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 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

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

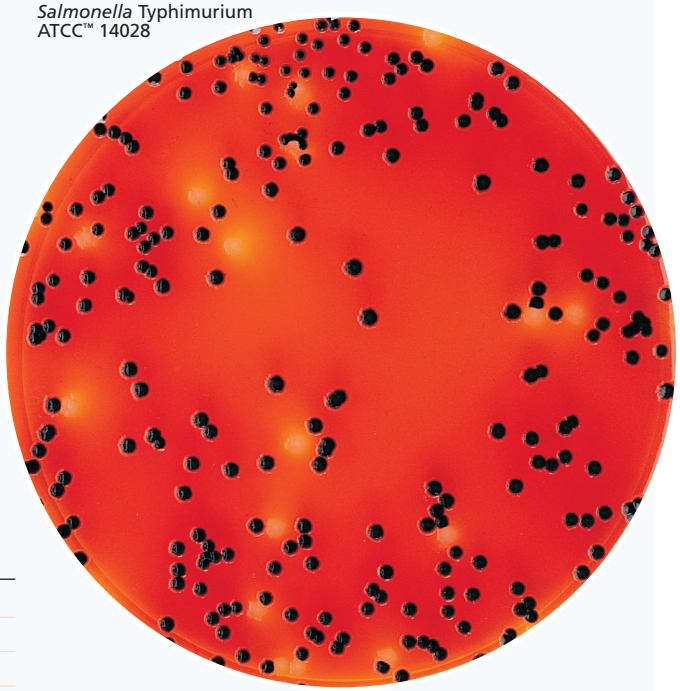
Difco™ XLT4 Agar Base with XLT4 Agar Supplement

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 enterica subsp. *enterica* 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



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, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th 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 – l00 mL