

References

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2. National Committee for Clinical Laboratory Standards. 1993. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A3. NCCLS, Villanova, Pa.
3. Wexler and Doern. 1995. In Murray, Baron, Pfaller, Tenover and Tenover (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
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Availability

Difco™ Wilkins-Chalgren Agar

NCCLS

Cat. No. 218051 Dehydrated – 500 g

Difco™ Anaerobe Broth MIC

NCCLS

Cat. No. 218151 Dehydrated – 500 g

XL Agar Base • XLD Agar

Intended Use

XLD Agar conforms with specifications of *The United States Pharmacopeia (USP)*.

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.

Summary and Explanation

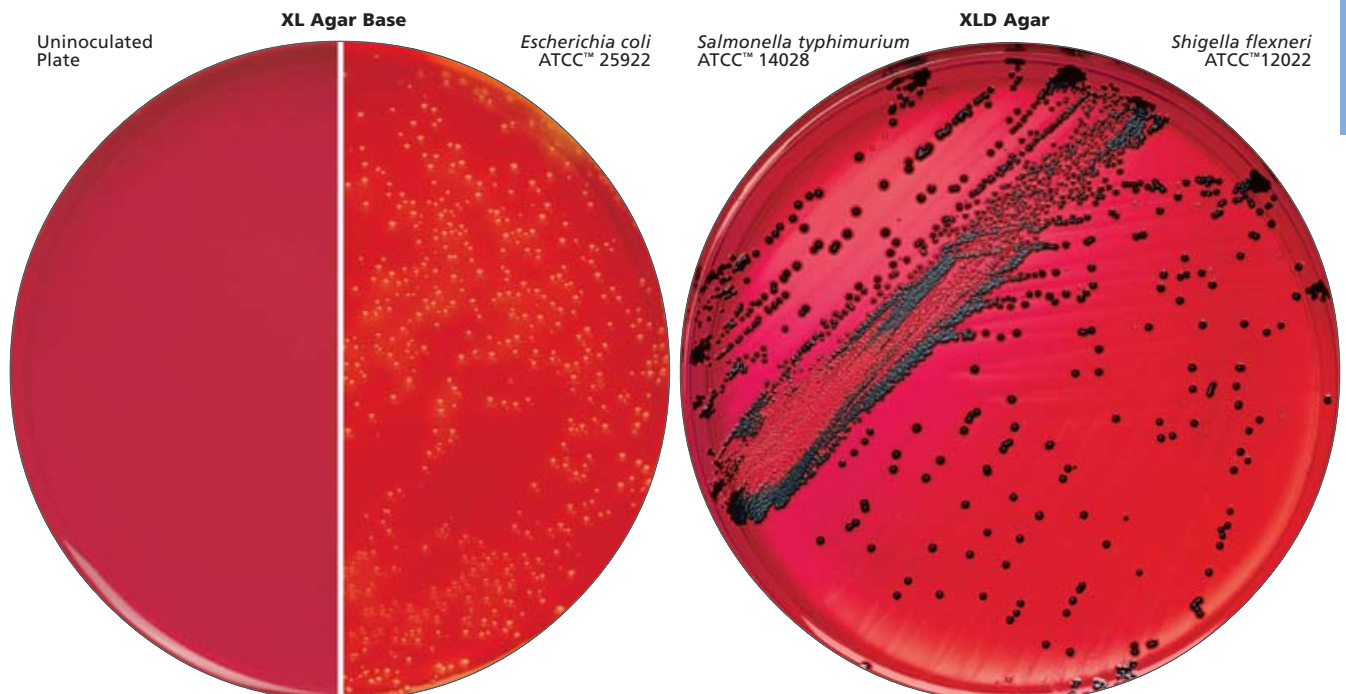
A wide variety of media have been developed to aid in the selective isolation and differentiation of enteric pathogens. Due to the large numbers of different microbial species and strains with varying nutritional requirements and chemical resistance patterns, investigators have developed various formulae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

XL Agar Base was developed by Taylor¹ 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.¹

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.¹

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



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™ XLD Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	5.7% solution, soluble in purified water upon boiling. Solution is red, very slightly to slightly opalescent.
Prepared Appearance:	Red, slightly opalescent.
Reaction of 5.7% 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 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Enterococcus faecalis</i>	29212	10 ² -2 × 10 ³	Partial inhibition	–
<i>Escherichia coli</i>	25922	10 ³ -2 × 10 ³	Partial inhibition	Yellow with or without bile precipitate
<i>Providencia alcalifaciens</i>	9886	10 ² -10 ³	Good	Red
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	10 ² -10 ³	Good	Red with black centers
<i>Shigella flexneri</i>	12022	10 ² -10 ³	Good	Red

Continued

primary isolation of *Shigella* and *Salmonella*.²⁻⁶ XLD Agar is included in the *USP* microbial limit test for screening specimens for the presence or absence of *Salmonella*⁷ and is recommended for the testing of foods, dairy products and water.⁷⁻¹²

Principles of the Procedure

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 sucrose (saccharose) were added to produce acid in excess.¹

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.¹

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	0.08	g
Agar	13.5	g

Difco™ XLD Agar

Approximate Formula* Per Liter

Xylose	3.75	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	15.0	g

BBL™ XLD Agar

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	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.

Identity Specifications**BBL™ XL Agar Base**

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

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.5% solution, soluble in purified water upon boiling. Solution is medium to dark, orange-red to rose-red, clear to slightly hazy.
Prepared Appearance:	Medium to dark, orange-red to rose-red, clear to slightly hazy.
Reaction of 5.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response**BBL™ XL Agar Base**

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (up to 48 hours if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good	Yellow without precipitate
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	10 ³ -10 ⁴	Good	Red to yellow without precipitate, black centers to predominately black
<i>Shigella flexneri</i>	12022	10 ³ -10 ⁴	Good	Red

BBL™ XLD Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Enterococcus faecalis</i>	29212	10 ⁴ -10 ⁵	Partial to complete inhibition	–
<i>Escherichia coli</i>	25922	10 ⁴ -10 ⁵	Partial to complete inhibition	If growth, yellow with or without bile precipitate
<i>Klebsiella pneumoniae</i>	33495	10 ³ -10 ⁴	Good	Yellow with or without bile precipitate
<i>Proteus vulgaris</i>	8427	10 ³ -10 ⁴	Good	Yellow with or without bile precipitate
<i>Pseudomonas aeruginosa</i>	10145	10 ³ -10 ⁴	Good	Red
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhi	19430	10 ³ -10 ⁴	Good	Red with black centers
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Paratyphi	9150	10 ³ -10 ⁴	Good	Red
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	10 ³ -10 ⁴	Good	Red with black centers
<i>Shigella boydii</i>	9207	10 ³ -10 ⁴	Good	Red
<i>Shigella flexneri</i>	12022	10 ³ -10 ⁴	Good	Red

Directions for Preparation from Dehydrated Product**BBL™ XL Agar Base**

- Suspend 45 g of the powder in 1 L of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Add brilliant green, if desired.
- Autoclave at 118°C for 10 minutes. Cool to 55-60°C.
- 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.

- Test samples of the finished product for performance using stable, typical control cultures.

Difco™ and BBL™ XLD Agar

- Suspend the powder in 1 L of purified water:
 - Difco™ XLD Agar** – 57 g;
 - BBL™ XLD Agar** – 55 g.
 Mix thoroughly.
- Heat with agitation just until the medium boils. DO NOT OVERHEAT. DO NOT AUTOCLAVE.
- Cool to 45-50°C in a water bath and use immediately. Overheating causes precipitation.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. 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 $35 \pm 2^\circ\text{C}$ 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:

<i>E. coli</i>	Large, flat, yellow; some strains may be inhibited
<i>Enterobacter/ Klebsiella</i>	Mucoid, yellow
<i>Proteus</i>	Red to yellow; most strains have black centers
<i>Salmonella</i>	Red-yellow with black centers
<i>Shigella, Salmonella</i>	
H ₂ S-negative	Red
<i>Pseudomonas</i>	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

1. Taylor. 1965. Am. J. Clin. Pathol. 44:471.
2. Taylor and Harris. 1965. Am. J. Clin. Pathol. 44:476.
3. Taylor and Harris. 1967. Am. J. Clin. Pathol. 48:350.
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11. Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
12. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.

Availability

BBL™ XL Agar Base

SMWW

Cat. No. 211836 Dehydrated – 500 g

Difco™ XLD Agar

AOAC BAM BS10 CMPH COMPF EP MCM7 SMD
SMWW USP

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

BBL™ XLD Agar

AOAC BAM BS10 CMPH COMPF EP MCM7 SMD
SMWW USP

Cat. No. 211838 Dehydrated – 500 g
212266 Dehydrated – 5 lb (2.3 kg)

United States and Canada

Cat. No. 221192 Prepared Plates – Pkg. of 20*
221284 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254055 Prepared Plates – Pkg. of 20*
254090 Prepared Plates – Ctn. of 120*

Japan

Cat. No. 251159 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.

XLT4 Agar Base • XLT4 Agar Supplement

Intended Use

XLT4 Agar Base is used with XLT4 Agar Supplement in isolating non-typhi *Salmonella*.

Summary and Explanation

Numerous media have been developed for isolating and differentiating enteric pathogens. The majority were designed to recover a broad spectrum of enteric pathogens.¹ Consequently, overgrowth of nuisance or contaminating organisms can be a major problem when recovery of a specific organism or species is desired. This is particularly true for *Salmonella*

isolation media where overgrowth of *Proteus*, *Providencia* and *Pseudomonas* can dramatically interfere with the detection and isolation of *Salmonella*.

In 1990, Miller and Tate described a new medium, XLT4 Agar, for isolating *Salmonella*.¹ The authors established the selectivity of XLT4 Agar using pure cultures of a variety of enteric organisms. They also evaluated its sensitivity in detecting and isolating *Salmonella* using fecal-contaminated farm samples containing high numbers of competing bacteria. In follow-up studies, Miller^{2,3} and Tate⁴ reported that XLT4 Agar significantly improved the