BD BBL™ XLD Agar

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QUALITY CONTROL PROCEDURES

I. INTRODUCTION

XLD Agar is a moderately selective and differential medium for the isolation, cultivation and differentiation of gram-negative enteric microorganisms from both clinical and non-clinical specimens.

II. PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Streak the plates for isolation. Use cultures diluted to yield 10^3–10^5 CFU/plate.
   b. Incubate the plates at 35 ± 2°C in an aerobic atmosphere.
   c. Include **Trypticase™** Soy Agar with 5% Sheep Blood plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for growth, reactions and selectivity.
3. Expected Results

CLSI Organisms	ATCC™	Recovery	Colony Color
**Salmonella choleraesuis* subsp. *choleraesuis* serotype Typhimurium	14028	Growth	Red with black centers
**Shigella flexneri*	12022	Growth	Red
**Enterococcus faecalis*	29212	Inhibition (partial)	
**Escherichia coli*	25922	Inhibition (partial to complete)	Yellow to yellow-red

*Recommended organism strain for User Quality Control.

III. ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.4 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 30 ± 1°C for 60 h and examine for microbial contamination.

PRODUCT INFORMATION

IV. INTENDED USE

XLD Agar is the complete Xylose Lysine Desoxycholate Agar recommended for isolation and differentiation of enteric pathogens, especially *Shigella* species.

V. SUMMARY AND EXPLANATION

A wide variety of media have been developed to aid in the selective isolation and differentiation of enteric pathogens. Due to the large number of different microbial species and strains with varying nutritional requirements and chemical resistance patterns, investigators have developed various formulae to meet general as well as specific needs relative to isolation and identification of these microorganisms.

XLD Agar was developed by Taylor in order to increase the efficiency of the isolation and identification of enteric pathogens, particularly *Shigella*.[1] The pathogens are differentiated not only from the nonpathogenic lactose fermenters but also from many nonpathogens which do not ferment lactose or sucrose. Additionally, the medium was formulated to increase the frequency of growth of the more fastidious pathogens,[1] which in other formulations often failed to grow due to the inclusion of excessively toxic inhibitors. The results obtained in a number of clinical evaluations have supported the claim for the relatively high efficiency of XLD Agar in the primary isolation of *Shigella* and *Salmonella*.[2-6]

VI. PRINCIPLES OF THE PROCEDURE

XLD Agar is both a selective and differential medium. It utilizes sodium desoxycholate as the selective agent and, therefore, it is inhibitory to gram-positive microorganisms. Xylose is incorporated into the medium since it is fermented by practically all enterics except for the shigellae and this property enables the differentiation of *Shigella* species. Lysine is included to enable the *Salmonella* group to be differentiated from the nonpathogens since without lysine, salmonellae rapidly would ferment the xylose and be indistinguishable from nonpathogenic species. After the salmonellae exhaust the supply of xylose, the lysine is attacked via the enzyme, lysine decarboxylase, with reversion to an alkaline pH which mimics the *Shigella* reaction. To prevent similar reversion by lysine positive coliforms, lactose and saccharose (sucrose) were added to produce acid in excess.[1]

To add to the differentiating ability of the formulation, an H_2S indicator system, consisting of sodium thiosulfate and ferric ammonium citrate, is included for the visualization of the hydrogen sulfide produced, resulting in the formation of colonies with black centers. The nonpathogenic H_2S-producers do not decarboxylate lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies.[1]

VII. REAGENTS

XLD Agar

Approximate Formula* Per Liter Purified Water

Xylose	3.5 g
L-Lysine	5.0 g
Lactose	7.5 g
Saccharose	7.5 g
Sodium Chloride	5.0 g
Yeast Extract	3.0 g
Phenol Red	0.08 g
Sodium Desoxycholate	2.5 g
Sodium Thiosulfate	6.8 g
Ferric Ammonium Citrate	0.8 g
Agar	13.5 g

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

Warnings and Precautions:

For *in vitro* Diagnostic Use in Taiwan

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[7-10] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

VIII. SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[11,12] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX. PROCEDURE

Material Provided: XLD Agar

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate plates, protected from light, at 35 ± 2°C for 18–24 h in an aerobic atmosphere. Colonies on XLD Agar may require 48 h incubation for full color development.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X. RESULTS

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Typical colonial morphology on XLD Agar is as follows:

E. coli	Large, flat, yellow. Some strains may be inhibited.
Enterobacter/Klebsiella	Mucoid, yellow
Proteus	Red to yellow. Most strains have black centers.
Salmonella.....	Red-yellow with black centers
Shigella, H2S-negative *Salmonella*.....	Red
Pseudomonas	Red
Gram-positive bacteria.....	No growth to slight growth

XI. LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[11-16]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII. AVAILABILITY

Cat. No.	Description
251159	BD **BBL™** XLD Agar, Ctn. of 100 plates

XIII. REFERENCES

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XIV. FURTHER INFORMATION

For further information please contact your local BD representative.

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