



Simmons Agar Base

M411

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

Directions

Suspend 21.28 grams in 900 ml distilled water. Heat to boiling 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.

Principle And Interpretation

These media are 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 (1) 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 (2) modified Kosers formulation by adding agar and bromo thymol blue (3). It is recommended by APHA (4).

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.

Quality Control

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

6.80-7.20

Cultural Response

M411: 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
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<i>Enterobacter aerogenes</i> ATCC 13048	50-100	good-luxuriant	positive reaction, blue colour
<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	inhibited	negative reaction, green colour
<i>Salmonella Typhi</i> ATCC 6539	50-100	fair-good	negative reaction, green colour
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good-luxuriant	positive reaction, blue colour
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	good-luxuriant	positive reaction, blue colour
<i>Shigella dysenteriae</i> ATCC 13313	$\geq 10^3$	inhibited	negative reaction, green colour

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

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, Baltimore.
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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