Formulae

Difco™ Wilkins-Chalgren Agar

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Peptone	10.0	g
Yeast Extract	5.0	g
Dextrose	1.0	g
Sodium Chloride	5.0	g
L-Arginine	1.0	g
Sodium Pyruvate	1.0	g
Hemin	5.0	mg
Vitamin K_1	0.5	mg
Agar	15.0	g

Difco™ Anaerobe Broth MIC

Consists of the same ingredients without the agar.

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Wilkins-Chalgren Agar – 48 g;
   Difco™ Anaerobe Broth MIC – 33 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion on susceptibility testing of anaerobic bacteria refer to appropriate procedures outlined in the references.[2-5]

Expected Results

Refer to appropriate references for acceptable ranges.

Limitation of the Procedure

Anaerobe Broth MIC is supplemented to a final concentration of 0.5 µg per mL of vitamin K_1 and 5.0 µg of hemin per mL. CLSI changed their recommendations to include use of broth with a final concentration of 1 µg of vitamin K_1 per mL.[2] To follow CLSI recommendations, the concentration of vitamin K_1 should be increased accordingly. A final concentration of 0.5 µg of vitamin K_1 per mL is sufficient, but some fastidious anaerobes may need a higher concentration of vitamin K_1.[5]

References

1. Wilkins and Chalgren. 1976. Antimicrob. Agents Chemother. *10*:926.
2. Clinical and Laboratory Standards Institute. 1993. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A3. CLSI, Wayne, Pa.
3. Clinical and Laboratory Standards Institute. 2001. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A5. CLSI, Wayne, Pa.
4. Wexler and Doern. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. Isenberg (ed.). 1995. Clinical microbiology procedures handbook, vol 1. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Wilkins-Chalgren Agar

CCAM

Cat. No. 218051 Dehydrated – 500 g

Difco™ Anaerobe Broth MIC

CCAM

Cat. No. 218151 Dehydrated – 500 g

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 Taylor[4] 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

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:	5.5% 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 ± 2°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™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	~10^3	Partial inhibition	–
Escherichia coli	25922	~10^3	Partial inhibition	Yellow
Providencia alcalifaciens	9886	100-300	Good	Red
Shigella flexneri	12022	100-300	Good	Red
*Escherichia coli**	8739	>100	Partial to complete inhibition (30-35°C)	Yellow
Salmonella enterica subsp. *enterica* 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

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 (prepared)

Appearance:	Medium orange red to red, trace hazy.
Reaction 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
Escherichia coli	25922	10^3-10^4	Good	Yellow
Salmonella enterica subsp. *enterica* 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 ± 2°C for 24 hours. Incubate (*) cultures at 30-35°C for 18-48 hours and (**) culture at 35-37°C for 18-48 hours.

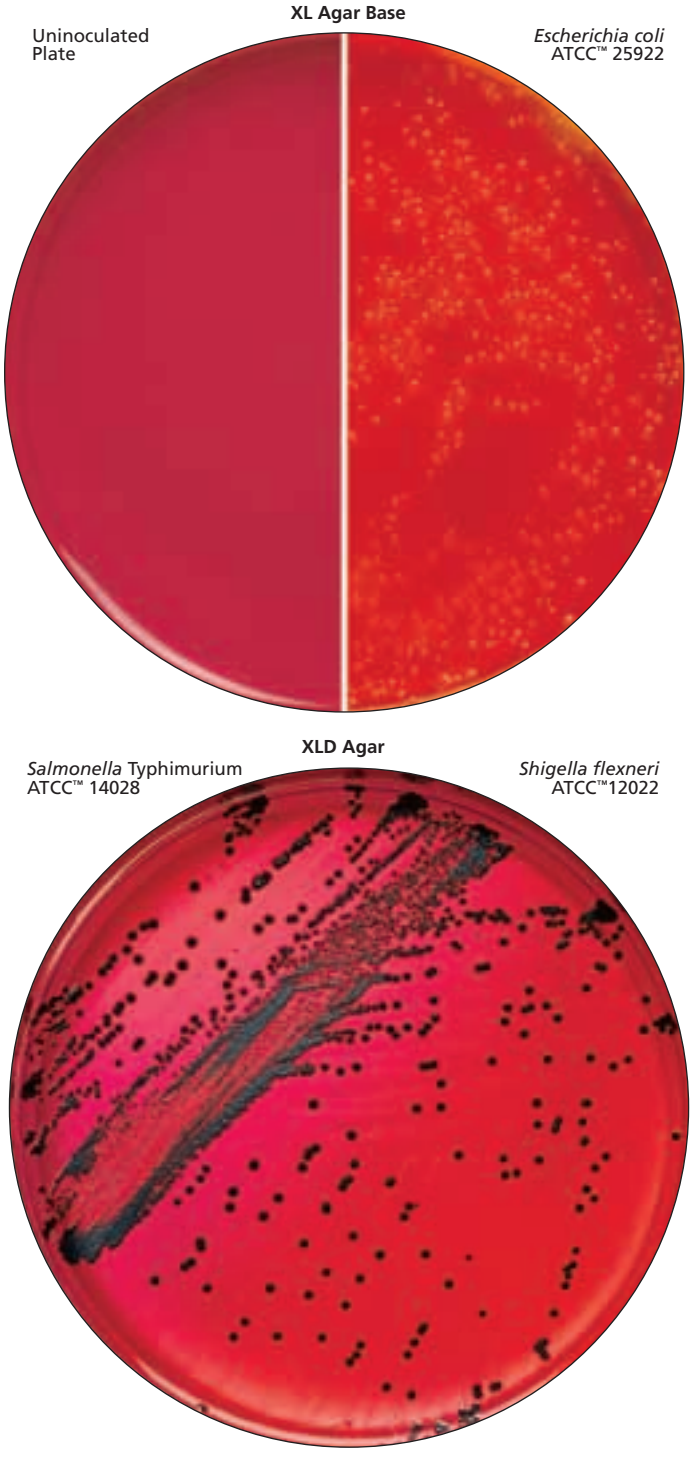
ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^4- 10^5	Partial inhibition	–
Escherichia coli	25922	10^4- 10^5	Partial to complete inhibition	Yellow to yellow-red
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Red with black centers
Shigella 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
Salmonella enterica subsp. *enterica* 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

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 non-pathogens. 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.

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.[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	0.08	g
Agar	13.5	g

Difco™ XLD Agar

Approximate Formula* Per Liter

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
Ferric Ammonium Citrate	0.8	g
Sodium Thiosulfate	6.8	g
Agar	13.5	g

**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 ± 2°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_2S-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

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Taylor. 1965. Am. J. Clin. Pathol. *44*:471.
5. Taylor and Harris. 1965. Am. J. Clin. Pathol. *44*:476.
6. Taylor and Harris. 1967. Am. J. Clin. Pathol. *48*:350.
7. Taylor and Schelhart. 1967. Am. J. Clin. Pathol. *48*:356.
8. Taylor and Schelhart. 1968. Appl. Microbiol. *16*:1387.
9. Pollock and Dahlgren. 1974. Appl. Microbiol. *27*:197.
10. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
11. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
12. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
13. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
14. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
15. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
16. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
17. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.

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 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. 252020 Prepared Plates – Pkg. of 20*
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.
†QC testing performed according to USP/EP/JP performance specifications.