

# IRIS *SALMONELLA*<sup>®</sup>

## DETECTION METHOD FOR *SALMONELLAE*

### 1 INTENDED USE

**IRIS *Salmonella*<sup>®</sup>** is an alternative research method of *Salmonellae* in human food and feeds, and environmental sample (except primary production samples).

Studies performed on **IRIS *Salmonella*<sup>®</sup>** Agar show a high specificity for the detection of *Salmonellae* including atypical species and serovars, which is a source of confusion on other medium.

Indeed, the detection of *Salmonella* Typhi and Paratyphi, lactose-positive *Salmonellae* (*Salmonella* Senftenberg and subspecies *S. arizonae* and *S. diarizonae*), saccharose-positive strains are ensured.

The media allows the detection of non-motile serovars (*S. Pullorum* and *S. Gallinarum*) or monophasic strains. **IRIS *Salmonella*<sup>®</sup>** Agar allows also the detection of strains which show a light or absence of esterase activity on other medium (*Salmonella bongori*, *Salmonella* Dublin and Atento, certain strains of *S. enterica*, *S. houtenae* and *S. diarizonae* subspecies).

**IRIS *Salmonella*<sup>®</sup>** method is officially certified by AFNOR Certification, under the reference number BKR 23/07-10/11, for all human and animal food products and production and production environment samples (excluding primary production environment samples). The certification validity runs until 07 July 2019.

**IRIS *Salmonella*<sup>®</sup>** is also validated for the detection of *Salmonellae* in samples from 50 g to 375 g for milk powder, including infant milk with and without probiotics and from 50 g to 125 g for flours and croquettes in animal feedstuffs.



BKR 23/07-10/11  
METHODES ALTERNATIVES D'ANALYSE  
POUR L'AGROALIMENTAIRE  
Certifié par AFNOR Certification <http://nf-validation.afnor.org/>

**IRIS *Salmonella*<sup>®</sup>** Agar may be used in the standard methods for the detection of *Salmonellae* as second isolation medium.

### 2 PRINCIPLES

The method allows the detection of motile and non-motile *Salmonellae*.

Analysis may be declared negative after 37 hours of enrichment (***Salmonella* Enrichment**) and differentiation (**IRIS *Salmonella*<sup>®</sup> Agar**) steps.

The 1/10 dilution step of the sample is performed in ***Salmonella* Enrichment** broth according to NF EN ISO 6579 recommendations.

The enrichment step is done with the addition of the **IRIS *Salmonella*<sup>®</sup> Selective Supplement** in the broth and sample mix. The obtained ***Salmonella* Enrichment** broth is incubated for 16 to 24 hours at 41.5 ± 1.0 °C for the general.

The differentiation step is performed by inoculation of 10 µL onto **IRIS *Salmonella*<sup>®</sup> Agar** and incubated for 21 hours at 37°C.

*Salmonella* colonies are magenta whereas other species are blue-violet or uncolored.

The selective agents permit the inhibition of Gram-positive and some Gram-negative bacteria.

An eventual confirmation step may be done by classical tests described in standard methods or by a Latex test directly from an isolated magenta colony from **IRIS *Salmonella*<sup>®</sup> Agar**

*Salmonella* give rise to magenta colonies.

The selective agents insure the inhibition of Gram positive and certain Gram negative bacteria.

The secondary flora presents blue, purple or uncolored colonies.

An eventual confirmation step can be performed by classical tests described in the normalized method or by a Latex test directly onto a magenta colony isolated from **IRIS *Salmonella*<sup>®</sup> Agar**.

### 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 buffer ..... 5,06 g

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

**Note:** The composition of **Salmonella Enrichment** conforms to that of Buffered Peptone Water.

#### **IRIS *Salmonella*<sup>®</sup> Supplement (Qsp 225 mL)**

For 1 tablet :

- Selective system ..... 0,1 g
- Coloring agent..... 0,005 g

#### **IRIS *Salmonella*<sup>®</sup> Supplement (Qsp 90 mL)**

For 1 tablet :

- Selective system ..... 0,04 g
- Coloring agent..... 0,002 g

#### **IRIS *Salmonella*<sup>®</sup> Liquid Supplement**

For 1 vial of 50 mL :

- Selective system ..... 2 g
- Coloring agent..... 0,1 g

#### **IRIS *Salmonella*<sup>®</sup> Agar**

For 1 liter of media :

- Peptone ..... 10,0 g
- Yeast extract .....5,0 g
- Sodium chloride ..... 5,0 g
- Phosphate buffer..... 7,0 g
- Selective agents..... 10,2 g
- Chromogenic mixture ..... 1,0 g
- Bacteriological agar..... 16,0 g
- Opacifying agents ..... 6,5 g

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

### 4 PREPARATION

#### **Preparation of dehydrated media *Salmonella* Enrichment :**

- Dissolve 20,0 g of dehydrated media (BK194) in 1 liter of distilled or demineralized water.
- Mix well, until complete dissolution.
- Distribute 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 of IRIS *Salmonella*<sup>®</sup> Agar dehydrated media :

- Dissolve 60.7 g of dehydrated media (BK212) in 1 liter of distilled or deionized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Maintain at boil for exactly 2 minutes.
- Do not overheat.
- Do not autoclave.
- Dispense the appropriate amount into Petri dishes.

✓ **Reconstitution :**  
60.7 g/L

✓ **Maintain at boil 2 minutes.**  
**Do not overheat**  
**Do not autoclave**

## 5 INSTRUCTIONS FOR USE

Always use good laboratory practices.  
Refer to standard NF EN ISO 7218.

### NF VALIDATION-certified protocol for samples up to 25g (human food, feedstuffs and environmental samples) :

- Introduce aseptically (x) g of the sample to be tested into 9 (x) mL of ready-to-use *Salmonella* Enrichment.
- Introduce the IRIS *Salmonella*<sup>®</sup> Liquid Supplement at the rate of 0.1 mL/g of sample (i.e. 2.5 mL for 25 g) for the liquid supplement (BS078).
- For 25 g of sample, introduce directly one tablet of IRIS *Salmonella*<sup>®</sup> Supplement (BS077) in 225 mL of broth.
- For 10 g of sample, introduce directly one tablet of IRIS *Salmonella*<sup>®</sup> Supplement (BS093) in 90 mL of broth.
- Mix well or use a stomacher if necessary.
- Incubate the broth at 41,5 ± 1,0 °C for **16 to 24 hours**.
- Re-streak 10 µL of the enrichment onto the surface of IRIS *Salmonella*<sup>®</sup> Agar.
- Incubate at 37 ± 1 °C for 24 hours ± 3 hours.

✓ **Enrichment :**  
1 :10 dilution,  
16-24 h at 41.5 °C

✓ **Detection :**  
Re-streak 10 µL,  
24 h at 37 °C

### Note on cold storage of media :

The enrichment broth, after incubation, can be kept up to 3 days at 2-8°C before re-streaking onto IRIS *Salmonella*<sup>®</sup> Agar. In the same way, after incubation, the IRIS *Salmonella*<sup>®</sup> Agar plates may be kept up to 3 days à 2-8°C before reading and subsequent confirmations.

In the context of NF VALIDATION, samples greater than 25 g have not been tested.

### NF VALIDATION-certified protocol for milk powder (including infant milk with and without probiotics) from 50 to 375 g

- Aseptically introduce (x) g of the sample to be tested into 9 (x) mL of *Salmonella* Enrichment preheated to 41,5°C.
- Introduce the IRIS *Salmonella*<sup>®</sup> Liquid Supplement (BS078) at the rate of 0.1 mL/g of sample (i.e. 37,5 mL for 375 g of sample) .
- Mix well or use a stomacher if needed.
- Incubate the broth at 41,5 ± 1,0 °C for **18 to 24 hours**.
- Re-streak 10 µL of the enrichment onto the surface of IRIS *Salmonella*<sup>®</sup> Agar.
- Incubate at 37 ± 1 °C for 24 hours ± 3 hours.

✓ **Enrichment :**  
1 :10 dilution,  
18-24 h at 41.5 °C

✓ **Detection :**  
Re-streak 10 µL,  
24 h at 37 °C

### Note on cold storage of media :

The enrichment broth, after incubation, can be kept up to 3 days at 2-8°C before re-streaking onto IRIS *Salmonella*<sup>®</sup> Agar. In the same way, after incubation, the IRIS *Salmonella*<sup>®</sup> Agar plates may be kept up to 3 days à 2-8°C before reading and subsequent confirmations

In the context of NF VALIDATION, only samples between 50 g and 375 g have been tested for the milk powder (including infant milk with or without probiotics).

### NF VALIDATION-certified protocol for flours and croquettes in animal feedstuffs, from 50 to 125 g

- Aseptically introduce (x) g of the sample to be tested into 9 (x) mL of *Salmonella* Enrichment preheated to 41,5°C.
- Introduce the IRIS *Salmonella*<sup>®</sup> Liquid Supplement (BS078) at the rate of 0.1 mL/g of sample (i.e. 12,5 mL for 125 g sample) .

- Mix well or use a stomacher if needed.
- Incubate the broth at  $41,5 \pm 1,0$  °C for **18 to 24 hours**.
- Re-streak 10 µL of the enrichment onto the surface of **IRIS Salmonella® Agar**.
- Incubate at  $37 \pm 1$  °C for 24 hours  $\pm$  3 hours.

✓ **Enrichment :**  
**1 :10 dilution,**  
**18-24 h at 41.5 °C**

✓ **Detection :**  
**Re-streak 10 µL,**  
**24 h at 37 °C**

**Note on cold storage of media :**

After incubation, the **IRIS Salmonella® Agar** plates may be kept up to 3 days à 2-8°C before reading and subsequent confirmations.

In the context of NF VALIDATION, only those samples between 50 g and 125 g have been tested for the flours and croquettes in animal feedstuffs.

## 6 RESULTS

Colony appearance on **IRIS Salmonella® Agar** is as follows :

Microorganisms	Characteristic colonies
<b>Salmonella spp.</b> (including <i>Salmonella</i> Typhi, Paratyphi, lactose-positive, saccharose-positive, immobile, monophasic, Dublin, <i>bongori</i> )	<b>Pink to Magenta</b>
<i>Escherichia coli</i>	Uncolored
<i>Enterobacter</i> spp., <i>Klebsiella</i> spp.	Blue-green to violet
<i>Proteus</i> spp.	Uncolored to brownish
Gram positive	Inhibited

See ANNEX 1 : PHOTO SUPPORT.

## 7 CONFIRMATION

In the context of NF VALIDATION, all positive results must be confirmed with one of the following protocols :

- Option 1 : Perform classical tests described in normalized methods CEN or ISO (including the purification step), starting from a magenta colony isolated on **IRIS Salmonella® Agar**.
- Option 2 : Perform the CONFIRM' *Salmonella* or de *Salmonella* Latex Test (Oxoïd) from an isolated magenta colony.

In the event of discordant results (presumptive positive by the alternative method, not confirmed by one of the above options, and in particular by the latex test(s), the laboratory must insure by any means possible the validity of the results they transmit. It is possible, for example, to proceed with biochemical tests or DNA probes as described in the standard NF EN ISO 7218.

## 8 QUALITY CONTROL

Typical culture response after 24 hours of incubation at 37 °C on **IRIS Salmonella® Agar** :

Microorganisms	Growth
<i>Salmonella</i> Typhimurium WDCM 00031	Good, magenta colonies
<i>Salmonella</i> Enteritidis WDCM 00030	Good, magenta colonies
<i>Enterobacter aerogenes</i> WDCM 00175	Good, blue colonies
<i>Escherichia coli</i> WDCM 00013	Partially inhibited, uncolored colonies
<i>Staphylococcus aureus</i> WDCM 00034	Inhibited
<i>Pseudomonas aeruginosa</i> WDCM 00025	Inhibited

## 9 STORAGE / SHELF LIFE

**Salmonella Enrichment :**

Dehydrated media : 2-30 °C.

Ready-to-use media in vials or flexible bags : 2-25 °C.

## **IRIS Salmonella<sup>®</sup> Supplement**

Liquid supplement : 2-8 °C.

Tablets : 2-8 °C.

## **IRIS Salmonella<sup>®</sup> Agar :**

Pre-poured media in Petri plates (Ø 90 mm) : 2-8 °C.

Dehydrated **IRIS Salmonella<sup>®</sup> Agar** : 2-8 °C.

Prepared medium in plates (benchmark value\*) : 1 month at 2-8 °C, shielded from light.

(\*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

## **CONFIRM' Salmonella :**

Kit : 2-8 °C.

The expiration dates are indicated on the labels.

## **10 PACKAGING**

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### **Salmonella Enrichment :**

500 g bottle .....	BK194HA
5 kg drum .....	BK194GC
10 x 225 mL vials .....	BM13608
3 x 3 L flexible bag .....	BM13708
2 x 5 L flexible bag .....	BM14408

### **Salmonella Enrichment + Tween<sup>®</sup> 80 :**

3 x 3 L flexible bag .....	BM16308
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### **IRIS Salmonella<sup>®</sup> Supplement :**

10 x 50 mL vials .....	BS07808
120 tablets Qsp 225 mL .....	BS07708
120 tablets Qsp 90 mL .....	BS09308

### **IRIS Salmonella<sup>®</sup> Agar :**

20 plates (Ø 90 mm) .....	BM16008
120 plates (Ø 90 mm) .....	BM16108
500 g bottle .....	BK212HA

### **Latex Agglutination test :**

CONFIRM' Salmonella .....	BT01108
Oxoid Salmonella Latex Test .....	

## **11 BIBLIOGRAPHY**

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NF EN ISO 6579-1. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1 : detection of Salmonella spp.

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.

## **12 ADDITIONAL INFORMATION**

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**IRIS Salmonella<sup>®</sup>** is a registered trademark of SOLABIA S.A.S.

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.

Document code : IRIS SALMONELLA\_ENv4  
Creation date : 02-2011  
Updated : 07-2017  
Origin of revision : Update of bibliography, addition of tablets Qsp 90 mL.

## IRIS *Salmonella*<sup>®</sup> Agar

Detection of *Salmonella*

Growth obtained after 24 hours of incubation at 37 °C.

