TECHNICAL DATA SHEET

IRON SULFITE AGAR (ISA)(ISO 15213-1)

ENUMERATION OF *CLOSTRIDIUM* SPP.

1 INTENDED USE

The Iron Sulfite Agar (ISA) (ISO 15213-1) is recommended for the enumeration of sulfite-reducing *Clostridium* spp.

Sulfite-reducing *Clostridium* spp. are obligate anaerobic, Gram-positive, spore-forming, rod-shaped bacteria. The most important species which belong to this group are *Clostridium (C.) perfringens*, *C. bifermentans* and *C. sporogenes*. Some species can cause foodborne illness. As ubiquitous bacteria they are predominantly found in nature. The *Clostridium* species inhabit soils and the intestinal tract of animals and humans.

Sulfite-reducing *Clostridium* spp., including *C. perfringens*, are widely used as microbial indicators of clostridial contamination in the manufacturing of foods (e.g. meat production). These have the capacity to produce heat-resistant spores.

The typical composition corresponds to that defined in the standard NF EN ISO 15213-1.

2 PRINCIPLES

Sulfite-reducing microorganisms *Clostridiums* spp. reduce the sodium sulfite to sulfide, which with ferric citrate forms a black iron sulfide precipitate around the colonies.

3 TYPE COMPOSITION

The composition can be adjusted to obtain optimal performance.

For 1 liter of media:

- Tryptone ..... 15,0 g
- Papaic digest of soybean meal ..... 5,0 g
- Yeast extract ..... 5,0 g
- Sodium metabisulfite ..... 0.5 g
- Ferric ammonium citrate ..... 1,0 g
- Bacteriological agar ..... 15,5 g

pH of the ready-to-use media at 25°C: 7,6 ± 0,2.

4 PREPARATION

Preparation from dehydrated media:

- Dissolve 42.0 g of dehydrated media (BK239) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in vials or in tubes.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the media at 44-47 °C.

✓ **Reconstitution:**
42,0 g/L

✓ **Sterilization:**
15 min at 121 °C

Use of ready-to-melt media in vials or in tubes:

- Melt the agar (if prepared in advance as above) or the ready-to-melt medium (BM235) for the minimum amount of time necessary to achieve complete liquefaction.
- Cool and maintain the media at 44-47 °C.

5 INSTRUCTIONS FOR USE

Prepare the initial suspension and its tenfold dilutions according to rules defined in corresponding ISO 6887 standards.

Enumeration of sulfite-reducing *Clostridium spp.* (ISO 15213-1)

Heat, if necessary, decimal dilution series to 80 °C for 10 min ± 1 min to destroy vegetative cells and activate the spores.

- Transfer 1 mL of inoculum and its serial dilutions to empty, sterile Petri plates.
- Pour about 15mL of medium.
- Mix well.
- Let solidify on a flat surface.
- Add a second layer of agar and let solidify.
- Incubate the plates in an anaerobic atmosphere for 48±2 hours at 37±1 °C.

6 RESULTS

Count black or grey to yellow-brown colonies.
Plates shall be counted within 30 min as the colour of the colonies can rapidly fade and disappear because of the oxidation of iron sulfite.
Proceed to confirmation test for the enumeration of *Clostridium* spp. If no confirmation is carried out, the results can be reported as “sulfite-reducing anaerobic bacteria”.

See ANNEXE 1: PHOTO SUPPORT.

7 QUALITY CONTROL

Dehydrated base media: beige powder, free-flowing and homogeneous.
Prepared media: amber agar.

Typical cultural response (Iron Sulfite Agar of ISO 15213-1) after 48 ± 2 hours of incubation at 37±1 °C (NF EN ISO 11133):

Microorganisms		Growth (Productivity ratio)	Characteristic colonies
Clostridium perfringens	WDCM 00007	$P_R \geq 50$ %	Black
Escherichia coli	WDCM 00013	-	White colonies, uncharacteristic

8 CONSERVATION

Dehydrated base media: 2-30 °C.
Ready-to-melt media: 2-25 °C.
The expiration dates are indicated on the label.

Prepared base media in vials or tubes (*): 180 days at 2-25 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer’s instructions.

9 PACKAGING

Dehydrated media:
500 g bottle..... BK239HA

Ready-to-melt media:
10 x 200 mL vials..... BM23508

10 BIBLIOGRAPHY

NF EN ISO 11133. July 2014. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

NF EN ISO 15213-1. February 2023. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Clostridium* spp. - Part 1: enumeration of sulfite-reducing *Clostridium* spp. by colony-count technique.

NF EN ISO 6887-1. June 2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: general rules for the preparation of the initial suspension and decimal dilutions.

NF EN ISO 6887-2. June 2017. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 2: Specific rules for the preparation of meat and meat products.

NF EN ISO 6887-3. June 2017. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 3: Specific rules for the preparation of fish and fishery products.

NF EN ISO 6887-4. June 2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 4: Specific rules for the preparation of miscellaneous products.

NF EN ISO 6887-5. May 2020. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 5: Specific rules for the preparation of milk and milk products.

NF EN ISO 6887-6. May 2013. Microbiology of food and animal feed - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 6: Specific rules for the preparation of samples taken at the primary production stage.

11 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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IRON SULFITE AGAR (ISO 15213-1)

Enumeration of sulfite-reducing *Clostridium* spp.

Results:

Growth obtained after 24 hours of incubation at 37 °C, under anaerobic conditions.