

# **Technical Information**

# **Simmons Agar Base**

### Product Code: DM 1411

**Application:** Simmons Agar Base is a synthetic medium recommended for differentiation between faecal coliform and members of the aerogenes group on the basis of citrate utilization.

Composition\*\*

Ingredients	Gms / Litre		
Magnesium sulphate	0.200		
Ammonium dihydrogen phosphate	0.200		
Sodium ammonium phosphate	0.800		
Sodium chloride	5.000		
Bromothymol blue	0.080		
Agar	15.000		
Final pH (at 25°C)	7.0±0.2		
**Formula adjusted, standardized to suit performance	parameters		

## Principle & Interpretation

This media is used for the differentiation between *Enterobacteriaceae* and the members of aerogenes group on the basis of citrate utilization as sole carbon source. Initially the citrate medium was developed by Koser <sup>(1)</sup> containing ammonium salt as the only nitrogen source and citrate as the only carbon source for differentiating *Escherichia coli* and *Enterobacter aerogenes* by IMViC tests. Later on Simmons <sup>(2)</sup> modified Kosers formulation by adding agar and bromo thymol blue <sup>(3)</sup>. It is also recommended by APHA <sup>(4)</sup>.

Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively. Microorganisms also use inorganic ammonium salts as their sole nitrogen source. Metabolism of these salts causes the medium to become alkaline, indicated by a change in colour of the pH indicator from green to blue. Bromothymol blue is the pH indicator. The medium should be freshly prepared because in dry conditions, changes in colour may appear even before inoculation, especially at the bottom of the slant.

# Methodology

Suspend 21.28 grams of powder media in 900 ml distilled water. Shake well & heat to dissolve the medium completely. Add 100 ml of 0.2% solution of sodium citrate. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

# **Quality Control**

#### **Physical Appearance**

Cream to yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Forest green coloured clear to slightly opalescent gel forms in tubes as slants

#### Reaction

Reaction of 2.13% w/v aqueous solution at 25°C pH: 7.0±0.2

pH range 6.80-7.20

#### Cultural Response/Characteristics

DM 1411: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours with added 0.2% solution of Sodium citrate.





Organism	Inoculum (CFU)	Growth	Citrate utilisation
Enterobacter aerogenes ATCC 13048	50-100	good-luxuriant	positive reaction, blue colour
Escherichia coli ATCC 25922	>=10³	inhibited	negative reaction, green colour
Salmonella Typhi ATCC 6539	50-100	fair-good	negative reaction, green colour
Salmonella Typhimurium ATCC 14028	50-100	good-luxuriant	positive reaction, blue colour
Salmonella Enteritidis ATCC 13076	50-100	good-luxuriant	positive reaction, blue colour
Shigella dysenteriae ATCC 13313	>=10³	inhibited	negative reaction, green colour

# Storage and Shelf Life

**Dried media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label. **Prepared Media:** 2-8<sup>0</sup> in sealable plastic bags for 2-5 days.

# **Further Reading**

- 1. Koser, 1923, J. Bact., 8:493.
- 2. Simmons, 1926, J. Infect. Dis., 39:209.
- 3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins,
- 4. Eaton A. D., Clesceri L. S., Rice E. W., and Greenberg A W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.

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