Anaerobe Isolation Agar (AIA)

DM630. A general purpose medium for the isolation of fastidious anaerobes.

**Typical Formulation**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Grams per litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone mixture</td>
<td>23.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.0</td>
</tr>
<tr>
<td>Soluble starch</td>
<td>3.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.5</td>
</tr>
<tr>
<td>Di potassium phosphate</td>
<td>10.0</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Magnesium phosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.2</td>
</tr>
<tr>
<td>Manganese sulphate</td>
<td>0.01</td>
</tr>
<tr>
<td>Cysteine hydrochloride</td>
<td>0.067</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.01</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.5</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

**pH approx. 7.2**

**Introduction**

Anaerobic bacteria are commonly found inhabiting the soft tissues of the body, particularly the oropharyngeal, intestinal and genitourinary tracts. They are opportunistic pathogens causing, often severe, infections in deeper tissues.

Although some anaerobic species are able to grow well on blood agar, various specifically enriched media have been recommended to produce optimal growth of the more fastidious anaerobes. MAST Anaerobe Isolation Agar (AIA) has been developed to allow isolation of a wide range of clinically significant anaerobic bacteria by the inclusion in the formulation of a carefully balanced mix of peptones and glucose to provide a nutritionally rich environment. The medium also contains a buffering system to maintain physiological pH, various minerals essential for growth and L-cysteine which decreases the redox potential of the medium and also stimulates the growth of a number of organisms.

As anaerobic bacteria are often present in mixed infections their isolation is greatly aided by the addition of selective antibiotics such as neomycin or nalidixic acid (MAST Selective Supplements MS8, MS8A, SV8, MS9, MS9A, SV9).

**Directions**

1. Suspend by swirling 59.3g of powder in 1 litre of deionised water.
2. Autoclave at 121°C (15 psi) for 15 minutes.
3. Cool to 50 - 55°C and add 5 - 7% sterile defibrinated horse blood and selective supplements if required.
4. Mix well before pouring.
5. Dry plates before use.

**In use**

Inoculate plates by streaking specimens over the surface of the medium and incubate at 37°C in an anaerobic atmosphere (typically 80% N, 10% H and 10% CO₂). It is advisable to set up duplicate plates, one for examination after 24 hours, and the other to remain undisturbed for 72-96 hours.

If plates are examined after 24 hours (for rapidly growing anaerobes such as *Cl. Perfringens* or *B. fragilis*), exposure to oxygen must be minimised before re-incubation. Plates incorporating selective supplements generally require extended incubation and should be used with a parallel non-selective medium.

After incubation the plates will darken, a normal effect of a reduced atmosphere on the blood. Colony size and appearance will vary from species to species.

**References**

Anaerobe Isolation Broth (AIB)

DM631. A general purpose liquid medium for the isolation of fastidious anaerobes.

Typical Formulation* grams per litre

Peptone mixture 23.0
Yeast extract 5.0
Soluble starch 0.5
Glucose 0.5
Sodium thioglycollate 0.5
Sodium bicarbonate 0.4
Resazurin 0.001
Magnesium sulphate 1.0
Sodium chloride 0.2
Mangene sulphate 0.01
Cysteine hydrochloride 0.5
Ferrous sulphate 0.01
Tween 80 0.5
Agar 0.5

pH approx. 7.2

Introduction
Anaerobic bacteria are commonly found inhabiting the soft tissues of the body. They are opportunistic pathogens causing, often severe, infections in deeper tissues. Other often extremely serious infections result from contact with anaerobes in the environment where they exist as saprophytes or as spores excreted in animal faeces. Gas gangrene for example, caused principally by *C. perfringens*, classically results from the contamination of wounds by soil.

Due to the oxygen sensitivity of anaerobic organisms the quality of specimen collection for laboratory diagnosis is critical and the most reliable specimens are pus or exudate from the depths of an open or a closed lesion. If swabs are used to send samples to a laboratory they should first be placed in a suitable medium such as MAST Amies Transport Medium (DM030).

MAST Anaerobe Isolation Broth (AIB), like MAST AIA, has been developed to permit the isolation of clinically significant fastidious anaerobes. The correct redox environment for recovery and growth of anaerobic bacteria is provided by a combination of reducing agents including sodium thioglycollate and cysteine, which also stimulates the growth of a variety of organisms. A small amount of agar limits absorption and diffusion of oxygen and resazurin is included as a visible redox indicator - when the medium is in a reduced state it is straw coloured, turning pink/red as it oxidises.

As anaerobic bacteria are often present in mixed infections, which can include facultatively anaerobic species, use of liquid media alone for primary anaerobic culture is not generally recommended. However during isolation from specimens where particularly slow growing or fastidious anaerobes are suspected to be present a broth medium is a useful adjunct to culture methods based on solid media.

Directions
1. Suspend by swirling 32.6g of powder in 1 litre of deionised water and dispense the required volume into screw capped vials.
2. Autoclave at 121°C (15 psi) for 15 minutes.
3. Allow to cool to 37°C and add selective supplements if required.

In use
Inoculate when the medium has cooled to 35 - 37°C and incubate at 37°C for 24 - 72 hours, or longer if required. Liquid enrichment media should be examined daily for evidence of growth particularly in the lower half of the tube for up to 14 days or according to procedures followed. If positive, the medium should be examined for the presence of organisms not found on primary isolation plates by Gram stain and subculture to a suitable solid medium such as MAST Anaerobe Isolation Agar (DM630).

On preparation, the medium may have a narrow purple/red band at the surface due to the action of oxygen on the resazurin. Tubes that have not been freshly prepared and may be pink in colour can be reheated, once only, by steaming for 15 minutes to remove any dissolved oxygen.

References

*Formulation may be modified to meet performance criteria