XL Agar Base • XLD Agar

Intended Use

XL (Xylose Lysine) Agar Base is used for the isolation and differentiation of enteric pathogens and, when supplemented with appropriate additives, as a base for selective enteric media.

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

XLD Agar meets United States Pharmacopeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP)¹⁻³ performance specifications, where applicable.

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.

XL Agar Base was developed by Taylor⁴ for the nonselective isolation and differentiation of gram-negative enteric bacilli. It is particularly recommended for obtaining counts of enteric organisms. This medium can be rendered moderately selective for enteric pathogens, particularly *Shigella*, by the addition of sodium desoxycholate (2.5 g/L) to make XLD Agar.⁴

XL Agar Base can be made selective for *Salmonella* by adding 1.25 mL/L of 1% aqueous brilliant green to the base prior to autoclaving. Its use is recommended for *Salmonella* isolation after selenite or tetrathionate enrichment in food analysis; both coliforms and *Shigella* are inhibited.⁴

XLD Agar was developed by Taylor in order to increase the efficiency of the isolation and identification of enteric pathogens, particularly *Shigella*.⁴ 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,⁴ 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*.⁵⁻⁹

XLD Agar is a selective and differential medium used for the isolation and differentiation of enteric pathogens from clinical specimens.¹⁰⁻¹² The value of XLD Agar in the clinical laboratory is that the medium is more supportive of fastidious enteric organisms such as *Shigella*.¹² XLD Agar is also recommended for the testing of food, dairy products and water in various industrial standard test methods.¹³⁻¹⁷ General Chapter <62> of the *USP* describes the test method for the isolation of *Salmonella* from nonsterile pharmaceutical products using XLD Agar as the solid culture medium.¹

Principles of the Procedure

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, 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 sucrose (saccharose) are added to produce acid in excess.⁴ 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.



User Quality Control NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both Difco[™] and BBL[™] brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications Difco™ XID Agar		
Dehydrated Appearance:	Pink, free-flowing, homogeneous.	
Solution:	5.5% solution, soluble in purified water upon boiling. Solution is red, very slightly to slightly opalescent.	
Prepared Appearance:	Red, slightly opalescent.	
Reaction of 5.5% Solution at 25°C:	pH 7.4 ± 0.2	

Cultural Response Difco[™] XLD Agar

Prepare the medium per label directions. Inoculate and incubate at $35 \pm 2^{\circ}$ C for 18-24 hours. Incubate (*) cultures at 30-35°C for 18-48 hours and (**) culture at 35-37°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUN CFU	1 RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	~103	Partial inhibition	_
Escherichia coli	25922	~103	Partial inhibition	Yellow
Providencia alcalifaciens	9886	100-300	Good	Red
Shigella flexneri	12022	100-300	Good	Red
Escherichia coli*	8739	>100 c	Partial to complete inhibitio (30-35°C)	Yellow n
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium*	14028	<100	Growth (30-35°C)	Red with black centers
Salmonella enterica subsp. enterica serotype Typhimurium**	14028	<100	Growth (35-37°C)	Red with black centers

<i>Identity Specification</i> BBL [™] XL Agar Base	s
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is dark medium to dark, red to rose-red, clear to slightly hazy.
Prepared Appearance:	Dark medium to dark, red to rose-red, clear to slightly hazy.
Reaction of 4.5%	
Solution at 25°C:	pH 7.5 ± 0.2
BBL [™] XLD Agar (prepa	red)
Appearance:	Medium orange red to red, trace hazy.
D 1 1 250C	

Reaction at 25°C: pH 7.4 \pm 0.2

Cultural Response BBL[™] XL Agar Base

Prepare the medium per label directions. Inoculate and incubate at $35 \pm 2^{\circ}$ C for 18-24 hours (up to 48 hours if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVER	COLONY Y COLOR
Escherichia coli	25922	10 ³ -10 ⁴	Good	Yellow
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ³ -10 ⁴	Good	Red to yellow with black centers to predominantly black
Shigella flexneri	12022	10 ³ -10 ⁴	Good	Red

BBL[™] XLD Agar (prepared)

Inoculate and incubate at $35 \pm 2^{\circ}$ C for 24 hours. Incubate (*) cultures at $30-35^{\circ}$ C for 18-48 hours and (**) culture at $35-37^{\circ}$ C for 18-48 hours.

ORGANISM	ATCC™	INOCULUN CFU	1 RECOVERY	COLONY COLOR
Enterococcus				
faecalis	29212	10 ⁴ - 10 ⁵	Partial inhibition	-
Escherichia coli	25922	10 ⁴ - 10 ⁵	Partial to complete inhibition	Yellow to yellow-red
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ³ -10 ⁴	Good	Red with black centers
Shigella flexneri	12022	10 ³ -10 ⁴	Good	Red
Escherichia coli*	8739	10 ² -10 ³	Partial to complete inhibition (30-35°C)	Yellow to red
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium*	14028	<100	Growth (30-35°C)	Red with black centers
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium**	14028	<100	Growth (35-37°C)	Red with black centers





To add to the differentiating ability of the formulation, an H₂S 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 H₂S producers do not decarboxylate lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies.⁴ 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.

Formulae

BBL[™] XL Agar Base

Approximate Formula* Per Liter		
Xylose	3.5	g
L-Lysine	5.0	g
Lactose	7.5	g
Sucrose	7.5	g
Sodium Chloride	5.0	g
Yeast Extract	3.0	g
Phenol Red	80.0	g
Agar 1	3.5	a

Difco[™] XLD Agar

Approximate Formula* Per Liter

Xylose		g
L-Lysine	5.0	g
Lactose	7.5	g
Saccharose	7.5	g
Sodium Chloride	5.0	g
Yeast Extract		g
Phenol Red		g
Sodium Desoxycholate	2.5	g
Ferric Ammonium Citrate		g
Sodium Thiosulfate	6.8	g
Agar	13.5	ģ
*Adjusted and/or supplemented as required to meet performance criteria		

Directions for Preparation from Dehydrated Product BBL[™] XL Agar Base

- 1. Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
- 2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Add brilliant green, if desired.
- 3. Autoclave at 118°C for 10 minutes. Cool to 55-60°C.
- 4. Add 20 mL of an aqueous solution containing 34% sodium thiosulfate and 4% ferric ammonium citrate. For XLD agar, add 25 mL of 10% aqueous sodium desoxycholate. Pour into plates.
- 5. Test samples of the finished product for performance using stable, typical control cultures.

Difco[™] XLD Agar

- 1. Suspend 55 g of the powder in 1 L of purified water. Mix thoroughly.
- 2. Heat with agitation just until the medium boils. DO NOT OVERHEAT. DO NOT AUTOCLAVE.



- 3. Cool to 45-50°C in a water bath and use immediately. Overheating causes precipitation.
- 4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.10-12

For food, dairy or water samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.¹³⁻¹⁷

For pharmaceutical samples, refer to the USP for details on sample collection and preparation for testing of nonsterile products.¹

Procedure

For clinical specimens, refer to appropriate standard references for details on testing protocol to obtain isolated colonies from specimens using XLD Agar.¹⁰⁻¹²

For food, dairy and water samples, refer to appropriate standard references for details on test methods using XLD Agar.¹³⁻¹⁷

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.¹

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 H ₂ S-negative	Red
Pseudomonas	Red
Gram-positive bacteria	No growth to slight
	growth

Limitations of the Procedure

- 1. Red, false-positive colonies may occur with some Proteus and Pseudomonas species.
- 2. Incubation in excess of 48 hours may lead to false-positive results.
- 3. S. Paratyphi A, S. Choleraesuis, S. pullorum and S. gallinarum may form red colonies without black centers, thus resembling Shigella species.
- 4. Some Proteus strains will give black-centered colonies on XLD Agar.

References

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Availability

BBL[™] XL Agar Base

SMWW

Cat. No. 211836 Dehydrated - 500 g

Difco[™] XLD Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP ISO JP MCM9 SMD SMWW USP

Cat. No.	278850	Dehydrated – 500 g ⁺
	278820	Dehydrated – 2 kg ⁺
	278830	Dehydrated – 10 kg ⁺

BBL[™] XLD Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP ISO JP MCM9 SMD SMWW USP

United Sta	tes and Ca	nada
Cat. No.	221192	Prepared Plates – Pkg. of 20* [†]
Europe	221204	
Cat. No.	254055 254090	Prepared Plates – Pkg. of 20* Prepared Plates – Ctn. of 120*
<i>Japan</i> Cat. No.	252020 251159	Prepared Plates – Pkg. of 20* Prepared Plates – Ctn. of 100*

BBL[™] XLD Agar//Hektoen Enteric Agar

Cat. No. 295646 Prepared I Plate[™] Dishes – Pkg. of 20* *Store at 2-8°C

tQC testing performed according to USP/EP/JP performance specifications.