

XLD AGAR

INTENDED USE

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

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 numbers 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 the 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 have 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

XLD Agar is a selective and differential medium used for the isolation and differentiation of enteric pathogens from clinical specimens.³ The value of XLD Agar in the clinical laboratory is that the medium is more supportive of fastidious enteric organisms such as *Shigella*.³ XLD Agar is also recommended for the testing of food, dairy products and water in various industrial standard test methods.⁴

PRINCIPLE

Xylose is incorporated into the medium because it is fermented by practically all enterics except for the *shigellae*. This property enables the differentiation of *Shigella* species. Lysine is included to enable the *Salmonella* group to be differentiated from the nonpathogens. Without lysine, *Salmonella* rapidly would ferment the xylose and be indistinguishable from nonpathogenic species. After the *Salmonella* 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 sucrose (saccharose) are added to produce acid in excess.¹ Degradation of xylose, lactose and sucrose generates acid products, which in the presence of the pH indicator phenol red, causes a color change in the medium from red to yellow.

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 Sodium chloride maintains the osmotic balance. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Agar is the solidifying agent.

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.

REAGENTS (FORMULA)

L-Lysine5.0gLactose7.5gSaccharose7.5gSodium Chloride5.0gYeast Extract3.0gPhenol Red0.08gSodium Desoxycholate2.5gFerric Ammonium Citrate0.8gSodium Thiosulfate6.8gAgar13.5gDeionized Water1000.0m	Xylose 3.5	g
Lactose7.5gSaccharose7.5gSodium Chloride5.0gYeast Extract3.0gPhenol Red0.08gSodium Desoxycholate2.5gFerric Ammonium Citrate0.8gSodium Thiosulfate6.8gAgar13.5gDeionized Water1000.0m	L-Lysine 5.0	g
Saccharose7.5gSodium Chloride5.0gYeast Extract3.0gPhenol Red0.08gSodium Desoxycholate2.5gFerric Ammonium Citrate0.8gSodium Thiosulfate6.8gAgar13.5gDeionized Water1000.0m	Lactose 7.5	g
Sodium Chloride5.0gYeast Extract3.0gPhenol Red0.08gSodium Desoxycholate2.5gFerric Ammonium Citrate0.8gSodium Thiosulfate6.8gAgar13.5gDeionized Water1000.0m	Saccharose7.5	g
Yeast Extract3.0gPhenol Red0.08gSodium Desoxycholate2.5gFerric Ammonium Citrate0.8gSodium Thiosulfate6.8gAgar13.5gDeionized Water1000.0m	Sodium Chloride 5.0	g
Phenol Red0.08gSodium Desoxycholate2.5gFerric Ammonium Citrate0.8gSodium Thiosulfate6.8gAgar13.5gDeionized Water1000.0m	Yeast Extract 3.0	g
Sodium Desoxycholate2.5gFerric Ammonium Citrate0.8gSodium Thiosulfate6.8gAgar13.5gDeionized Water1000.0m	Phenol Red 0.08	g
Ferric Ammonium Citrate0.8gSodium Thiosulfate6.8gAgar13.5gDeionized Water1000.0m	Sodium Desoxycholate 2.5	g
Sodium Thiosulfate6.8gAgar13.5gDeionized Water1000.0m	Ferric Ammonium Citrate 0.8	g
Agar	Sodium Thiosulfate 6.8	g
Deionized Water 1000.0 m	Agar 13.5	g
	Deionized Water 1000.0	ml

PROCEDURE

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using XLD Agar.³

For food, dairy and water samples, refer to appropriate standard references for details on test methods using XLD Agar.⁴

For pharmaceutical samples, refer to USP General Chapter <62> for details on the examination of nonsterile products and the isolation of *Salmonella* using XLD Agar.⁵

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. Incubate plates, protected from light, at $35 \pm 2^{\circ}$ C for 18-24 hours. Colonies on XLD agar may require 48 hours incubation for full color development.

EXPECTED RESULTS

Degradation of xylose, lactose and sucrose generates acid products, causing a color change in the medium from red to yellow.

Hydrogen sulfide production under alkaline conditions causes colonies to develop black centers. This reaction is inhibited by the acid conditions that accompany carbohydrate fermentation.

Lysine decarboxylation in the absence of lactose and sucrose fermentation causes reversion to an alkaline condition and the color of the medium changes back to red.

Typical colonial morphology and reactions on XLD Agar are 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*, *Salmonella* H2S-negative: Red *Pseudomonas*: Red Gram-positive bacteria: No growth to slight growth

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Escherichia coli ATCC 25922	$35 \pm 2^{\circ}$ C for 18-24 hours	Growth, Yellow
Shigella flexneri ATCC 12022	$35 \pm 2^{\circ}$ C for 18-24 hours	Growth, Red

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