# Mitis Salivarius Agar Base

**M259**

## Intended Use:

Recommended for the isolation of streptococci, especially *Streptococcus mitis, Streptococcus salivarius* and *Enterococcus faecalis* from grossly contaminated specimens.

## Composition**

| Ingredients | g / L |
|---|---|
| Tryptone | 15.000 |
| Peptone | 5.000 |
| Dextrose (Glucose) | 1.000 |
| Sucrose | 50.000 |
| Dipotassium hydrogen phosphate | 4.000 |
| Trypan blue | 0.075 |
| Crystal violet | 0.0008 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 7.0±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 90.07 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 1 ml of sterile PTe 1% Selective Supplement (1 ml per vial) (FD052). **DO NOT REHEAT** the medium after the addition of tellurite solution. Mix well and pour into sterile Petri plates.

## Principle And Interpretation

Streptococcus species are mostly commensal residents of the mouth and throat, though several may act as opportunistic pathogens and a few as primary pathogens (1). Streptococcus "viridans" group consists of *Streptococcus salivarius* and *Streptococcus mitis*. They exhibit different types of haemolysis when grown on Blood Agar Base. Therefore it is difficult to differentiate these organisms found in saliva from the other accompanying flora. Mitis Salivarius Agar Base is used for the isolation of *S.mitis, S. salivarius* and *Enterococcus faecalis* from mixed cultures. *E. faecalis* is the most common member of the Enterococci to cause infections in humans and is also a cause of human endocarditis (2). Mitis Salivarius Agar is formulated as per Chapman (3,4,5). This medium (with 1% potassium tellurite) is a highly selective medium, which enables to isolate streptococci from highly contaminated specimens like exudates from body cavities and faeces etc., as it inhibits a wide variety of bacteria. Some authors have also used sodium azide in this medium to inhibit the growth of gram-negative bacteria like *Proteus* (6).

Tryptone and peptone in the medium provide the essential growth nutrients. Dextrose and sucrose are the fermentable carbohydrates. Dipotassium phosphate buffers the medium. Trypan blue is an acidic, blue diazo dye while crystal violet is a basic dye and also a bacteriostatic agent, which inhibits many gram-positive organisms. Potassium tellurite also helps to make the medium selective for streptococci. Occasionally *Streptococcus mutans* strains may be inhibited on Mitis Salivarius Agar Base due to the high concentration of trypan blue in the medium. Also some *S. mitis* strains may be more easily distinguished with longer incubation (7).

## Type of specimen

Clinical samples - Feaces (Enterococci), Dental plaque and carious lesions swabs; saliva

## Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations :

1. Molds exhibit growth after 2 days of incubation (7).
2. Do not heat medium after addition of PTe 1% Selective Supplement (1 ml per vial) (FD052); solution is heat-labile (7).

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

**Appearance**

Light yellow to light blue homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**

Dark blue coloured clear to slightly opalescent gel forms in Petri plates

**Reaction**

Reaction of 9.0% w/v aqueous solution at 25°C. pH : 7.0±0.2

**pH**

6.80-7.20

**Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours with added 1% Potassium Tellurite (FD052).

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of colony |
|---|---|---|---|---|
| *Enterococcus faecalis* ATCC 29212 (00087*) | 50-100 | good-luxuriant | >=50% | blue - black |
| *Escherichia coli* ATCC 25922 (00013*) | >=$10^4$ | inhibited | 0% | |
| *Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*) | >=$10^4$ | inhibited | 0% | |
| *Streptococcus intermedius* ATCC 9895 | 50-100 | good-luxuriant | >=50% | blue |
| *Streptococcus pyogenes* ATCC 19615 | 50-100 | good-luxuriant | >=50% | blue |
| *Streptococcus salivarius* ATCC 13413 | 50-100 | good-luxuriant | >=50% | blue (gum drop) |

Key : *Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

## Reference

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HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro*** **diagnostic medical device**

30°C
10°C **Storage temperature**

EC REP CEpartner4U, Esdoornlaan 13,
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**CE Marking**

**Do not use if package is damaged**

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