Helicobacter Pylori Selective Supplement (Dent)

PRODUCT INFORMATION

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

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

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

T011-5g - Trimethoprim, Powder, 5g

T011-25g - Trimethoprim, Powder, 25g

T011-100g - Trimethoprim, Powder, 100g

C058-100mg - Cefsulodin Sodium, Powder, 100 mg

C058-250mg - Cefsulodin Sodium, Powder, 250 mg

C058-1g - Cefsulodin Sodium, Powder, 1g

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 Helicobacter Pylori Selective Supplement (Dent) is a selective medium for the isolation of *Helicobacter pylori* from clinical specimens.

BACKGROUND

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

Trimethoprim is a bacteriostatic antibiotic which belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors.

Cefsulodin is a third generation cephalosporin antibiotic that has very specific activity against Pseudomonas aeruginosa. It has no significant activity against other Gram-negative bacteria and very limited activity against Gram-positive bacteria and anaerobic bacteria.

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

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*).

Trimethoprim acts by interfering with the action of bacterial dihydrofolate reductase, inhibiting synthesis of tetrahydrofolic acid. Tetrahydrofolic acid is an essential precursor in the de novo synthesis of the intermediate Thymidine monophosphate (dTMP), precursor of DNA metabolite Thymidine triphosphate. Bacteria are unable to take up folic acid from the environment (i.e. the infection host) and are thus dependent on their own de novo synthesis. Inhibition of the enzyme starves the bacteria of nucleotides necessary for DNA replication causing, in certain circumstances, cell lethality due to thymineless death.

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

Helicobacter pylori is associated with a number of gastric conditions, chiefly gastritis and peptic ulcers.

Helicobacter pylori Selective Supplement (Dent) was developed from Dent's selective medium described for the isolation of *H. pylori* from gastric biopsies. This is a modification of Skirrow's medium in which polymixin B is replaced by cefsulodin and amphotericin B is added to inhibit *Candida* species.

When used routinely in the laboratory for 100 gastric biopsies, Dent's medium achieved a higher isolation rate for *H. pylori* and lower contamination by other organisms when compared with Skirrow's medium

and chocolate blood agar. The provision of a good selective medium for *H. pylori* will help establish the role of this organism in the aetiology of gastric disease

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	
Helicobacter Pylori Selective Supplement (Dent)	
Vancomycin	10
Trimethoprim	5
Cefsulodin	5
Amphotericin B	5
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Columbia Blood Agar Base and Helicobacter Pylori Selective Supplement (Dent)

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, Add 35 ml per 500 ml of laked horse blood and mix well before pouring into sterile Petri dishes.

Protocol

1. Prepare the medium as directed. The plates can be stored at 4 °C for three weeks but it is essential that they are kept moist. This can be achieved simply by keeping the plates in a plastic bag.

2. Smear the specimen on to the medium.

Note - the recovery of *H. pylori* from gastric biopsies is improved by direct cultivation as soon as possible after collection. If transportation is necessary, then place the biopsy against the neck of a small, sterile glass bottle containing 0.1 ml of sterile saline. The biopsy should adhere to the glass but be protected from dehydration by water vapour.

3. Incubate at 35°C for three to five days under microaerophilic conditions.

4. Examine for the presence of discrete, translucent and non-coalescent colonies. Note that colonies of *H. pylori* do not resemble those of *Campylobacter* species.

5. Confirm the identity of the isolates with the following tests: Gram negative, curved or spiral bacillus. Growth at 35°C, no growth at 25°C, variable growth at 42°C. Urease positive, Catalase positive, Oxidase positive, Hippurate negative.

Quality control

Positive control:

Helicobacter pylori ATCC® 43526: Good growth; colourless colonies.

Negative control:

Candida albicans ATCC® 10231: Inhibited or no growth

REFERENCES

1. Marshall B. K., Warren J. R., Blincow E. D., Phillips M., Goodwin C. S., Murray R., Blackbourne S. J. and Waters T. E. (1988) The Lancet, December 24/31, No 8626/8627.

2. Dent J. C. and McNulty C. A. M. (1988) Eur. J. Clin. Microbiol. Infec. Dis. 7. 555±568.

3. Buck G. E. (1988) Laboratory Management, 26, No.9.

4. Skirrow M. B. (1977) BMJ, 1. 9-11.