Fluid medium R
(Lactose-sulfite medium)

356-4914

DEFINITION
Broth for the detection and confirmation of spores of *Clostridium perfringens* in food products after incubation at 46°C.

STANDARDS
• ***European Pharmacopeia* 6.0** - Biological methods - **2.6.13.**: Microbiological test of non-sterile products (Detection of specified micro-organisms).

PRINCIPLE
The principle of the Lactose-sulfite broth relies on the ability of *Clostridium perfringens* to ferment lactose while producing gas (observable in the Durham bell jar) and to reduce sulphite to sulphide at 46°C (black iron sulphide precipitate in base of the tube.

PRESENTATION
• **Dehydrated**
100 g **code 356-4914**

STORAGE
• Dehydrated: + 15° to 25°C, in carefully-sealed bottles in a cool, dry place.
• Expiration date and batch number are shown on the package.

TYPICAL FORMULA

Peptone	5 g
Yeast extract	2.5 g
Sodium chloride	2.5 g
Lactose	10 g
Cystein hydrochloride	300 mg
Distilled water	1,000 ml

Final pH (25°C) = 7.1 ± 0.1

Remark: the formula has been adapted to attain the required performance criteria.

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
• Diluent(s)
• Distilled water
• Sterile 1.2% solution of sodium metabisulfite or sterile 1.2% solution of sodium sulfite
• Sterile 1% solution of ferric ammonium citrate (III)

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)
• Scales
• Sterile weighing bags
• Test tubes (16 x 160 mm) with autoclave-proof stoppers and corresponding Durham bell jars
• Flasks fitted with corresponding Durham bell jars
• Sterile pipettes (10 ml,…)
• Sterile Pasteur pipettes **(code 355-0751)**
• Water-bath precise to ± 1°C
• Autoclave
• All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM
Always shake well before use
Dissolve 20.3 g of powder in 1 liter of distilled water.
Heat gently until completely dissolved.
Dispense 8 ml per tube fitted with a Durham bell jar (utilization of broth as confirmation medium for *Clostridium perfringens*).
Dispense 80 ml per flask fitted with a Durham bell jar (utilisation of broth for the detection of *Clostridium perfringens* spores in gelatine for foodstuffs).
Sterilize in an autoclave for 15 minutes at 121°C ± 1°C.

Immediately prior to inoculation:
Eliminate any bubbles in the Durham bell jar by turning the tubes (or flasks) over.
Slightly loosen the caps on the tubes (or flasks), then place them in a boiling waterbath for 5 minutes (regeneration). Tighten the caps.

Cool the tubes to room temperature. Add 0.5 ml of sterile 1.2% solution of sodium metabisulfite (or sodium sulfite*) to each tube and 0.5 ml of sterile 1% solution of ferric ammonium citrate III. In the case of flasks of 80 ml of broth, add 5 ml of each solution.

• The results obtained with sodium sulfite (growth of *C. perfringens*, intensity of coloration of iron sulpfide precipitation) are generally superior to those obtained with sodium metabisulphite.

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PROTOCOL

• **Preparation of samples**

According to the standards applicable to the product concerned

• **Inoculation and incubation**

Utilization of Lactose-Sulfite broth as confirmation medium for *Clostridium perfringens:*

Inoculate a tube of Lactose-Sulfite broth with 4 to 5 drops of an 18 – 24 hour culture in thioglycolate broth of the bacteria to be identified (suspected *Clostridium perfringens*). Tighten the caps. Incubate at 46°C for 24 hours (± 2 h).

Utilization of Lactose-Sulfite broth as medium for the detection of spores of *Clostridium perfringens* :

Inoculate a flask of Lactose-Sulfite broth with 10 ml of solution of the gelatine to be analyzed. Tighten the caps. Incubate at 46°C for 24 hours (± 2 h), possibly prolonging incubation up to 48 hours (± 2 h).

READING AND INTERPRETATION

Utilization of Lactose-sulfite broth as confirmation medium for *Clostridium perfringens* :

Clostridium perfringens: tubes presenting black iron sulphide precipitate **and** with Durham bell jar at least 1/3 full of gas.

Utilization of Lactose-sulfite broth as medium for the detection of spores of *Clostridium perfringens* :

The sample contains at least 1 spore of *Clostridium perfringens* if the flask presents iron sulphide precipitate **and** if the Durham bell jar contains gas.

PRECAUTIONS

- Before regeneration, eliminate any bubbles of gas present in the Durham bell jar by turning the tubes (or flasks) over.
- Re-oxygenation of the base broth after regeneration should be avoided. The inoculum is mixed with the broth using circular movements, avoiding the inclusion of air in the broth. A mechanical shaker (e.g. Vortex type) should not be used.
- Comply with Good Laboratory Practice.

QUALITY CONTROL

In view of the current harmonization of pharacopeias, we recommend that you refer to the certificates of analysis for procedures relating to the quality control (performance and selectivity) of media produced by Bio-Rad.

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

Lactose-sulfite/*Clostridium perfringens* spores/ Food products/ Detection/ Confirmation /Lactose/Gas/Durham bell jar/ MPN/Broth.

BIBLIOGRAPHY

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