

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.

The term of validity is set at 04/12/2023.



BKR 23/04-12/07
METHODES ALTERNATIVES D'ANALYSE
POUR L'AGROALIMENTAIRE
Certifié par 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. This liquid medium allows a proper osmotic balance and its buffered formula enables an optimal resuscitation 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 inoculation onto **COMPASS® Salmonella Agar** with incubation requiring 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.

5 PROTOCOL CERTIFIED NF VALIDATION

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

Note on cold storage:

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

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 several 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 streaking on COMPASS® Salmonella Agar.
Incubate at 37 ± 1 °C for 24 ± 3 hours.
Salmonella produce magenta colonies on COMPASS® *Salmonella* Agar.

Notes on the use of COMPASS® *Salmonella* Agar

Certain strains of serovars 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** : though the use of 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 : for example, an enrichment medium common to both.

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
<i>Salmonella</i> Enteritidis + <i>Escherichia coli</i> + <i>Pseudomonas aeruginosa</i>	WDCM 00030 WDCM 00013 WDCM 00025	White culture, opaque ≥ 30mm	Magenta colonies
<i>Salmonella</i> Typhimurium + <i>Escherichia coli</i> + <i>Pseudomonas aeruginosa</i>	WDCM 00031 WDCM 00012 WDCM 00025	White culture, opaque ≥ 30mm	Magenta colonies
<i>Escherichia coli</i>	WDCM 00013	Inhibited	
<i>Enterococcus faecalis</i>	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

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 (Ø 90 mm)	BM14108
120 plates (Ø 90 mm)	BM15008

COMPASS® Salmonella Agar:

20 plates (Ø 90 mm)	BM06608
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9 BIBLIOGRAPHY

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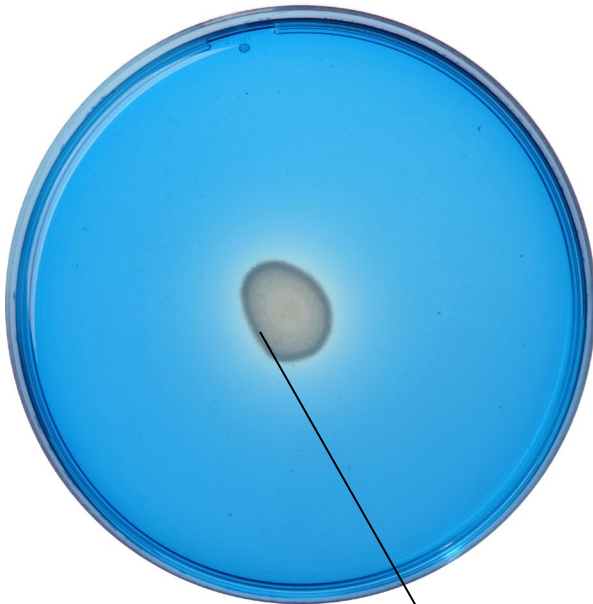
10 ADDITIONAL INFORMATION

COMPASS® & SESAME Salmonella TEST® are trademarks of SOLABIA S.A.S.

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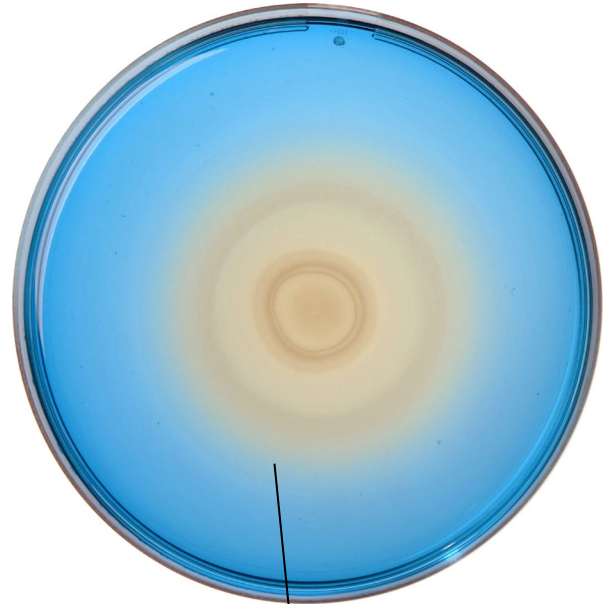
SESAME® SALMONELLA DETECTION

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)