Meat Liver Sulfite Iron/Agar

356-9654 / 355-4777
355-4770 / 355-4794

DEFINITION
Agar medium used to detect and enumerate spores of **sulfite-reducing anaerobia** in food products and water.

STANDARDS
WATER
- **ISO/DIS 6461-2 (June 2005):** Water quality - Detection and enumeration of *Clostridium perfringens*. - Part 2: Membrane filtration method (ISO 6461-2: 1986 review).

- **NF EN 26461-2 (July 1993):** Water quality - Detection and enumeration of spores of sulfite-reducing anaerobic micro-organisms *(Clostridia)*. Part 2: Membrane filtration method.

- **NF T90-415 (October 1985):** Detection and enumeration of spores of sulfite-reducing anaerobic bacteria and of sulfite-reducing *Clostridium* - General method by incorporation into agar and in deep tubes.

- **NF T90-461/A2 (May 2007)**: Water quality - Microbiology - Quality control for culture media.

PRINCIPLE
The principle of the meat-liver sulfite iron medium relies on the ability of sulfite-reducing anaerobic bacteria to reduce ammonium sulfite to iron sulfide, responsible for the blackening of colonies, in anaerobiosis at 37℃.
Incubation at 46℃ allows preferential culture of *Clostridium perfringens*.

PRESENTATION
- **Ready-to-use**

100 ml x 6 bottles	**code 355-4770**
20 ml x 25 tubes	**code 355-4777**
7.5 ml x 25 tubes	**code 355-4794**

- **Dehydrated**

500 g	**code 356-9654**

STORAGE
- Ready-to-use: + 2℃ to 8℃.
- Dehydrated: + 15℃ to 25℃, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Meat-liver base	30 g
Glucose	2 g
Starch	2 g
Agar	11 g
Distilled water	1,000 ml
Sodium sulfite	2.5 g
Iron salts	0.5 g

Final pH (25℃) before autoclaving = 7.7 ± 0.1

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- Test tubes (16 x 160 mm) with autoclave-proof stoppers
- Bottles with autoclave-proof stoppers
- Sterile Petri dishes (Ø= 90 mm)
- Anaerobiosis cloche
- Anaerobiosis catalyser
- Sterile Pasteur pipettes **(code 355-0751)**
- Water-bath precise to ± 1℃
- Thermostatically-controlled incubator or incubation room, precise to ± 1℃
- Autoclave
- All usual laboratory equipment.

PREPARATION OF DEHYDRATED MEDIUM
Always shake well before use.
Dissolve 46.5 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix thoroughly until a homogenous suspension is obtained. Heat gently, swirling frequently, then bring to boiling point until completely dissolved. Dispense 100 ml of medium per bottle or 20 ml or 7.5 ml per tube and sterilize in autoclave at 121℃ (± 3 ℃) for 15 minutes.

Reconstitution ratio: 46.5 g/l.
500 g of powder makes approximately 10.7 liters of medium.

PROTOCOL
- **Preparation of samples / Destruction of vegetative forms**
  According to standards applicable to the product concerned.

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To destroy of vegetative forms, the specimen must be placed in water-bath at 80℃ ± 2℃ for 10 minutes and quickly cooled to 44℃ - 49℃.

- **Regeneration of tubes**
  Regenerate the tubes at 100℃ for 20 minutes, then maintain under super-cooling conditions between 44℃ and 49℃ before inoculation.

- **Inoculation**
  In 20 ml tubes
  Inoculate 5 ml of test specimen into a 20 ml tube of complete medium and homogenize using twisting up-and-down movements.

  In 7.5 ml tubes
  Inoculate the tubes by means of an inoculating loop and homogenize using twisting up-and down movements.

  In Petri dishes
  Deposit 1 ml of test specimen on the dish. Add 18 ml of super-cooled complete medium. Mix. Leave to solidify, then add a second layer of a few ml of agar.

- **Incubation**
  After solidification, the tubes and dishes are incubated at 37℃ ± 1℃ and/or 46℃ ± 1℃ for 24 and 48 hours (in anaerobiosis for Petri dishes).

READING AND INTERPRETATION

- Spores of sulfite-reducing anaerobic bacteria are black, surrounded by a black halo.

- It is essential to take a reading after 24 hours of incubation. In the presence of numerous colonies, diffusion of halos can lead to a uniform black coloration of the tube, and enumeration becomes impossible after 48 hours of incubation.

- Conversely, if there are a small number of colonies at the first reading, new colonies may develop over the next 24 hours.

Identification of sulfite–reducing *Clostridium* can be carried out by sub-culturing these colonies which are surrounded by black halo (*refer to standard NF T90-415 for details of procedure*).

PRECAUTIONS

- To avoid re-oxygenation of the medium, it should be stirred gently.
  N.B.: Regenerated media may present very slight turbidity, but this does not affect their culture performance.

- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Result after 24 h culture at 37℃	
	GROWTH	COLOR
*Clostridium perfringens** ATCC 13124	+	Black
Clostridium perfringens CIP 60.60	+	Black
E. coli RIVM WR1 **	+	No typical growth

*** Relative yield: R = [66% - 150%]**
**** RIVM WR1 equivalent to NCTC 13167**

STRAINS	Result after 24 h culture at 46℃	
	GROWTH	COLOR
Clostridium perfringens CIP 60.60	+	Black

QUALITY CONTROL OF MANUFACTURER

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

Meat-liver sulfite iron / Sulfite-reducing anaerobes / Spores / *Clostridium* / Food products / Water / Detection / Enumeration / Medium.

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