XLT4 Agar (NCM0100)

Revision: 1 Effective Date: 9/22/2021

Intended Use
XLT4 Agar is used with Tergitol 4 for the isolation of non-typhi *Salmonella* spp and is not intended for use in the diagnosis of disease or other conditions in humans.

Description
XLT4 Agar is a selective differential isolation medium for the specific detection of *Salmonella* spp. from environmental and food samples. Due to its highly selective nature, XLT4 Agar is particularly effective when used with samples where overgrowth of contaminating flora is expected, for example, fecally-contaminated agricultural samples.

Developed to perform as per Miller & Tate in 1990, this medium was found to improve the recovery of non- typhi *Salmonella* from chicken and farm environmental samples. Dusch and Altwegg further established the application of XLT4 Agar to salmonellae detection in clinical samples, with the notable exceptions of *Salmonella* typhi and *Salmonella* paratyphi. The presence of peptone and yeast extract provides sufficient nutrients to allow the optimal growth of *Salmonella* spp.

Selectivity is provided by the anionic surfactant Tergitol-4, NCM4079 XLT4 Supplement. This compound acts as an effective selective agent which is active against Gram-positive and many Gram-negative organisms, including *Proteus* spp.

Differentiation is based on fermentation of the sugars xylose, lactose and sucrose in addition to the decarboxylation of lysine. The inclusion of the pH indicator, phenol red, provides visual evidence of a pH decrease (yellow) or increase (red) in the medium. Ammonium iron (III) citrate is present to distinguish hydrogen-sulfide (H_2S) producing from non-H_2S producing organisms.

Most enteric organisms, except *Shigella*, will ferment xylose to produce acid. However, the salmonellae will also decarboxylate the lysine to keep the pH neutral to alkali, thus maintaining red coloration. At near-neutral pH *Salmonella* can produce H_2S from the reduction of ammonium iron (III) citrate and thiosulphate ions producing black or black-centered colonies.

Non H_2S-producing salmonellae will be red without a black center.

Other Enterobacteriaceae (non-salmonellae) which are not inhibited by NCM4079 XLT4 Supplement, will ferment xylose, lactose and/or sucrose but will not decarboxylate lysine. This fermentation activity causes a decrease in pH, resulting in a color change within the colonies from red to yellow.

Typical Formulation

Proteose Peptone	1.6 g/L
Yeast Extract	3.0 g/L
L-Lysine	5.0 g/L
Xylose	3.75 g/L
Lactose	7.5 g/L
Sucrose	7.5 g/L
Ammonium Iron (III) Citrate	0.08 g/L
Sodium Thiosulfate	6.8 g/L
Sodium Chloride	5.0 g/L
Phenol Red	0.8 g/L
Agar	18.0 g/L

Final pH: 7.4 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

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Supplement
NCM4079 XLT4 Supplement

Precaution
Refer to SDS

Preparation
1. Suspend 59 grams of the medium in one liter of purified water.
2. Add 4.6 mL of NCM4079-100 XLT4 Supplement.
3. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
4. DO NOT OVERHEAT OR AUTOCLAVE THIS MEDIUM.
5. Cool to 45-50°C.

Test Procedure
Inoculate a suitable *Salmonella* enrichment broth, e.g. Tetrathionate Broth, and incubate at 35°C for 18 - 24 hours. Following enrichment, subculture onto XLT4 Agar. Streak for isolation. Incubate plates aerobically at 35 ± 2°C. Examine for growth after 18 - 24 and 48 hours incubation.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige to light pinkish.

Prepared Appearance: Prepared medium is clear to slightly hazy, red with no precipitate.

Expected Cultural Response: Cultural response on XLT4 Agar, with the addition of NCM4079 XLT4 Supplement, incubated aerobically at 37 ± 1°C and examined for growth after 18 - 48 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
Enterococcus faecalis ATCC® 29212	> 10^4	Complete Inhibition	N/A
Escherichia coli ATCC® 25922	> 10^4	Partial to complete Inhibition	N/A
Staphylococcus aureus ATCC® 25923	> 10^4	Complete Inhibition	N/A
Salmonella enteritidis ATCC® 13076	50-200	> 50%	Translucent/red colony with black center
Salmonella typhimurium ATCC® 14028	50-200	> 50%	Translucent/red colony with black center
Proteus mirabilis ATCC® 29906	> 10^4	Complete Inhibition	N/A

The organisms listed are the minimum that should be used for quality control testing.

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 pink-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* spp. are partially inhibited and colonies appear red.

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Expiration
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures
1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. XLT4 Agar is intended for detecting *Salmonella* based on selectivity and colonial characteristics. Presumed *Salmonella* colonies must be confirmed biochemically and/or immunologically.

Storage
Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Dusch, H. and Altwegg, M. (1995). Evaluation of five new plating media for the isolation of
2. *Salmonella* species. *Journal of Clinical Microbiology*. **33.** No.4. 802-804.
3. Miller, R.G. and Tate, C.R. (1990). A highly selective plating medium for the isolation of
4. *Salmonella. The Maryland Poultryman,* April: 2-7.
5. Miller, R.G., Tate, C.R., Mallinson, E.T. and Scherrer, J.A. (1991). Xylose-Lysine-Tergitol 4: An improved selective agar for the isolation of *Salmonella*. *Poultry Science* **70.** 2429-2432.
6. Miller, R.G., Tate, C.R., Mallinson, E.T. and Scherrer, J.A. (1992). *Erratum*. Xylose-Lysine- Tergitol 4: An improved selective agar for the isolation of *Salmonella*. *Poultry Science* **71.** 398.
7. Tate, C.R., Miller, R.G. and Mallinson, E.T. (1992). Evaluation of two isolation and non-isolation methods for detecting naturally occurring salmonellae from broiler flock environmental drag- swab samples. *J. Food Prot.* **55.** 964-967.

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