Simmons Citrate Agar (NCM0168)

Revision: 0 Effective Date: 11/19/2021

Intended Use

Simmons Citrate Agar is used for the differentiation of microorganisms on the basis of citrate utilization in a laboratory setting. Simmons Citrate Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Koser first developed a liquid medium for differentiating coliforms from fecal coliforms. Fecal coliforms were unable to use citrate as the sole source of carbon and inorganic ammonium salt as a sole source of nitrogen. Non-fecal coliforms, such as *Enterobacter aerogenes* could use citrate in such a medium with resultant alkalinity. Liquid medium had the disadvantage of appearing turbid when a large inoculum was used, although no growth had taken place. This observation led Simmons to devise a solid medium that eliminated the problem with turbidity.

Simmons Citrate Agar is a modification of Koser's medium, with the addition of bromothymol blue and 1.5% agar. Organisms able to metabolize citrate grow luxuriantly. The medium is alkalinized and changes from green to deep blue in 24 – 48 hours. *Escherichia coli* either do not grow at all on this medium or grow so sparsely that no change in reaction is apparent. Simmons Citrate Agar is recommended for differentiation of enteric Gram-negative bacilli from laboratory specimens, water samples, and food samples.

Typical Formulation

Ammonium Dihydrogen Phosphate	1.0 g/L
Dipotassium Phosphate	1.0 g/L
Sodium Chloride	5.0 g/L
Sodium Citrate	2.0 g/L
Magnesium Sulfate	0.2 g/L
Bromothymol Blue	0.08 g/L
Agar	15.0 g/L

Final pH: 6.9 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 24.2 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense into tubes and autoclave at 121°C for 15 minutes.
4. After autoclaving, allow medium to solidify in a slanted position.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and yellow with green tinge to green with yellow tinge.

Prepared Appearance: Prepared medium is forest green and trace to slight haze.

Expected Cultural Response: Cultural response on Simmons Citrate Agar incubated at the appropriate atmosphere and temperature and examined for growth after 18 - 48 hours

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Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Recovery	Reactions
Citrobacter freundii ATCC® 43864	Direct inoculation	Growth	Blue medium
Enterobacter aerogenes ATCC® 13048	Direct inoculation	Growth	Blue medium
Escherichia coli ATCC® 25922	Direct inoculation	Partial to Complete Inhibition	Green medium
Proteus mirabilis ATCC® 29906	Direct inoculation	Growth	Blue medium
Salmonella enteritidis ATCC® 13076	Direct inoculation	Growth	Blue medium
Salmonella typhimurium ATCC® 14028	Direct inoculation	Growth	Blue medium
Shigella flexneri ATCC® 12022	Direct inoculation	Partial to Complete Inhibition	Green medium
Shigella sonnei NCTC 8574	Direct inoculation	Partial to Complete Inhibition	Green medium

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Obtain a pure colony of the test organism.
2. Streak only the surface of the slant with a light inoculum.
3. Incubate tubes at 37 ± 1°C for 18 – 48 hours with loose caps

Results

A positive reaction is indicated by growth on the slant with an intense blue color (alkaline reaction). A negative reaction is indicated by inhibition to poor growth without change in color (medium remains green).

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Some citrate positive organisms require a 48-hour incubation or longer for a pH change to occur.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

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