

Instructions for Use

XLD AGAR

Cat. no. J71	Urine Quad Plate (EMB/Modified XLD/Simmons Citrate/Blood Agar, 5%), 15x100mm Plate, 20ml	10 plates/bag
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INTENDED USE

Hardy Diagnostics Modified XLD Agar is recommended for use as a selective and differential medium for the isolation of gram-negative enteric pathogens from specimens.

SUMMARY

Xylose Lysine Deoxycholate (XLD) Agar was developed by Taylor for the differentiation, isolation, and identification of enteric pathogens, and to support the growth of more fastidious enteric organisms.⁽⁶⁾ XLD Agar was especially designed to allow the growth of *Shigella* species, and is a proven medium for the isolation of this organism. It has also been found to be an excellent medium for isolating *Salmonella* species as well.

The selective agent in XLD Agar is sodium deoxycholate, which inhibits the growth of gram-positive organisms. The carbohydrate source is xylose which is fermented by most enterics except for *Shigella* species, and these colonies appear red on this medium as a result. A second differential mechanism for *Salmonella* is employed by the addition of lysine. Lysine decarboxylation reverts the pH of the medium to an alkaline condition. To avoid this reversal to a *Shigella* reaction, lactose and sucrose are added in excess. The addition of sodium thiosulfate and ferric ammonium citrate as a sulfur source and indicator, respectively, allows hydrogen sulfide forming organisms to produce colonies with black centers, under alkaline conditions. Organisms which ferment xylose, are lysine decarboxylase-negative, and do not ferment lactose or sucrose cause an acid pH in the medium, and form yellow colonies. Examples of such organisms are *Citrobacter* spp., *Proteus* spp., and *Escherichia coli*.

Modified XLD Agar contains a reduced amount of sodium deoxycholate in order to permit the growth of a wider variety of enteric organisms normally inhibited on traditional XLD Agar.

FORMULA

Ingredients per liter of deionized water:*

Lactose	7.5gm
Sucrose	7.5gm
Sodium Thiosulfate	6.8gm
L-Lysine	5.0gm
Sodium Chloride	5.0gm
Xylose	3.75gm

Yeast Extract	3.0gm
Sodium Deoxycholate	0.5gm
Ferric Ammonium Citrate	0.8gm
Phenol Red	0.08gm
Agar	15.0gm

Final pH 7.4 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, 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 blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory 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.^(1-3,5) 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 media and refrigerated until inoculation.

Method of Use: Allow the plates to warm to room temperature, and the agar surface to dry before inoculating. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically at 35-37°C. for 18-24 hours. Examine colonial morphology, characteristics, and hemolytic reactions.

It is recommended that selective enrichment broths, such as GN Broth or Selenite Cystine Broth (Cat. no. K39, or K69, respectively), be used in conjunction with other selective plating medias, such as HE Agar (Cat. no. G63). This recommendation is made in order to maximize the recovery of enteric pathogens.

INTERPRETATION OF RESULTS

Salmonella spp. appear as red colonies with black centers. Lysine-positive organisms appear red. *Shigella* spp. also appear red. Other lysine-negative fermenters, such as *E. coli*, *Citrobacter* and *Proteus* spp. appear yellow. Consult listed references for the identification of colony morphology and further biochemical tests required for identification.^(1-3,5)

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.

Some species of *Salmonella* may form red colonies without a black center, which resemble *Shigella* colonies. In addition, a few species of *Shigella* ferment lactose, and *Salmonella* that fail to decarboxylate lysine would not be detected on this medium.

Processing delays of over 2-3 hours of unpreserved stool specimens greatly jeopardizes the recovery of many enteric pathogens, as these organisms are very susceptible to the acidic changes that occur with a temperature drop of the feces.

Red, false-positive colonies may occur with *Proteus* and *Pseudomonas*.

Incubation in excess of 48 hours may lead to 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, slides, staining supplies, other culture media, microscopes, 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	
<i>Salmonella enterica</i> ATCC® 14028	A	24hr	35°C	Aerobic	Growth; red colonies with black centers
<i>Shigella flexneri</i> ATCC® 12022	A	24hr	35°C	Aerobic	Growth; red to pink colonies
<i>Escherichia coli</i> ATCC® 25922	B	24hr	35°C	Aerobic	Growth; yellow to yellow red colonies
<i>Enterococcus faecalis</i> ATCC® 29212	B	24hr	35°C	Aerobic	Partial to complete inhibition; clear, pinpoint colonies

* 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

Modified XLD Agar should appear a clear, and red in color, and may have a slight precipitate.

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. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.
5. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.
6. Taylor, E.I. 1965. *Am. J. Clin. Path.* ; 44:471.
7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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

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