Brucella Selectavial™

**Introduction**

Members of the genus *Brucella* are intracellular parasites which can cause localised infection of bone, tissue or organ systems in humans. *Brucellae* are Gram negative coco-bacilli which are non-motile and aerobic, although some strains require CO₂ for growth. Organisms may be isolated from unpasteurised dairy products, infected animals and clinical specimens. The three principle *Brucella* species are pathogenic in a wide range of mammals although each has a preferred host. *B.abortus* is predominantly isolated from cattle, *B.suis* normally infects swine and *B.melitensis* is found in goats and sheep. Each species mentioned above has been found to be infective to humans. In mammals other than man the brucellae will accumulate in the mammary glands, causing infection of the milk, and in the genital organs, particularly the pregnant uterus, often leading to abortion.

As *Brucella* spp. are slow growing organisms, with inoculated plates needing incubation periods of 10 days, unsupplemented plates tend to be overgrown with contaminants present in the samples. The use of a selective supplement is recommended to suppress the growth of such contaminants. Some Brucella supplements previously described contain bacteriostatic dyes, which have been found to be inhibitory to fastidious strains of Brucella, particularly *B.abortus* biotype 2.¹ ² The supplement SV38 is based upon Farrell’s highly selective antibiotic formulation³ and omits the use of dyes. Work by Farrell and Robertson⁴ confirmed the ability of the formulation to recover and support the growth of all Brucellae including *B.abortus* biotype 2 strains.

Selective plates may be used for direct culture of *Brucella* spp. from milk and other samples. The plates should be incubated at 35-37°C in 10-20% (v/v) carbon dioxide and examined every two days for ten days. Colonies appear on the agar surface after 3 days of incubation and reach 1.5-2.5mm in diameter. Colonies are entire with a smooth glistening surface.

**Description**

Each Brucella Selectavial contains accurately assayed quantities of antibiotics in a soluble, non-interfering carrier substance.

<table>
<thead>
<tr>
<th>Brucella Selectavial</th>
<th>Concentration in 1 litre medium</th>
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<tbody>
<tr>
<td>Polymyxin B</td>
<td>5,000iu 5,000iu/litre</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>25,000iu 25,000iu/litre</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>100mg 100mg/litre</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>5mg 5mg/litre</td>
</tr>
<tr>
<td>Nystatin</td>
<td>100,000iu 100,000iu/litre</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>20mg 20mg/litre</td>
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</tbody>
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**Directions**

1. Sterilise the medium, Mast Brucella Medium (DM107), cool to 55°C and hold in a water bath at this temperature.

2. Reconstitute the contents of one vial using 10ml of 50% methanol. The best method is to aseptically add the diluent using a sterile needle and syringe. Draw the diluent into the syringe and after removing the plastic cap of the vial, inject through the rubber stopper of the vial. The lyophilised supplement will rapidly suspend and may be withdrawn into the syringe.

3. Add the antibiotic solution to 1 litre of medium and discard the needle into an approved container. DO NOT TRY TO RE-SHEATH AN EXPOSED NEEDLE. Unused reconstituted supplement should be discarded and not frozen.

4. Mix gently but thoroughly to evenly distribute the selective agents.

5. Supplement the medium with 5% inactivated horse serum (to inactivate hold at 56°C for 30 minutes). Pour culture plates and allow to set.

6. Prepared culture plates may be used immediately or stored in plastic bags at 2-8°C for up to one week before use.
References


