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 (with the exception of those from animal production).

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 hour incubation.

SESAME *Salmonella* TEST® is officially certified NF VALIDATION, under the reference number BKR 23/04-12/07, of which the validity runs until December 4th , 2019.

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 18 ± 2 hours.
This liquid medium allows a proper osmotic balance and its buffered formula enables an optimal resuscitation of *Salmonella* strains.

The differentiation or detection stage is possible through the inoculation of the enrichment media into **SESAME Salmonella Detection®**, followed by incubation for 24 ± 3 hours.
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. Do Not Autoclave

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 18 ± 2 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,
18 h at 37°C

✓ **Detection :**
Reinoculate 3 drops
24 h at 41,5 °C

Note on cold storage :
SESAME® *Salmonella* Detection can be stored for 72 h at 4 °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 steaking.
  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 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.
Rare strains of *Enterobacter* may present the esterase activity and produce blue-green colonies surrounded with a magenta coloration.

- **Option 3** : though the use of another certified NF VALIDATION method, that uses a different principle of detection from that used in *SESAME® Salmonella TEST*. 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**
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 (*) : 180 days at 2-25 °C

SESAME ***Salmonella*** **Detection**
Dehydrated media : 2-30 °C.
Ready-to-melt media in vials : 2-8 °C.
Pre-poured media in Petri plates (∅ 90 mm) : 2-8 °C.
Prepared media in plates (*) : 15 days at 2-8 °C.

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

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

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

9 BIBLIOGRAPHY

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HUMBERT, F., LALANDE, F., ROSE, V., et SALVAT, G.. 1998. Evaluation d'un nouveau milieu d'isolement pour la mise en évidence des salmonelles dans les élevages et les denrées d'origine animale. 5ème congrès de la Société Française de Microbiologie. 128.

NF EN ISO 6579. Décembre 2002. Microbiologie des aliments - Méthode horizontale pour la Recherche des *Salmonella* spp. Modifiée en Octobre 2007 par l'amendement A1 : annexe D : recherche de *Salmonella* spp. dans les matières fécales des animaux et dans des échantillons environnementaux au stade de la production primaire.

NF EN ISO 16140. Octobre 2003. Microbiologie des aliments. Protocole pour la validation des méthodes alternatives. Modifiée en Octobre 2011 par l'amendement A1.

NF EN ISO 7218. Octobre 2007. Microbiologie des Aliments. Exigences générales et recommandations. Modifiée en Décembre 2013 par l'amendement A1.

10 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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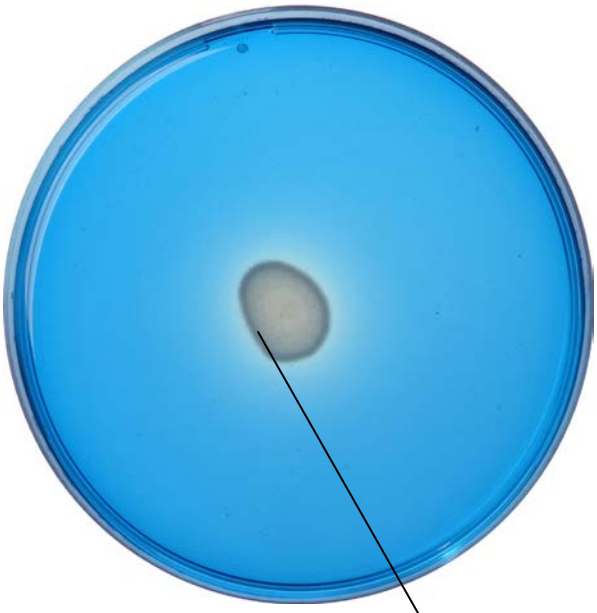
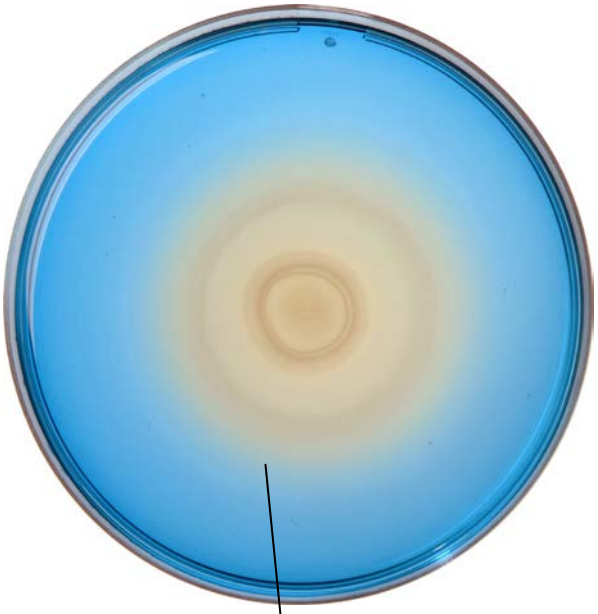
SESAME® *SALMONELLA* DETECTION

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)