Specification

Solid medium for verifying citrate utilization by enterobacteria according to the ISO standards.

Formula * in g/L

Magnesium sulfate..... 0,20
Monoammonium phosphate.....1,00
Dipotassium phosphate.....1,00
Sodium citrate..... 2,00
Sodium chloride..... 5,00
Bromothymol blue..... 0,08
Agar.....15,00

Final pH 6,8 ±0,2 at 25 ºC

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

Directions

Dissolve 24 g of powder in 1 L of distilled water. Bring to the boil. Dispense in tubes and sterilize in the autoclave at 121° C for 15 minutes. Allow to solidify with a long slant.

Description

Simmons Citrate Agar is the solid version of the classical Koser citrate medium, and can be used in plates format as well as in slanted tubes. Slant tubes can be inoculated by surface streaking or by a deep stab. Although it was originally described as an isolation and identification medium for certain fungi, Edwards and Ewing recommended it for the IMViC (Indol, Methyl red, Vogues Proskauer and Citrate) test. It has the advantage over Koser's medium that readings can be made by the indicator colour change, instead of the turbidity of the medium, which is sometimes difficult to detect.

Technique

To ensure an accurate result use an inoculum as small as possible and unsure the medium is freshly prepared, because if it is very dry, a false result (colour change) may appear, even before inoculation, especially at the bottom of the slant.
The basis of this medium is in the capacity of microorganisms to use citrate as a sole carbon source and ammonium compounds as the only nitrogen source for their growth. Among enterobacteria, these properties are possessed by the following genera: Enterobacter, Klebsiella, Serratia, Citrobacter and some species of Salmonella such as S. schottumelleri, S. typhimurium, S. arizona etc. Escherichia, Shigella, Salmonella typhi and S. paratyphi are unable to growon this media.
Although the test result must be read as growth proceeds, the presence of an indicator makes it easier, as citrate degradation results in an alkaline reaction, which is indicated by the indicator turning an intense blue. This is evident even when the growth is at an early stage.

Quality control

Incubation temperature: 35°C ±2,0 **Incubation time:** 24-48 h
Inoculum: Pure culture is inoculated by surface streaking

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC® 27853	Good - very good	Blue medium
Enterobacter aerogenes ATCC® 13048	Good - very good	Blue medium
Escherichia coli ATCC® 25922	Inhibited	Green medium
Shigella flexneri ATCC® 12022	Inhibited	Green medium
Salmonella typhimurium ATCC® 14028	Good - very good	Blue medium
Salmonella abony NCTC® 6017	Good - very good	Blue medium
Escherichia coli ATCC® 8739	Inhibited	Green medium

References

· APHA-AWWA-WEF (1998) Standard Methods for the examination of water and wastewater. APHA. Washington DC. USA.
· DIN 38411-6 (1991) Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung; Mikrobiologische Verfahren (Gruppe K); Nachweis von Escherichia coli und coliformen Keimen (K6)
· FDA (Food and Drug Adminstrations) (1998). Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg Md. USA.
· HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC International. Gaithersburgs. Md. USA.
· ISO 10273 Standard. (1994) General guidance for the detection of presumptive pathogenic Yersinia enterocolitica.
· ISO/TS 22964 (2006) Milk and milk products.- Detection of Enterobacter sakazakii.
· SIMMONS J.S. (1926) A culture medium for differentiating organisms of typhoid-colon aerogenes group and for isolating certain fungi. J.Inf. Dis. 39:209.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).