Modified Brucella Selective Supplement

PRODUCT INFORMATION

P007-1MU - Polymyxin B Sulfate, Powder, 1MU

P007-10MU - Polymyxin B Sulfate, Powder, 10MU

P007-100MU - Polymyxin B Sulfate, Powder, 100MU

B002-5g - Bacitracin USP, Powder, 5g

B002-25KU - Bacitracin USP, Powder, 25KU

B002-25g - Bacitracin USP, Powder, 25g

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

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

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

N010-500KU - Nystatin, Powder, 500KU

N010-5MU - Nystatin, Powder, 5MU

N010-25MU - Nystatin, Powder, 25MU

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

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

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

N002-50mg - Natamycin, Powder, 50mg

DESCRIPTION

Brucella Medium Base with Modified Brucella Selective Supplement is a serum-dextrose-antibiotic medium for the cultivation and isolation of *Brucella*.

BACKGROUND

Polymyxin B is an antibiotic primarily used for resistant gram-negative infections. It is derived from the bacterium *Bacillus polymyxa*. Polymyxin B is a mixture of two closely related compounds, polymyxin B1 and polymyxin B2. It has a bactericidal action against almost all gram-negative bacilli except the Proteus group.

Bacitracin is a mixture of related cyclic polypeptides produced by organisms of the licheniformis group of *Bacillus subtilis* var Tracy, isolation of which was first reported in 1945.

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.

Nystatin is a polyene antifungal drug to which many molds and yeast infections are sensitive, including Candida.

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

Natamycin is a naturally occurring antifungal agent produced during fermentation by the bacterium *Streptomyces natalensis*, commonly found in soil. Natamycin has a very low solubility in water, due to the amphiphilic nature of the molecule. However, natamycin is effective at very low levels. Most molds have an MIC (minimum inhibitory concentration) of less than 10 ppm.

Mechanism of action

Polymyxins bind to the cell membrane and alter its structure, making it more permeable. The resulting water uptake leads to cell death.

Bacitracin interferes with the dephosphorylation of the C55-isoprenyl pyrophosphate, a molecule that carries the building-blocks of the peptidoglycan bacterial cell wall outside of the inner membrane.

Like amphotericin B and natamycin, nystatin binds to ergosterol, a major component of the fungal cell membrane. When present in sufficient concentrations, it forms pores in the membrane that lead to K+ leakage and death of the fungus. Ergosterol is fairly unique to fungi, so the drug does not have such catastrophic effects on animals.

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 BRUCELLA MEDIUM BASE

Brucella Medium Base may be used to prepare the serum-dextrose-antibiotic medium described by Jones and Brinley Morgan for the cultivation and isolation of *Brucella*, including fastidious types. Brucella medium with antibiotics has advantages over the media described by Kuzdas and Morse and Renoux in that it will support growth of fastidious types and it is more effective as a selective medium. During investigations, Jones and Brinley Morgan showed that serum-glucose agar with antibiotics gave excellent growth of all *Brucella* strains and permitted better growth of *Brucella abortus* biotype 2 - a strain which had been difficult or impossible to cultivate.

Content concentrations

Typical Formula*	**mg/litre**
Brucella Medium Base	
Peptone	10
`Lab-Lemco' powder	5
Glucose	10
Sodium chloride	5
Agar	15
Final pH 7.5 ± 0.2 @ 25°C	
Modified Brucella Selective Supplement	
Polymyxin B	5,000 IU
Bacitracin	25,000 IU
Nalidixic acid	5
Nystatin	100000 IU
Vancomycin	20
Natamycin	50
* Adjusted as required to meet performance standards	

Table 1 typical formula for Brucella Medium Base and Modified Brucella Selective Supplement

METHOD

Preparation

Suspend appropriate amount of Brucella medium base in of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add 5% of inactivated horse serum. Reconstitute supplement as directed. Incubate for 10-15 minutes at 35°C. Mix thoroughly and immediately add the vial contents to sterile Brucella medium base, cooled to 50°C together with 5-10% v/v sterile inactivated horse serum and 1-5% w/v of a filter-sterilised 10% solution of dextrose. Mix well and pour into sterile petri dishes.

Protocol

The addition of dyes (i.e. malachite green and gentian violet) as selective agents, is not recommended, as it may result in poor growth of many Brucella strains. Where a non-selective medium is required, the medium may be employed with the addition of serum only (i.e. without antibiotics): for subsequent differentiation between strains of Brucella. This medium is recommended for use in conjunction with the Cruickshank dyestrip method:

1. Impregnate filter paper strips with 1:200 Basic Fuchsin or 1:600 Thionin. Dry.

2. Place the strips parallel on the surface of the serum-dextrose agar and then cover with a thin layer of the same medium. Then allow the medium to solidify.

3. Make stroke inoculations of the Brucella strains to be tested, at right angles to the strips.

4. Incubate in 10% carbon dioxide for 2-3 days at 35°C.

5. Examine. Resistant strains grow right across the strip, but sensitive strains show inhibition of growth up to 10mm from the strip. Typical growth patterns are then as follows:

	Basic Fuchsin 1:200	Thionin 1:600
Brucella abortus	growth	no growth
Brucella melitensis	growth	growth
Brucella suis	no growth	growth

However, there are exceptions to the above and it is therefore advisable to base identification on many characteristics.

The slow growth of Brucella species, combined with their requirement for highly nutritious media means that selective agents must be incorporated to prevent overgrowth of contaminant organisms from milk or veterinary tissues.

Media containing bacteriostatic dyes are inhibitory to strains of *Brucella abortus* biotype 2 and other fastidious strains. Antibiotics used in place of dyes enabled growth of all biotypes *Brucella* species to appear on selective media. However, Leech et al. showed that the serum-glucose-antibiotic formulation was not sufficiently selective and was less efficient than guinea-pig inoculation.

Barrow and Peel modified a selective medium devised

by Mair. This contained both antibiotics and gentian violet. Failure of some strains of Brucella abortus to grow confirmed their sensitivity to very low concentrations of the dye recognised by Mair.

Farrell developed a highly selective antibiotic-containing nutrient medium which incorporated bacitracin 25 iu/ml, vancomycin 20 mg/ml, cycloheximide 100 mg/ml and nystatin 100 iu/ml, in a serum-glucose agar base.

In comparative trials10 the medium was shown to give a rate of isolation equivalent to that achieved by guinea-pig inoculation. It also supported the growth of Brucella abortus biotype 2 strains.

Brucella selective supplement is based on the superior formulation of Farrell. Its greater efficiency at suppressing bacterial contamination than either serum glucose agar or Barrow and Peel's Medium was shown in a further trial.

1. For direct culture of *Brucella* species from milk transfer the samples to sterile tubes and hold overnight at 40°C.

2. Withdraw an aliquot of gravity cream with a spiral wire and spread over a plate of supplemented agar with a bent sterile glass rod.

3. Incubate the plates at 35°C in an atmosphere containing 10-20% (v/v) carbon dioxide and examine every two days for ten days.

4. *Brucella* colonies appear as 1-2 mm diameter convex colonies with round entire edges, and may be identified by slide agglutination.

Quality control

Positive control:

Bordetella bronchiseptica ATCC® 4617*:* Good growth; small clear colonies

Negative control:

Staphylococcus aureus ATCC® 25923: No growth

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