IFU

Instructions for Use

SIMMONS CITRATE AGAR

Cat. no. L80	Simmons Citrate Agar, 16x100mm Tube, 5.5ml Slant	20 tubes/box
Cat. no. J71	Urine Quad Plate (Eosin Methylene Blue (EMB) Agar, Levine / Xylose Lysine Deoxycholate (XLD) Agar, Modified / Simmons Citrate / Tryptic Soy Agar (TSA) Blood, 5%, 15x100mm Quadplate, 5ml/section	10 plates/bag

INTENDED USE

Hardy Diagnostics Simmons Citrate Agar is recommended for use in the differentiation of gram-negative enteric bacilli based on citrate utilization.

SUMMARY

In the early 1920s, Koser developed a liquid medium formulation for the differentiation of fecal coliforms from the coliform group.[5] Simmons later modified this formulation to produce a solid medium that eliminated potential errors when interpreting growth.[9]

Simmons Citrate Agar contains ammonium dihydrogen phosphate, which supplies the only source of nitrogen, and sodium citrate, which serves as the sole source of carbon. Organisms capable of utilizing ammonium dihydrogen phosphate and citrate will grow unrestricted on this medium. Bromothymol blue acts as a pH indicator, causing the medium to change from green (neutral) to blue (alkaline) with increasing pH. Citrate utilization produces an alkaline carbonate, resulting in a deep blue color change within the agar. The medium will remain green if organisms are not able to metabolize sodium citrate.

FORMULA

Ingredients per liter of deionized water:*

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

Final pH 6.9 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. Products should not be used if there are any signs of contamination, deterioration, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[2-4,6,7] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium and refrigerated until inoculation.

Method of use: This medium is not intended to be used for primary isolation. Using a light inoculum, inoculate medium with growth from a pure culture. Inoculate the slant by streaking the surface in a serpentine manner. Replace caps loosely on the tube(s). Incubate aerobically for 24-96 hours at 35 degrees C. Examine daily for growth and color change.

INTERPRETATION OF RESULTS

A positive reaction is indicated by growth with development of a deep blue color reaction within the medium. A negative reaction is evidenced by no growth or growth with the medium remaining green in color.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Failure to incubate tubes with loose caps may result in false-negative results.

Use a light inoculum to streak the slant; a heavy inoculum may result in false-positive results.

When inoculating multiple biochemicals from the same culture, inoculate this medium first, or flame inoculating needle prior to streaking this medium. Carryover of glucose or other nutrients onto this medium may result in false-positive results.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Salmonella enterica ATCC® 14028	E	24-48hr	35°C	Aerobic	Growth; deep blue color in medium
Klebsiella pneumoniae ATCC® 13883	E	24-48hr	35°C	Aerobic	Growth; deep blue color in medium
Escherichia coli ATCC® 25922	E	24-48hr	35°C	Aerobic	Inhibited; no color change to blue

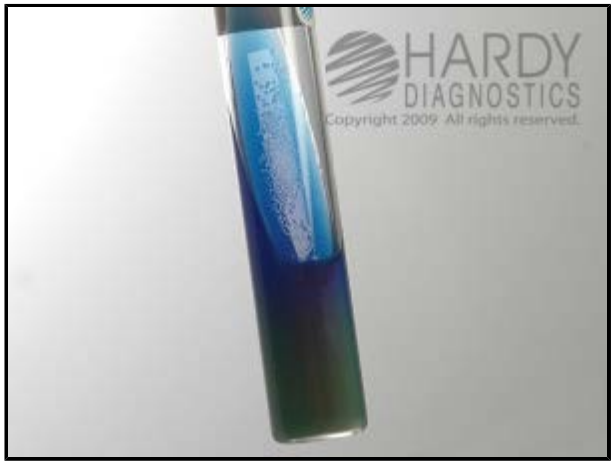
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Simmons Citrate Agar should appear slightly opalescent with possible slight precipitate, and dark green in color.



Salmonella enterica (ATCC® 14028) colonies growing on Simmons Citrate Agar (Cat. no. L80). The blue color change is indicative of citrate utilization. Incubated aerobically for 24 hours at 35ºC.

Escherichia coli (ATCC® 25922) growth inhibited on Simmons Citrate Agar (Cat. no. L80). Incubated aerobically for 24 hours at 35ºC.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

4. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

5. Koser, S.A. 1923. Utilization of the salts of organic acids by the colon-aerogenes group. *J. Bacteriol.*, 8:493.

6. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

7. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

8. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

9. Simmons, J.S. 1926. A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. *J. Infect. Dis.*, 39:209.

ATCC is a registered trademark of the American Type Culture Collection.

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