

# Mitis Salivarius Agar Tellurite Solution 1%

## Intended Use

Mitis Salivarius Agar is used with Tellurite Solution 1% in isolating *Streptococcus mitis*, *S. salivarius* and enterococci, particularly from grossly contaminated specimens.

## Summary and Explanation

*S. mitis*, *S. salivarius* and *Enterococcus* species are part of the normal human flora. *S. mitis* and *S. salivarius* are known as viridans streptococci. These organisms play a role in cariogenesis and infective endocarditis and cause an increasing number of bacteremias.<sup>1</sup> Enterococci cause urinary tract infections, wound infections and bacteremia.<sup>2</sup> These organisms can colonize the skin and mucous membranes.

Chapman<sup>3-5</sup> investigated methods for isolating streptococci and formulated Mitis Salivarius Agar. The medium facilitates isolation of *S. mitis* (*Streptococcus viridans*), *S. salivarius* (nonhemolytic streptococci) and enterococci from mixed cultures.<sup>6</sup>

## Principles of the Procedure

Mitis Salivarius Agar contains peptones as sources of carbon, nitrogen, vitamins and minerals. Dextrose and saccharose are carbohydrate sources. Crystal violet and potassium tellurite (from Tellurite Solution 1%) inhibit most gram-negative bacilli

and most gram-positive bacteria except streptococci. Trypan blue gives the colonies a blue color. Agar is the solidifying agent.

## Formulae

### Difco™ Mitis Salivarius Agar

Approximate Formula* Per Liter	
Pancreatic Digest of Casein .....	6.0 g
Proteose Peptone No. 3 .....	9.0 g
Proteose Peptone .....	5.0 g
Dextrose .....	1.0 g
Saccharose .....	50.0 g
Dipotassium Phosphate .....	4.0 g
Trypan Blue .....	75.0 mg
Crystal Violet .....	0.8 mg
Agar .....	15.0 g

### BBL™ Tellurite Solution 1%

Sterile 1% solution of Potassium Tellurite.

\*Adjusted and/or supplemented as required to meet performance criteria.

## Directions for Preparation from Dehydrated Product

1. Suspend 90 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.

## User Quality Control

### Identity Specifications

#### Difco™ Mitis Salivarius Agar

Dehydrated Appearance: Bluish-beige, free-flowing, homogeneous.

Solution: 9.0% solution, soluble in purified water upon boiling. Solution is deep royal blue, very slightly opalescent.

Prepared Appearance: Deep royal blue, slightly opalescent.

Reaction of 9.0%

Solution at 25°C: pH 7.0 ± 0.2

#### BBL™ Tellurite Solution 1%

Appearance: Colorless and clear to trace hazy.

### Cultural Response

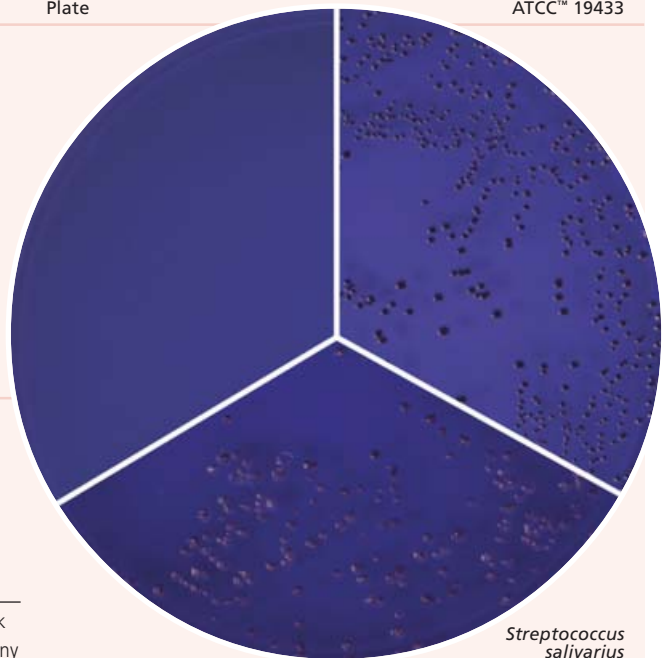
#### Difco™ Mitis Salivarius Agar with BBL™ Tellurite Solution 1%

Prepare the complete medium per label directions. Inoculate and incubate under 5-10% CO<sub>2</sub> at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Enterococcus faecalis</i>	19433	10 <sup>2</sup> -10 <sup>3</sup>	Good	Blue/black
<i>Escherichia coli</i>	25922	10 <sup>3</sup>	Partial to complete inhibition	Brown, if any
<i>Staphylococcus aureus</i>	25923	10 <sup>3</sup>	Partial to complete inhibition	–
<i>Streptococcus mitis</i>	9895	10 <sup>2</sup> -10 <sup>3</sup>	Good	Blue
<i>Streptococcus salivarius</i>	9758	10 <sup>2</sup> -10 <sup>3</sup>	Good	Blue "gum drop" shape

Uninoculated  
Plate

*Enterococcus faecalis*  
ATCC™ 19433



*Streptococcus  
salivarius*  
ATCC™ 9758

4. Add 1 mL of Tellurite Solution 1%. DO NOT HEAT THE COMPLETE MEDIUM.
5. Test samples of the finished product for performance using stable, typical control cultures.

### Procedure

See appropriate references for specific procedures.

### Expected Results

*S. mitis* produces small or minute blue colonies. These colonies may become easier to distinguish with longer incubation. *S. salivarius* produces blue, smooth or rough “gum drop” colonies, 1-5 mm in diameter depending on the number of colonies on the plate. *Enterococcus* species form dark blue or black, shiny, slightly raised, 1-2 mm colonies.

### Limitations of the Procedure

1. If coliforms grow on the medium, they produce brown colonies.

2. Molds will grow on the medium after two days incubation.
3. *Erysipelothrix rhusiopathiae* produces colorless, circular, convex colonies.
4. Beta-hemolytic streptococci produce colonies that resemble *S. mitis*.

### References

1. Ruoff, Whiley and Beighton. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
2. Facklam, Sahm and Teixeira. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
3. Chapman. 1944. J. Bacteriol. 48:113.
4. Chapman. 1946. Am. J. Dig. Dis. 13:105.
5. Chapman. 1947. Trans. N.Y. Acad. Sci. (Series 2) 10:45.
6. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

### Availability

#### Difco™ Mitis Salivarius Agar

Cat. No. 229810 Dehydrated – 500 g

#### BBL™ Tellurite Solution 1%

Cat. No. 211917 Tube – 20 mL

## Modified mTEC Agar

(See *mTEC Agar, Modified*)

## Moeller Decarboxylase Broths

(See *Decarboxylase Differential Media*)

## Moeller KCN Broth Base

### Intended Use

Moeller KCN Broth Base, when supplemented with a solution of potassium cyanide, is used in the differentiation of enteric bacilli on the basis of their ability to grow promptly in the presence of cyanide.

### Summary and Explanation

In 1954, Moeller reported on the use of a medium containing cyanide as an aid in the differentiation of members of the *Enterobacteriaceae*.<sup>1</sup> Edwards and Ewing<sup>2</sup> modified Moeller Cyanide Broth and it is this modified formulation that is supplied as BBL™ brand Moeller KCN Broth Base prepared in a tube.

This medium, when supplemented with potassium cyanide, is used in the differentiation of members of the genus *Salmonella* from *Citrobacter freundii*.<sup>3</sup> It is particularly useful in differentiating cultures of *C. freundii* that either fail to ferment lactose or ferment it slowly (i.e., strains formerly classified as Bethesda-Ballerup bacteria).<sup>3</sup> Except for strains of groups IV and V, members of the genus *Salmonella* do not grow in KCN medium, whereas species of *Citrobacter* with the exception of *C. (diversus) koseri* do grow in this medium.<sup>3,4</sup>

### Principles of the Procedure

The addition of 0.15 mL of a 0.5% solution of potassium cyanide to each of the prepared tubes of the nutritive base enables differentiation of members of various genera within the *Enterobacteriaceae* family.

### Procedure

Prior to use, add 0.15 mL of a 0.5% solution (0.5 g in 100 mL of COLD sterile distilled water) of potassium cyanide to each tube containing 10 mL of base and close tightly.

**Precaution: Do not mouth pipette. Extreme care should be taken at all times in handling and disposing of potassium cyanide. Work should be performed within a chemical hood.**

Inoculate Moeller KCN Broth with a loopful of an 18- to 24-hour culture of the test organism. Incubate at 35 ± 2°C in an aerobic atmosphere and examine daily for 2 days.

### Expected Results

Except for a few infrequently-isolated species, members of the genera *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Morganella*, *Proteus* and *Providencia* are KCN positive; i.e., they grow in the presence of KCN. With certain exceptions, other *Enterobacteriaceae* are negative (inhibited) in the KCN test.<sup>4</sup>