TECHNICAL DATA SHEET

SESAME *SALMONELLA* TEST®

METHOD OF SALMONELLA DETECTION

1 INTENDED USE

SESAME *Salmonella* TEST® represents an alternative method for the detection of *Salmonella* in human and animal products, as well as environmental samples.

This method is destined to the detection of motile Salmonella and is not adapted to non-motile Salmonella (non-motile strains or that have lost their mobility).

The analyses can be declared negative in 37 hours after only two steps of pre-enrichment (***Salmonella* Enrichment**) and differentiation (**SESAME *Salmonella* Detection**).

The confirmation of presumptive positive samples is achieved using **COMPASS® *Salmonella* Agar**, which requires an additional 21 hours incubation.

SESAME *Salmonella* TEST® is certified NF VALIDATION, under Attestation N° BKR 23/04-12/07, according to the validation protocol NF EN ISO 16140-2 of 2016 for all human food and feed products, as well as samples from the industrial production environment. The reference method used for the validation is standard NF EN ISO 6579-1 of 2017.
Refer to the certificate available on the NF VALIDATION website for the expiry date of the method.

BKR 23/04-12/07
ALTERNATIVE ANALYSIS METHOD
FOR FOOD INDUSTRY
Certified by AFNOR Certification http://nf-validation.afnor.org/

In the context of NF VALIDATION, test portions weighing more than 25 g have not been tested.

2 PRINCIPLES

The 1:10 dilution in ***Salmonella* Enrichment** broth is realized following the recommendations established in the EN ISO 6579 standard.

Pre-enrichment is performed by inoculating into ***Salmonella* Enrichment** and incubating for 16 to 22 hours.
Osmotic balance and buffered broth enable an optimal revivification of *Salmonella* strains.

Differentiation is performed by inoculation and incubation for 24 ± 3 hours of **SESAME *Salmonella* Detection**.
The excellent capacity of this semi-solid media to promote migration of *Salmonella*, combined with judicious selective agents allows the rapid identification of presumptive positive samples by simple visualization on the surface of the Petri plate.

Any needed confirmation steps are performed by inoculating onto **COMPASS® *Salmonella* Agar** and incubating for 24 ± 3 hours.

The principle of this solid chromogenic, selective culture media is based on the specific revelation of esterase and β-glucosidase, which for the former is exclusive to *Salmonella* and for the latter which is lacking for the vast majority of strains.

3 TYPICAL COMPOSITION

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

***Salmonella* Enrichment**
For 1 liter of media:

- Peptone ..... 10.00 g
- Sodium chloride ..... 5.00 g
- Phosphate buffers..... 5.06 g

pH of the ready-to-use media at 25 °C: 7.0 ± 0.2.

Note: The ***Salmonella* Enrichment** formula is compliant with Buffered Peptone Water.

SESAME *Salmonella* Detection
For 1 liter of media:

- Peptone ..... 4.59 g
- Acid hydrolysate of casein ..... 4.59 g
- Sodium chloride ..... 7.34 g
- Monopotassium phosphate..... 1.47 g
- Selective agents ..... 10.98 g
- Bacteriological agar ..... 2.70 g

pH of the ready-to-use media at 25 °C: 5.2 ± 0.2

COMPASS® *Salmonella* Agar
For 1 liter of media:

- Peptone ..... 10.00 g
- Sodium chloride ..... 5.00 g
- Phosphate buffer ..... 7.00 g
- Selective agents ..... 9.00 g
- Chromogenic mixture..... 1.40 g
- Bacteriological agar ..... 15.00 g

pH of the ready-to-use media at 25°C: 7.0 ± 0,2.

4 PREPARATION

Preparation from dehydrated media *Salmonella* Enrichment®:

- Dissolve 20.0 g of dehydrated media (BK194) in 1 liter of distilled or demineralized water.
- Mix slowly, until complete dissolution.
- Dispense into tubes of 9 mL or in vials of 225 mL.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution:**
20.0 g/L

✓ **Sterilization:**
15 min at 121°C

Preparation from dehydrated media SESAME *Salmonella* Detection:

- Dissolve 31.7 g of dehydrated media (BK195) 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 44-47 °C.
- Pour into sterile Petri plates.
- Let solidify on a cold, flat surface.
- Do not dry the plates after cooling.

✓ **Reconstitution:**
31.7 g/L

✓ **Sterilization:**
Boiling.

Notes on the use of ready-to-melt SESAME *Salmonella* Detection media

- Melt the agar (BM138) for the minimum amount of time necessary to achieve total liquefaction.
- Do not repeat this operation more than once.

Respect good laboratory practices (refer to NF EN ISO 7218).

INSTRUCTIONS FOR USE

- Introduce aseptically 25 g of the sample to be tested into 225 mL of ***Salmonella*** **Enrichment**.
- Homogenize or use a stomacher if needed.
- Incubate the broth at 37.0 ± 1.0 °C for 16 to 22 hours.
- Inoculate 3 drops (roughly 0.1 mL) of the culture coming from the ***Salmonella*** **Enrichment**, in the center of a plate of **SESAME *Salmonella* Detection**.
- Incubate at 41.5 ± 1.0 °C, for 24 ± 3 hours, without inverting the plates.

✓ **Enrichment:**
Dilution 1:10,
16 to 22h at 37°C

✓ **Detection:**
Reinoculate 3 drops
21 to 27 h at 41.5 °C

Notes:
SESAME *Salmonella* Detection can be stored for 72 h at 2-8°C after inoculation and incubation before performing subsequent confirmations.

Refer to different parts of ISO 6887 :

- Use ***Salmonella*** **Enrichiment with Tween®** for the initial suspension and enrichment of matrices containing more than 20% fat.
- Use ***Salmonella*** **Enrichment double strength** or ***Salmonella*** **Enrichment** for acidic and acidifying matrices.
- Add 0.1 g/L α-amylase for infant cereals.

RESULTS

- The appearance of a white, opaque halo with a diameter equal or superior to 30 mm, at the inoculation point indicates the presumptive presence of *Salmonella*.

CONFIRMATION

In the context of NF VALIDATION, positive results must be confirmed by one of following methods:

Standardized methods or ISO 16140-6 validated methods

- Use the classic tests described in the methods standardised by CEN or ISO (including the purification step), starting from a characteristic colony isolated on **SESAME *Salmonella* Detection**.

- Use methods validated acc. ISO 16140-6, starting from a characteristic colony isolated on SESAME *Salmonella* Detection

NF Validation certified methods

- **Option 1:** performing classical tests described in standardized methods such as CEN, ISO or AFNOR (including purifications steps), take a fraction of bacterial culture in the migration area obtained on SESAME *Salmonella* Detection.

- **Option 2:** by using **COMPASS® *Salmonella* Agar**.

  Take a fraction of bacterial culture in the migration area obtained on SESAME *Salmonella* Detection and inoculate by steaking on COMPASS® *Salmonella* Agar.
  Incubate at 37 ± 1 °C for 24 ± 3 hours.
  Salmonella produces magenta colonies on COMPASS® *Salmonella* Agar.

Notes on the use of COMPASS® *Salmonella* Agar
Certain strains of servovars Dublin & Atento, as well as some from the subspecies *S. houtenae*, *S. bongori* and *S. diarizonae*, can present a weak to nil magenta pigmentation, resulting from the weak esterase activity that characterizes these strains.

- **Option 3:** using another certified NF VALIDATION method that uses a different principle of detection. The validated protocol used in this second method must be followed in its entirety; all the steps preceding the start off point for the confirmation step must be common to the two methods.
  The two certified methods (one used for detection, the other for confirmation) must therefore have a common core.

In the event of discordant results (positive with the alternative method but lacking confirmation with one of the options listed above), the laboratory must perform the necessary steps to assure the validity of the results.

6 QUALITY CONTROL

Typical culture response after 18 h of incubation at 37 °C in ***Salmonella*** **Enrichment**, followed by 24 h of incubation at 41.5 °C on **SESAME *Salmonella* Detection**, and subculture on **COMPASS® *Salmonella* Agar** for 24 h at 37 °C:

Microorganisms		Growth on **SESAME *Salmonella* Detection**	Cultural characteristics after subculturing on **COMPASS® *Salmonella* Agar**
Salmonella Enteritidis + *Escherichia coli* + *Pseudomonas aeruginosa*	WDCM 00030 WDCM 00013 WDCM 00025	White culture, opaque ≥ 30mm	Magenta colonies
Salmonella Typhimurium + *Escherichia coli* +*Pseudomonas aeruginosa*	WDCM 00031 WDCM 00012 WDCM 00025	White culture, opaque ≥ 30mm	Magenta colonies
Escherichia coli	WDCM 00013	Inhibited	
Enterococcus faecalis	WDCM 00087	Inhibited	

7 STORAGE / SHELF LIFE

Salmonella **Enrichment:**
Dehydrated media: 2-30 °C.
Ready-to-use media in vials or flexible bags: 2-25 °C.

Prepared media (*): 6 months at 2-25 °C

SESAME *Salmonella* Detection
Dehydrated media: 2-25 °C.
Ready-to-melt media in vials: 2-8 °C, out of light.
Pre-poured media in Petri plates (∅ 90 mm): 2-8 °C, out of light.
Prepared media in plates (*): 15 days at 2-8 °C, out of light.

COMPASS *Salmonella* Agar
Pre-poured media in Petri plates (∅ 90 mm): 2-8 °C, out of light.

The expiration dates are indicated on the labels.
(*)Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

8 PACKAGING

Salmonella **Enrichment:**
500 g bottle..... BK194HA
5 kg drum BK194GC
10 x 225 mL vials..... BM13608
3 x 3 L flexible bags..... BM13708
2 x 5 L flexible bags..... BM14408
40 x 5 L flexible bags..... BM23708

***Salmonella* Enrichment + Tween® 80:**

3 x 3 L flexible bags BM16308
2 x 5 L flexible bags BM19808
10 vials of 225mL BM21608

***Salmonella* Enrichment Double-Strength Buffered**

500 g bottle BK225HA
5 kg drum BK225GC
2 x 5 L flexible bags BM20008
10 vials of 225 mL BM20108

SESAME *Salmonella* Detection:

500 g bottle BK195HA
10 x 200 mL vials BM13808
20 plates (∅ 90 mm) BM14108
120 plates (∅ 90 mm) BM15008

COMPASS® *Salmonella* Agar:

20 plates (∅ 90 mm) BM06608
120 plates (∅ 90 mm) BM23008

9 BIBLIOGRAPHY

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NF EN ISO 6579-1. April 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.

NF EN ISO 16140-2. July 2016. Microbiology of the food chain - Method validation - Part 2 : protocol for the validation of alternative (proprietary) methods against a reference method.

NF EN ISO 7218. October 2007. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.

NF EN ISO 7218/A1. October 2013. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations - Amendment 1.

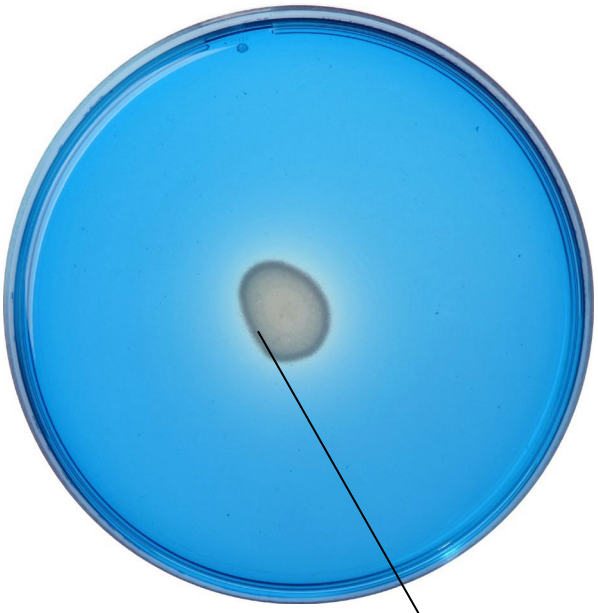
ISO 6887. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1 to 6.

10 ADDITIONAL INFORMATION

Document code : SESAME SALMONELLA_EN_V11
Creation date : 04-2003
Updated : 10-2023
Origin of revision : Re-formatting related to renewal, additions and deletions of packaging.

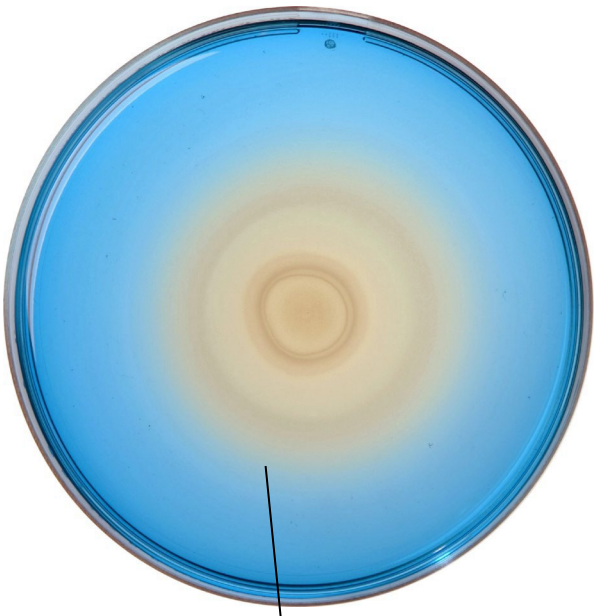
SESAME *SALMONELLA* DETECTION

Detection of mobile *Salmonella*



Non characteristic aspect

If growth, only at the point of inoculation. No opaque halo.



Salmonella sp.

Characteristic aspect

White culture and opaque halo centered on the point of inoculation.

SESAME *Salmonella* Detection

Incubation 24 hours at 41.5°C (inoculation point at the center of the plate) Characteristic aspect forming an opaque halo centered on the point of inoculation (Capacity to migrate in a gel matrix)