TECHNICAL DATA SHEET

XLT4 AGAR

DETECTION OF *SALMONELLA*

1 INTENDED USE

XLT4 (Xylose-Lysine-Tergitol 4) Agar is a selective isolation medium for the detection of *Salmonella*, except for *Salmonella* Typhi and Paratyphi.
XLT4 agar can be used as the second media of choice in the normalized methods of *Salmonella* detection in food microbiology. It is also used as media of choice in animal health for *Salmonella* detection.

The typical composition corresponds to that defined in the standards NF U47-102.

2 HISTORY

In 1991, Miller and Tate demonstrated that the use of XLT4 Agar increased the frequency of detection of non-Typhi *Salmonella* in poultry samples containing a high secondary microflora, and that the medium allowed a good differentiation between *Salmonella* and *Citrobacter*. The medium described by these authors incorporated Tergitol 4 in a modified Xylose-Lysine base, in order to inhibit a wide spectrum of competitive flora (*Proteus*, *Pseudomonas*, *Providencia*) which previously had interfered with *Salmonella* detection..
Further studies performed by Dusch and Altwegg established that XLT4 Agar could be used for *Salmonella* detection in clinical samples, with the exception of *Salmonella* Typhi and *Salmonella* Paratyphi.

3 PRINCIPLES

Xylose is fermented by enteropathogenic bacteria, with the exception of *Shigella*, which are therefore differentiated from other bacteria. After having exhausted the xylose, *Salmonella* decarboxylate lysine (via lysine decarboxylase) to cadaverine, which provokes an increase in the pH. In an alkaline (basic) environment *Salmonella* forms red colonies in the presence of the pH indicator, phenol red.

Black colonies, due to the appearance of iron sulfide in the colony center, are formed through the reduction of ferric ammonium citrate by pathogenic hydrogen sulfide producers.

The medium contains two additional sugars, lactose and sucrose. Fermentation of either or both sugars results in acidification of the medium and leads to the formation of yellow colonies in the presence of phenol red indicator.

Non-pathogenic strains that do not decarboxylase lysine produce an acidification from the sugar fermentation. The resulting decrease in pH prevents the blackening of the colonies.

Tergitol 4 is the commercial name of a 26-28% solution of an anionic detergent, 7-ethyl 2-methyl 4-undecyl sulfate, in a sodium salt form. It inhibits contaminating Gram-positive flora and numerous Gram-negative strains, notably *Proteus.*

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of complete media :

- Peptone ..... 1,6 g
- Yeast extract ..... 3,0 g
- L-Lysine ..... 5,0 g
- Lactose ..... 7,5 g
- Sucrose ..... 7,5 g
- Xylose ..... 3,75 g
- Sodium chloride ..... 5,0 g
- Sodium thiosulfate ..... 6,8 g
- Ferric ammonium citrate ..... 0,8 g
- Red phenol ..... 80,0 mg
- Tergitol 4 ..... 4,6 mL
- Bacteriological agar ..... 18,0 g

pH of the ready-to-use media at 25 °C : 7,4 ± 0,2.

For 59 g of dehydrated base media BK156

- Peptone ..... 1,6 g
- Yeast extract ..... 3,0 g
- L-Lysine ..... 5,0 g
- Lactose ..... 7,5 g
- Sucrose ..... 7,5 g
- Xylose ..... 3,75 g
- Sodium chloride ..... 5,0 g
- Sodium thiosulfate ..... 6,8 g
- Ferric ammonium citrate ..... 0,8 g
- Phenol red ..... 80,0 mg
- Bacteriological agar ..... 18,0 g

For one vial of supplement BS039

- Tergitol 4 ..... 50 mL

5 PREPARATION

- Dissolve 59,0 g of dehydrated media (BK156) in 1 liter of distilled or demineralized water.
- Add 4.6 mL of Tergitol 4 Selective Supplement (BS039).
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Do not overheat, do not autoclave.
- Cool and maintain in a molten state at 44-47 °C.
- Pour into sterile Petri plates and let solidify on a cold, flat surface.

✓ **Reconstitution :**
59,0 g/L
+ 4,6 mL of Tergitol 4

✓ **Sterilization :**
Do not autoclave

6 INSTRUCTIONS FOR USE

- Dry the plates in an incubator, covers partially removed.
- To the surface of plates prepared as above, or to ready-to-use plates (BM036) brought to room temperature, inoculate by streaking on the surface of the medium, using enrichment media used for the detection of *Salmonella*.
- Incubate at 37 ± 2 °C for 24 ± 3 hours.

✓ **Inoculation :**
On surface

✓ **Incubation :**
24 h at 37 °C

NOTE :
In animal health, for the detection of *Salmonella* Abortusovis, an incubation of 48 h is necessary.

7 RESULTS

Typical (H_2S positive) *Salmonella* colonies are red with a black center. They may present a yellow halo after 24 hours incubation. In the event of a prolonged incubation, the colonies become red to pink with a black center or entirely black.
H_2S-negative *Salmonella* appear red to pink without the black center.

Citrobacter, *Klebsiella* and *Enterobacter cloacae* produce yellow colonies.
Growth of *Enterobacter aerogenes* and *Escherichia coli* is partially inhibited, colonies present on the medium are yellow.
Proteus, Pseudomonas and Providencia are partially to completely inhibited.
Shigella produces slow growth and pink colonies.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated base media : pinkish powder, free-flowing and homogeneous.
Tergitol 4 Supplement : slightly yellow solution, limpid.
Prepared (complete) media : red-orange agar.

Typical culture response after 24 hours of incubation at 37 °C.

Microorganisms		Growth	Characteristics
Salmonella Typhimurium	WDCM 00031	Good, score 2	Red colonies with black center
Salmonella Enteritidis	WDCM 00030	Good, score 2	Red colonies with black center
Escherichia coli	WDCM 00013	Weak, score 0-1	Yellow colonies
Enterococcus faecalis	WDCM 00087	Inhibited, score 0	-
Staphylococcus aureus	WDCM 00034	Inhibited, score 0	-

9 STORAGE / SHELF LIFE

Dehydrated base media (without Tergitol 4) : 2-30 °C.
Tergitol 4 Selective Supplement : 2-25 °C.
Pre-poured media in Petri plates : 2-8 °C.
The expiration dates are indicated on the labels.

Prepared (complete) media in plates (with supplement) (*): 15 days at 2-8 °C.
Prepared media in vials (*) : Not recommended.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated base media (without Tergitol 4) :
500 g bottle BK156HA

Tergitol 4 Selective Supplement:
50 mL vial BS03908

Pre-poured complete media in Petri plates (Ø 90 mm) :
20 plates BM03608

11 BIBLIOGRAPHY

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Tate, C.R., Miller, R.G., and Mallinson E.T.. 1992. Evaluation of two isolation and two no-isolation methods for detecting naturally occurring *Salmonellae* from broiler flock environmental drag-swab samples. Journal of Food Protection, **55** : 964-967.

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12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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ANNEX 1 : PHOTO SUPPORT

XLT4 Agar

Detection of *Salmonella* spp..

Results :

Growth obtained after 24 hours of incubation at 37 °C.