quoted in

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4. Taylor E. and Phillips I. (1983) J. Med. Microbiol. 16. 83-92.

5. Totton P. A., Amsel R., Hale J., Piot P. and Holmes K. K. (1972) J. Clin. Microbiol. 15. 141-147.

6. Bailey R. K., Voss J. L. and Smith R. F. (1979) J. Clin. Microbiol. 9. 65-71.

7. Greenwood J. R. and Picket M. J. (1979) J. Clin. Microbiol. 9. 200-204.