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.

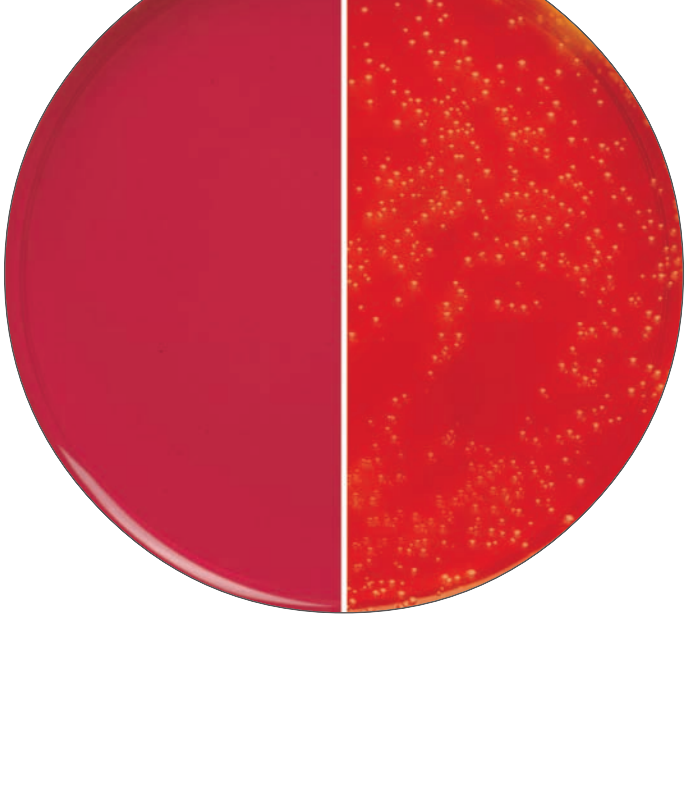
Summary and Explanation

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 Taylor[1] 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
Enterococcus faecalis	29212	10^3-2×10^3	Partial inhibition	–
Escherichia coli	25922	10^3-2×10^3	Partial inhibition	Yellow with or without bile precipitate
Providencia alcalifaciens	9886	10^2-10^3	Good	Red
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^2-10^3	Good	Red with black centers
Shigella flexneri	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 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]

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
Escherichia coli	25922	10^3-10^4	Good	Yellow without precipitate
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^3-10^4	Good	Red to yellow without precipitate, black centers to predominately black
Shigella flexneri	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
Enterococcus faecalis	29212	10^4-10^5	Partial to complete inhibition	–
Escherichia coli	25922	10^4-10^5	Partial to complete inhibition	If growth, yellow with or without bile precipitate
Klebsiella pneumoniae	33495	10^3-10^4	Good	Yellow with or without bile precipitate
Proteus vulgaris	8427	10^3-10^4	Good	Yellow with or without bile precipitate
Pseudomonas aeruginosa	10145	10^3-10^4	Good	Red
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhi	19430	10^3-10^4	Good	Red with black centers
Salmonella choleraesuis subsp. *choleraesuis* serotype Paratyphi	9150	10^3-10^4	Good	Red
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^3-10^4	Good	Red with black centers
Shigella boydii	9207	10^3-10^4	Good	Red
Shigella flexneri	12022	10^3-10^4	Good	Red

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™ and BBL™ XLD Agar

1. Suspend the powder in 1 L of purified water:
   Difco™ XLD Agar – 57 g;
   BBL™ XLD Agar – 55 g.
   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.

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 ± 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. 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.
4. Taylor and Schelhart. 1967. Am. J. Clin. Pathol. *48*:356.
5. Taylor and Schelhart. 1968. Appl. Microbiol. *16*:1387.
6. Pollock and Dahlgren. 1974. Appl. Microbiol. 27:197.
7. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
8. U.S. Food and Drug Adminsitration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
9. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, Md.
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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 Tate[4] reported that XLT4 Agar significantly improved the

User Quality Control

Identity Specifications

Difco™ XLT4 Agar Base

Dehydrated Appearance:	Pink, free flowing, homogeneous.
Solution:	5.9% solution, soluble upon boiling in purified water containing 4.6 mL/L of XLT4 Agar Supplement. Solution is red, slightly opalescent.
Prepared Appearance:	Reddish-orange, slightly opalescent.
Reaction of Final Medium at 25°C:	pH 7.4 ± 0.2

Difco™ XLT4 Agar Supplement

Appearance:	Colorless to slightly yellow, clear, slightly viscous solution.

Cultural Response

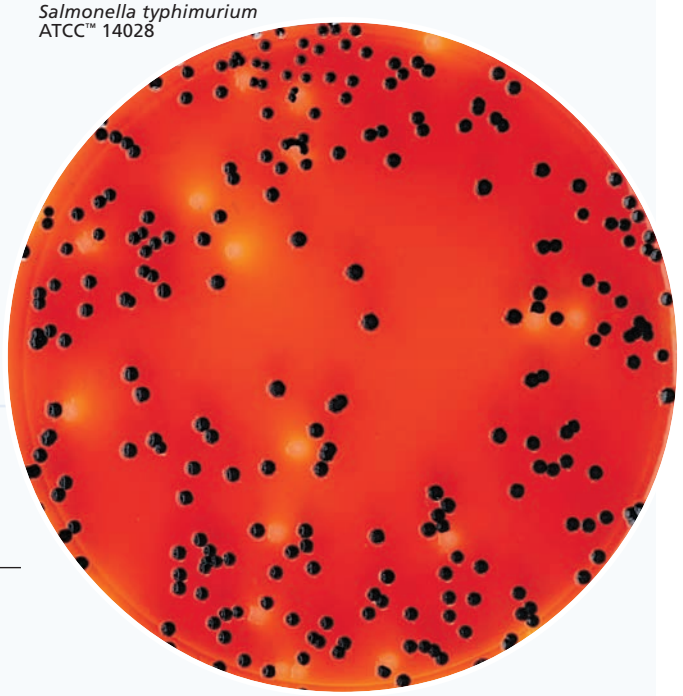
Difco™ XLT4 Agar Base with XLT4 Agar Supplement

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^3	Marked inhibition	–
Escherichia coli	25922	10^3	Partial inhibition	Yellow
Proteus mirabilis	25933	10^3	Inhibition	–
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	10^2-10^3	Good	Yellow to red with black centers
Staphylococcus aureus	25923	10^3	Inhibition	–

XLT4 Agar Base with XLT4 Supplement

Salmonella typhimurium
ATCC™ 14028



recovery of non-typhi *Salmonella* from chicken and farm environmental drag-swab samples.

Principles of the Procedure

XLT4 Agar Base contains peptone as a source of complex nitrogen compounds. Yeast extract is added as a source of vitamins and other cofactors. Differentiation of *Salmonella* from other organisms that also grow on this medium is based on fermentation of xylose, lactose and sucrose, decarboxylation of lysine and the production of hydrogen sulfide. Hydrogen sulfide production is detected by the addition of ferric ions. Sodium thiosulfate is added as a source of inorganic sulfur. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. Phenol red is added as an indicator of pH changes resulting from fermentation and decarboxylation reactions. XLT4 Agar Supplement is added to inhibit growth of non-*Salmonella* organisms.

Formulae

Difco™ XLT4 Agar Base

Approximate Formula* Per Liter

Proteose Peptone No. 3	1.6	g
Yeast Extract	3.0	g
L-Lysine	5.0	g
Xylose	3.75	g
Lactose	7.5	g
Saccharose	7.5	g
Ferric Ammonium Citrate	0.8	g
Sodium Thiosulfate	6.8	g
Sodium Chloride	5.0	g
Agar	18.0	g
Phenol Red	0.08	g

Difco™ XLT4 Agar Supplement

A 27% solution (approximate) of the surfactant Tergitol™** 4 (7-ethyl-2-methyl-4-undecanol hydrogen sulfate, sodium salt).

**Adjusted and/or supplemented as required to meet performance criteria.*
***Tergitol is a trademark of Union Carbide Chemicals & Plastics Technology Corporation.*

Directions for Preparation from Dehydrated Product

1. Suspend 59 g of the powder in 1 L of purified water.
2. Add 4.6 mL XLT4 Agar Supplement. Mix thoroughly.
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Avoid overheating. DO NOT AUTOCLAVE.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Inoculate a suitable *Salmonella* enrichment broth (such as Tetrithionate Broth) and incubate at 35°C for 18-24 hours.
2. Following enrichment, subculture onto XLT4 Agar. Streak for isolation.
3. Incubate plates aerobically at 35 ± 2°C. Examine for growth after 18-24 and 48 hours incubation.

Expected Results

Typical *Salmonella* colonies (H_2S-positive) appear black or black-centered with a yellow periphery after 18-24 hours of incubation. Upon continued incubation, the colonies become entirely black or pink to red with black centers.

Colonies of H_2S-negative *Salmonella* strains appear pinkish-yellow.

Most *Citrobacter* colonies that grow on this medium are yellow without evidence of blackening. Growth of *Enterobacter aerogenes* and *Escherichia coli* is markedly inhibited; colonies that do grow appear yellow without evidence of blackening. Growth of *Proteus*, *Pseudomonas*, *Providencia*, *Alteromonas putrefaciens*, *Yersinia enterocolitica* and *Acinetobacter calcoaceticus* is markedly to completely inhibited on XLT4 Agar. *Shigella* species are partially inhibited and colonies appear red.

Limitations of the Procedure

1. XLT4 Agar is intended for detecting and isolating *Salmonella* based on selectivity and colonial characteristics. Presumed *Salmonella* colonies must be confirmed by biochemical and/or immunological methods. Consult appropriate references for further information.[5-7]
2. Non-*Salmonella* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Salmonella*. Consult appropriate references.[5-7]
3. Freshly inoculated plates and plates held over several days may develop multicolored, metallic looking crystals/flecks on the surface. These crystals/flecks do not interfere with the performance of the medium.

References

1. Miller and Tate. 1990. The Maryland Poultryman April:2.
2. Miller, Tate, Mallinson and Schemer. 1991. Poultry Science *70*:2429.
3. Miller, Tate, Mallinson and Schemer. 1992. Poultry Science *71*:398.
4. Tate, Miller and Mallinson. 1992. J. Food Prot. *55*:964.
5. U.S. Department of Agriculture. 1998. Microbiology laboratory guidebook, 3rd ed., Food Safety and Inspection Service, USDA, Washington, D.C.
6. Murray, Baron, Pfaller, Tenover and Yolken (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Downes and Ito (ed.) 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ XLT4 Agar Base

USDA

Cat. No. 223420 Dehydrated – 500 g

Difco™ XLT4 Agar Supplement

USDA

Cat. No. 235310 Bottle – 100 mL