TECHNICAL DATA SHEET

LACTOSE-SULFITE (LS) BROTH

CONFIRMATION OF *CLOSTRIDIUM PERFRINGENS*

1 INTENDED USE

Lactose-Sulfite Broth is a confirmation medium allowing the selective detection of both the vegetative cells and spores of *Clostridium perfringens* in food products and biological samples of animal origin, without the usual confirmatory tests.

The typical composition responds to that defined in the standard NF EN ISO 7937, for the enumeration of *Clostridium perfringens* in food microbiology.

2 HISTORY

Using the past work by Put (1961) relative to the sensitivity of *Clostridium* to sulfite, the optimal growth of *Clostridium perfringens* at 46°C, as well as its capacity to ferment lactose, Beerens *et al*. in 1982 successfully formulated and developed LS broth. In particular, they recommended its use for the detection of small quantities of *Clostridium perfringens* in food products in which there was a heavy secondary contamination of other sulfito-reducing bacteria.

3 PRINCIPLES

The specificity of the medium to *Clostridium perfringens* is due principally to the sulfite resistance of microorganism and its ability to ferment lactose with gas production.

The concentration of metabisulfite inhibits the development of the majority of clostridia other than *Clostridium perfringens.*

Incubation at 46°C assures the specific culture of *Clostridium perfringens*, which reduces the sodium metabisulfite to sulfide, provoking with ferric citrate a black iron sulfide precipitate that settles in the bottom of the culture tube.

4 TYPICAL COMPOSITION

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

For 1 liter of media:

- Tryptone ..... 4.44 g
- Yeast extract ..... 2.22 g
- L-Cysteine hydrochloride ..... 0.27 g
- Lactose ..... 8.89 g
- Sodium chloride ..... 2.22 g
- Sodium metabisulfite ..... 0.67 g
- Ferric ammonium citrate ..... 0.56 g

pH of the ready-to-use media at 25 °C: 7,1 ± 0,2.

5 PREPARATION

Use of dehydrated medium:

- Dissolve 19,3 g of dehydrated media (BK140) in 1 liter of distilled or demineralized water.
- Slowly stir until complete dissolution.
- Dispense 9 mL into appropriately-sized tubes containing a Durham tube.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution:**
19.3 g/L

✓ **Sterilization:**
15 min at 121 °C

Use of ready-to-melt media:

- Before use, regenerate the media by heating the tubes for 10 minutes at 100°C.
- Cool to ambient temperature.

6 INSTRUCTIONS FOR USE

- Re-inoculate 5 black colonies noires from TSC agar into tubes of Thioglycollate medium with Resazurin (BK017, BM082).
- Incubate the tubes under anaerobic conditions for 18 to 24 heures at 37 °C.
- Inoculate 5 drops of the culture obtained in the Thioglycollate tubes into each tube of LS broth prepared as described.
- Eliminate the air in the Durham tubes by turning upside down the tubes.
- Incubate at 46 °C for 18 to 24 hours in a conrolled water bath.

✓ **Inoculation:**
5 drops of inoculum

✓ **Incubation:**
18 to 24 h at 46 °C

7 RESULTS

Verify that the caps are slightly unscrewed before reading the results.
The fermentation of lactose is indicated by the presence of gas in the Durham tubes (minimum volume equal to ¼ of the volume of the Durham tube itself) in 24 hours, as well as simultaneous appearance of a black iron sulfide precipitate in the culture tubes indicating the presence of *Clostridium perfringens.*

Note: For ready-to-use medium in tubes, the color of iron sulfide precipitate can be from grey to black.

See ANNEX 1: PHOTO SUPPORT .

8 QUALITY CONTROL

Dehydrated media: cream powder, free-flowing and homogeneous.

Prepared media: amber solution, limpid.

Typical culture response after 24 hours of incubation at 46 °C:

Microorganisms		Growth	Gas production	Blackening
Clostridium perfringens	WDCM 00007	Good, score 2	Positive	Positive
Clostridium perfringens	WDCM 00080	Good, score 2	Positive	Positive
Clostridium sporogenes	ATCC® 19404	Weak, score 1	Negative	Possible

9 STORAGE / SHELF LIFE

Dehydrated media: 2-30 °C.
Ready-to-use media: 2-8°C, shielded from the light.
The expirations are indicated on the labels.

Prepared media in tubes (*) : use on the same day as preparation.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media:
500 g bottle BK140HA

Ready-to-use media:
Pack of 7 tubes of 9 mL with Durham tube BM19208

11 BIBLIOGRAPHY

Beerens, H., Romond, C.H., Lepage, C., and Criquelion, J. 1982. A Liquid Medium for the Enumeration of *Clostridium perfringens* in Food and Faeces. Isolation and Identification Methods for Foods Poisoning Organisms. Edited by Academic Press London, 137-149.

NF EN ISO 7937. Février 2005. Microbiologie des aliments. Méthode horizontale pour le dénombrement de *Clostridium perfringens*. Technique par comptage des colonies.

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

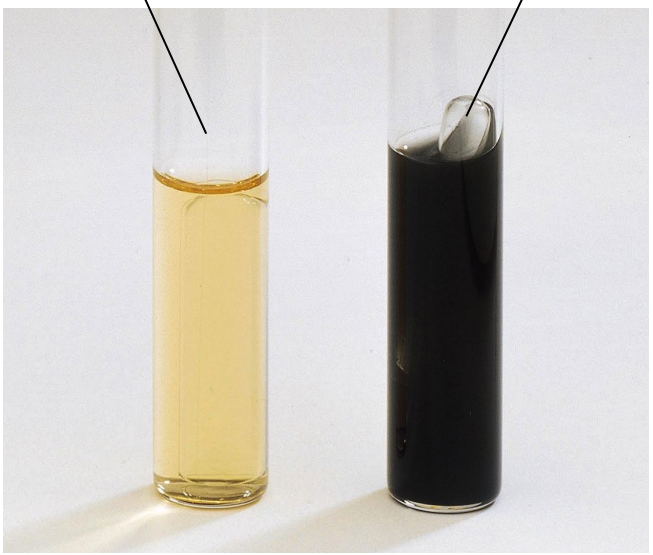
Lactose-Sulfite (LS) BROTH

Detection & confirmation of *Clostridium perfringens*.

Results:

Growth obtained after 24 hours of incubation at 46 °C.

Negative control



Positive sample

Characteristics :
Blackening of the media and gas presence in the Durham tubes.