

PRODUCT SPECIFICATION SHEET

Koser Citrate Medium (DM128)

Intended Use

Koser Citrate Medium (DM128) is recommended for the differentiating *Escherichia coli* from *Enterobacter aerogenes* based on citrate utilization.

Product Summary and Explanation

Coliform bacteria serve as bacterial indicators of sanitary quality of food and water. These bacteria are normally found in the intestinal tract of humans and many warm-blooded animals.⁽¹⁾ Coliforms encompasses mostly of *Enterobacteriaceae* from the genera *Enterobacter*, *Klebsiella*, *Escherichia*, and *Citrobacter*. The characteristics of the members of *Enterobacteriaceae* are that they are gram-negative rods and ferment glucose to form acid along with gas production.⁽²⁾ Two important members of the *Enterobacteriaceae* family are *Escherichia coli* and *Enterobacter aerogenes*. Both can be differentiated on the basis of IMViC test. *Enterobacter* species are able to utilize sodium citrate as the sole carbon source while *E.coli* fail to do so. This property is used to differentiate the coli-aerogenes group.⁽³⁾ Koser demonstrated in 1923 that coli-aerogenes bacteria could be differentiated by their use of certain salts of organic acids.⁽³⁾ Koser found that the sodium salt of citric acid (sodium citrate) is used as a source of carbon by *E. aerogenes* and not by *E. coli*. Biochemical identification schemes for identifying *E. coli* frequently include Koser citrate. *E. coli* is an important member of the coliform group of bacteria. The coliforms are described as aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Procedures to detect, enumerate and presumptively identify coliforms are used in testing foods and dairy products. 2-5 Presumptive identification is confirmed by performing biochemical tests that specifically identify *E. coli*. Koser Citrate Medium is used as a base for studying citrate utilization tests. This medium is recommended by APHA, and others, to presumptively identify coliforms encountered in the food and dairy industry.⁽³⁻⁷⁾

Principles of the Procedure

The various salts used serve as source of carbon and nitrogen to the organisms. Citric acid or its sodium salt is utilized as a sole source of carbon and ammonium salt as the sole source of nitrogen by *E. aerogenes* while *E. coli* does not utilize these salts and hence fail to grow. Koser Citrate Medium is prepared with chemically pure salts and tested to determine that no sources of carbon (other than sodium citrate) or nitrogen (other than ammonium salts) are present. Koser Citrate Medium may be used in place of Simmon Citrate Agar (DM244). Inoculating into Koser Citrate Medium further identifies coli-like colonies from Endo or EMB Agar plates. After 24-48 hours incubation, tubes showing marked turbidity may be assumed to contain organisms from aerogenes group and if the medium remains clear it may be considered as coli. Presumptive positive organisms identified on this medium should be further confirmed by performing the biochemical tests.

Formula / Liter

Ingredients	Gms / Liter
Sodium ammonium phosphate	1.50
Monopotassium phosphate	1.00
Magnesium sulphate	0.20
Sodium citrate	3.00
Final pH: 6.7 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 5.7 grams of the medium in one liter of distilled water.
2. Dispense into tubes.
3. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

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Quality Control Specifications

Dehydrated Appearance	White to cream, homogeneous free flowing powder
Prepared Medium	Colourless, clear solution without any precipitate
Reaction of 0.57% solution	pH 6.7 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Citrate Utilisation
1.	<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	positive reaction, turbidity
2.	<i>Enterobacter cloacae</i> ATCC 23355	50-100	luxuriant	positive reaction, turbidity
3.	<i>Escherichia coli</i> ATCC 25922	50-100	none-poor	negative reaction, no turbidity
4.	<i>Klebsiella pneumoniae</i> ATCC 13883	50-100	luxuriant	positive reaction, turbidity

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

Test Procedure

1. Transfer growth from a single colony or a loopful of liquid suspension and inoculate the broth medium.
2. Incubate at 35 ± 2°C for 18-24 hours.
3. Refer to appropriate references for standard test procedures.

Results

Refer to appropriate references and standard test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

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

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Koser Citrate Medium

Product Code : DM 128

Available Pack sizes : 100gm / 500gm

References

1. Alcamo I. E., 2001, Fundamentals of Microbiology, 6th Edition, Jones and Bartlett Publishers.
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5. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.
6. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
7. Wehr H. M. and Frank J. H., (Eds.), 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
8. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
9. Horwitz and Garcia (ed.). 2004 (update, 2007). Official methods of analysis of AOAC International, 18th ed. AOAC International, Gaithersburg, Md.

Further Information

For further information please contact your local MICROMASTER Representative.



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