TECHNICAL DATA SHEET

BISMUTH SULFITE AGAR (ISO 6579-1)

DETECTION OF *SALMONELLA* TYPHI, PARATYPHI AND OTHER *SALMONELLAE*

1 INTENDED USE

Bismuth Sulfite Agar ISO 6579-1 is a selective medium used to isolate *Salmonella* in water, dairy products and other food products.

This medium is particularly used for the detection of *Salmonella enterica* subspecies *enterica* serovars Typhi and Paratyphi in human consumption and the feeding of animals, environmental samples and samples from the primary production stage.

Bismuth Sulfite agar can be used in the normalized methods for *Salmonella* detection as the second isolation media.

The typical composition corresponds to that defined in the standards NF EN ISO 6579-1.

2 HISTORY

In 1926, Wilson and Blair combined bismuth and sodium sulfite in a medium destined to isolate *Salmonella* of the typhi and paratyphi groups. In 1956, Hajna and Damon described a modified formula which was recommended by the United States Pharmacopoeia.

3 PRINCIPLES

The concentrations of brilliant green and bismuth sulfite inhibit accompanying Gram-positive flora and most enterobacteria, except for *Salmonella* and several *Shigella*.

Using the sulfur compounds in the medium, *Salmonella* releases hydrogen sulfide which produces a metallic precipitate in the presence of ferrous sulfate, giving the colonies a black or sometimes green color.

It is particularly recommended to first enrich using Tetrathionate, Selenite or Rappaport-Vassiliadis Broths and to simultaneously inoculate onto other less selective media: MacConkey, XLD or Hektoen Enteric Agars, for example.

Because of its elevated inhibitory power, this medium enables a highly contaminated inoculum to be used.

4 TYPICAL COMPOSITION

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

For 1 liter of media:

- Enzymatic digest of animal tissues ..... 10.00 g
- Meat extract..... 5.00 g
- Dextrose ..... 5.00 g
- Disodium hydrogen phosphate (anhydrous) (Na2HPO4) ..... 4.00 g
- Ferrous sulfate ..... 0.30 g
- Bismuth sulfite ..... 8.00 g
- Brilliant green ..... 25.0 mg
- Bacteriological agar..... 20.00 g

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

5 PREPARATION

- Dissolve 52,3 g of dehydrated media (BK223) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Do not autoclave.
- Cool and maintain the media in a molten state at 47-50 °C.
- Homogenize well in order to disperse the precipitate.
- Pour into sterile Petri plates.
- Let solidify on a cold, flat surface.
- Dry in an incubator with the covers partially removed.

✓ **Reconstitution:**
52.3 g/L

✓ **Sterilization:**
Bring to boil

Note:
Immediately after its preparation, the medium has optimal selectivity which gradually decreases with time. This is why it is not recommended to store the ready-to-use medium more than 4 days at 2-8°C.

6 INSTRUCTIONS FOR USE

- Inoculate by streaking the medium with the enrichment media used.
- Incubate between 34 and 38°C for 24 and 48 hours.

✓ **Inoculation:**
On surface

✓ **Incubation:**
24 h and 48 h between 34 and 38°C

7 RESULTS

Characteristic colonies of *Salmonella* Typhi appear black, flat, dry colonies surrounded by a black-brown zone with metallic reflections.
Green to brownish colonies without dark zones are characteristic of *Salmonella* Enteritidis, *Salmonella* Gallinarium, *Salmonella* Choleraesuis, and *Salmonella* Paratyphi.
Coliforms, *Proteus* and *Shigella* are strongly inhibited but may sometimes yield small greenish or brownish colonies.

See ANNEX 1: PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media: greenish-beige powder, free-flowing and homogeneous.
Prepared media in plates: green-beige agar.

Typical culture response after 48 hours of incubation at 37 °C:

Microorganisms		Growth	Characteristics
Salmonella Typhimurium	WDCM 00031	Good	Black colonies with metallic reflection
Salmonella Enteritidis	WDCM 00030	Good	Greenish brown colonies
Escherichia coli	WDCM 00013	Partially inhibited	Green colonies
Enterococcus faecalis	WDCM 00087	Inhibited	-

9 STORAGE / SHELF LIFE

Dehydrated base media: 2-30 °C.
The expiration date is indicated on the label.

Prepared media in plates (*)**:** 4 days at 2-8 °C, shielded from light.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media:
500 g bottle BK223HA

11 BIBLIOGRAPHY

Wilson, J.W., and Blair, E.M. 1931. Further experience of the Bismuth Sulphite Media in the isolation of *Bacillus typhosus* and *Bacillus paratyphosus* B from faeces, sewage and water. J. Hyg., 31: 138.

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NF EN ISO 19250. Juin 2013. Water quality — Detection of Salmonella spp.
NF EN ISO 6579-1. Avril 2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp.
NF EN ISO 6579-1/A1. March 2020. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1 : detection of Salmonella spp. - Amendment 1 Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC

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

BISMUTH SULFITE Agar (modified Wilson Blair)

Detection of *Salmonella* Typhi and other *Salmonellae*

Results :

Growth obtained after 24 hours of incubation at 37 °C (surface inoculation).

***Salmonella* Typhi**

Characteristic colony :
Black color with metallic reflects, flat and dry, surrounded by a brown-black halo.