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# 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)*<sup>1-3</sup> 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<sup>4</sup> 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.<sup>4</sup>

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.<sup>4</sup>

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

XLD Agar is a selective and differential medium used for the isolation and differentiation of enteric pathogens from clinical specimens.<sup>10-12</sup> The value of XLD Agar in the clinical laboratory is that the medium is more supportive of fastidious enteric organisms such as *Shigella*.<sup>12</sup> XLD Agar is also recommended for the testing of food, dairy products and water in various industrial standard test methods.<sup>13-17</sup> 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.<sup>1</sup>

## 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.<sup>4</sup> 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™ 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           |
|--|-------|------------------|--|------------------------|
| <i>Enterococcus faecalis</i>   | 29212 | ~10 <sup>3</sup> | Partial inhibition                       | –                      |
| <i>Escherichia coli</i>  | 25922 | ~10 <sup>3</sup> | Partial inhibition                       | Yellow                 |
| <i>Providencia alcalifaciens</i>   | 9886  | 100-300          | Good                                     | Red                    |
| <i>Shigella flexneri</i>   | 12022 | 100-300          | Good                                     | Red                    |
| <i>Escherichia coli</i> *  | 8739  | >100             | Partial to complete inhibition (30-35°C) | Yellow                 |
| <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 |

### 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

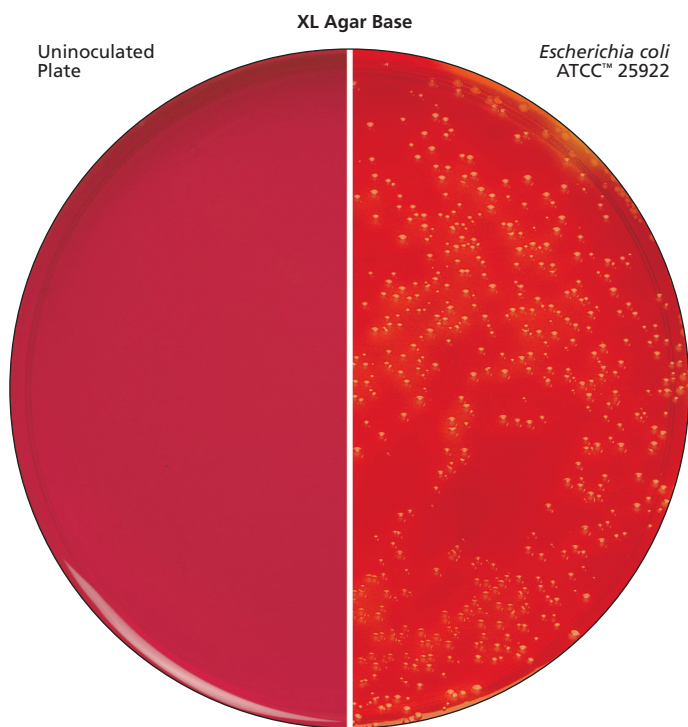
Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (up to 48 hours if necessary).

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

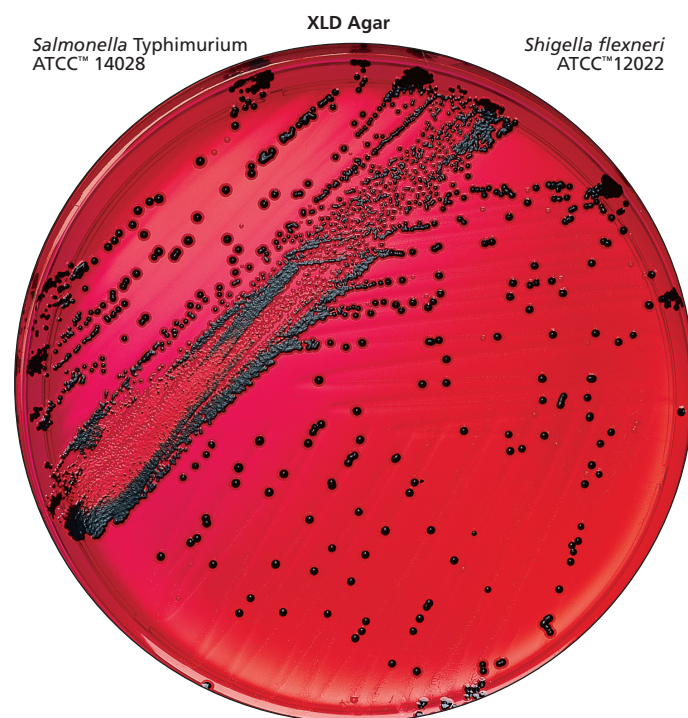
#### BBL™ XLD Agar (prepared)

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           |
|--|-------|-----------------------------------|--|------------------------|
| <i>Enterococcus faecalis</i>   | 29212 | 10 <sup>4</sup> - 10 <sup>5</sup> | Partial inhibition                       | –                      |
| <i>Escherichia coli</i>  | 25922 | 10 <sup>4</sup> - 10 <sup>5</sup> | Partial to complete inhibition           | Yellow to yellow-red   |
| <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium   | 14028 | 10 <sup>3</sup> -10 <sup>4</sup>  | Good                                     | Red with black centers |
| <i>Shigella flexneri</i>   | 12022 | 10 <sup>3</sup> -10 <sup>4</sup>  | Good                                     | Red                    |
| <i>Escherichia coli</i> *  | 8739  | 10 <sup>2</sup> -10 <sup>3</sup>  | 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 |



*Escherichia coli*  
ATCC™ 25922



*Salmonella Typhimurium*  
ATCC™ 14028

*Shigella flexneri*  
ATCC™ 12022

To add to the differentiating ability of the formulation, an H<sub>2</sub>S 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<sub>2</sub>S producers do not decarboxylate lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies.<sup>4</sup> 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.

- 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.

## Sample Collection and Handling

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

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

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

## Procedure

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

For food, dairy and water samples, refer to appropriate standard references for details on test methods using XLD Agar.<sup>13-17</sup>

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.<sup>1</sup>

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:

|   |  |
|---|--|
| <i>E. coli</i> .....                                      | Large, flat, yellow; some strains may be inhibited |
| <i>Enterobacter / Klebsiella</i> .....                    | Muroid, 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 <sub>2</sub> S-negative ... | Red  |
| <i>Pseudomonas</i> .....                                  | Red  |
| Gram-positive bacteria .....                              | No growth to slight growth                         |

## Limitations of the Procedure

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

## References

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- Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
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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 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.