



PRODUCT SPECIFICATION SHEET

Christensen Citrate Sulphite Agar (DM1057)

Intended Use

Christensen Citrate Sulphite Agar (DM1057) is recommended for differentiation of enteric bacilli based on citrate utilization and hydrogen sulphide production.

Product Summary and Explanation

Christensen Citrate Sulphite Agar was described by Christensen⁽¹⁾ to verify citrate utilization and H₂S production to differentiate Coliforms and enteric pathogens. This medium is a modification of the Christensen Iron Agar.⁽¹⁾ This modification was described by Edwards and Ewing.⁽²⁾ Christensen reported that all members of genera *Escherichia*, *Enterobacter*, *Citrobacter* and *Salmonella* as well as *Alkalescens-Dispar* were capable of utilizing citrate as a source of energy while *Shigella* species failed to utilize citrate.

Principles of the Procedure

Christensen Citrate Sulphite Agar contains yeast extract which provides essentially nitrogenous compounds and vitamins for growth of organisms. L-Cysteine hydrochloride is a reducing agent. Dextrose is the fermentable carbohydrate. Sodium citrate is the energy source for citrate utilizing organisms. The reduction of ferric ammonium citrate to iron sulphide by H₂S producing organisms is indicated by blackening of the medium. Sodium thiosulphate enhances H₂S production. Organisms that metabolize citrate as a sole source of carbon with the help of citritase enzyme cleave citrate to oxaloacetate and acetate. Oxaloacetate is then converted to pyruvate and CO₂ via another enzyme, oxaloacetate decarboxylase. Further, this CO₂ combines with sodium and water to form sodium carbonate, an alkaline compound.⁽³⁾ As a result, the pH of medium rises and the indicator, phenol red changes from orange red to cerise. Presence of the cerise colour indicates a positive finding for citrate utilization.

Formula / Liter

| Ingredients | Gms / Liter |
|--|-------------|
| Sodium citrate | 3.00 |
| Dextrose | 0.20 |
| Yeast extract | 0.50 |
| L-Cysteine hydrochloride | 0.10 |
| Ferric ammonium citrate | 0.40 |
| Potassium phosphate | 1.00 |
| Sodium chloride | 5.00 |
| Sodium thiosulphate | 0.08 |
| Phenol red | 0.012 |
| Agar | 14.00 |
| 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.
3. Care should be taken while inoculating, as, a too heavy inoculum may give a false positive result.⁽⁴⁾

Directions

1. Suspend 24.29 grams of the medium in one litre distilled water.
2. Heat to boiling to dissolve the medium completely. Dispense into test tubes.
3. Autoclave at 118 to 121°C, 12 to 15 lbs pressure for 15 minutes.
4. Cool the tubes in slanted position to give slants with generous butts.





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Quality Control Specifications

| | |
|----------------------------|--|
| Dehydrated Appearance | Light yellow to light pink homogeneous free flowing powder |
| Prepared Medium | Orange red coloured, very slightly opalescent gel forms in tubes as slants |
| Reaction of 2.42% Solution | pH : 6.7 ± 0.2 at 25°C |
| Gel Strength | Firm, comparable with 1.4% Agar gel |

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 | H ₂ S |
| 1. | <i>Enterobacter aerogenes</i> ATCC 13048 | 50-100 | good-luxuriant | positive reaction, cherry colour | negative reaction, no colour change |
| 2. | <i>Escherichia coli</i> ATCC 25922 | 50-100 | good-luxuriant | negative reaction, no colour change | negative reaction, no colour change |
| 3. | <i>Salmonella Typhimurium</i> ATCC 14028 | 50-100 | good-luxuriant | positive reaction, cherry colour | positive reaction, blackening of medium |
| 4. | <i>Salmonella Enteritidis</i> ATCC 13076 | 50-100 | good-luxuriant | positive reaction, cherry colour | positive reaction, blackening of medium |
| 5. | <i>Klebsiella pneumoniae</i> ATCC 13883 | 50-100 | good-luxuriant | weakly positive, orange-pink colour | negative reaction, no colour change |
| 6. | <i>Shigella flexneri</i> ATCC 12022 | 50-100 | good-luxuriant | negative reaction, no colour change | negative reaction, no colour change |
| 7. | <i>Shigella sonnei</i> ATCC 25931 | 50-100 | good-luxuriant | negative reaction, no colour change | negative reaction, no colour change |

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

Test Procedure

Refer to appropriate references for standard test procedures.

Results

Refer to appropriate references 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. Strong positive cultures upon prolonged incubation turn the entire butt black.
2. Some members of *Salmonella* like *Salmonella* Typhi are weakly positive and require 2-5 days for hydrogen sulphide production.





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

Packaging

Product Name : Christensen Citrate Sulphite Agar

Product Code : DM1057

Available Pack sizes : 500gm

References

1. Christensen W.B., 1949, Research Bull., Weld County Health Dept., Greeley Co., 1:3.
2. Edwards P.R. and Ewing W. H., 1955 and 1962, Identification of Enterobacteriaceae Minneapolis, Burgess Publishing Co., pg. 179 and 242.
3. Horward B., 1994, Clinical and Pathogenic Microbiology, 2nd ed., Mosby Year Book, Inc.
4. Branson D., 1972, Methods in Clinical Bacteriology, Springfield, III: C. Thomas, 15.

Further Information

For further information please contact your local MICROMASTER Representative.



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DM1057PSS,QAD/FR/024,Rev.00/01.01.2018

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