

# IRIS SALMONELLA®

## DETECTION METHOD FOR SALMONELLAE

### 1 INTENDED USE

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

Studies performed on **IRIS Salmonella®** 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®** 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®** is NF VALIDATION certified, according to the validation protocol NF EN ISO 16140-2 of 2016 for all human food and animal food products, as well as samples of the industrial production environment. The reference method used for validation is the NF EN ISO 6579-1 standard of 2017.

**IRIS Salmonella®** 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.

The method is also NF VALIDATION certified with the CSD supplement for the following categories:

- Infant milk powders, with and without probiotics; ingredients for test samples of up to 50 g, with a 1/10th dilution.
- Infant milk powders, with and without probiotics for test samples of 50 to 375 g, with a dilution to 1/4
- Samples of the production environment

The term of validity is 07 October 2023.



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

**IRIS Salmonella® 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® 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® Selective Supplement** in the broth and sample mix. The obtained **Salmonella Enrichment** broth is incubated for 16 to 24 hours at  $41.5 \pm 1.0$  °C for the general.

The differentiation step is performed by re-streaking the broth on IRIS Salmonella® Agar and incubating for 21 hours at 37 ° C.

*Salmonella* colonies are magenta.

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

The secondary flora presents blue, purplish or colourless colonies.

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® Agar**

### 3 TYPICAL COMPOSITION

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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:  $7.0 \pm 0.2$ .

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

#### **Salmonella Enrichment double-strength buffered**

For 1 liter of media:

- Peptone .....	10.00 g
- Sodium chloride .....	5.00 g
- Phosphate buffer .....	10.12 g

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

#### **IRIS Salmonella® 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:  $7.0 \pm 0.2$ .

### 4 PREPARATION

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#### **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.
- Divide according to the intended use into tubes or vials so that the mother suspension can be made up to 1/10th or ¼.
- 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 dehydrated *Salmonella* Enrichment double-strength buffered:

- Dissolve 25.1 g of dehydrated media (BK225) in 1 liter of distilled or demineralized water.
- Mix well, until complete dissolution.
- Divide according to the intended use into tubes or vials so that the mother suspension can be made up to 1/10th or ¼.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution:**  
25.1 g/L

✓ **Sterilization:**  
15 min at 121°C

### Preparation of IRIS *Salmonella*® 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.
- Cool to room temperature and pour into Petri dishes.
- Cool on a flat surface.

✓ **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.

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

#### Notes :

- Use *Salmonella* Enrichment with Tween for mother suspension and enrichment of matrices with more than 20% fat.
- Use *Salmonella* Enrichment double-strength buffered or *Salmonella* Enrichment for acidic and acidifying matrices.
- Introduce the **IRIS *Salmonella*® 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*® Supplement** (BS077) in 225 mL of broth.  
For 10 g of sample, introduce directly one tablet of **IRIS *Salmonella*® 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*® Agar**.
- Incubate at 37 ± 1 °C for 24 hours ± 3 hours.

✓ **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*® Agar** for all human food and samples of the industrial production environment. In the same way, 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, 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® 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® 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® Agar**.
- In the same way, after incubation, the **IRIS Salmonella® Agar** plates may be kept up to 3 days à 2-8°C before reading and subsequent confirmations

**Note on acid matrices**

In the context of NF VALIDATION, it is possible to use Salmonella Enrichment or Salmonella Enrichment double-strength buffered for the mother suspension of acidic and acidifying matrices.

**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® 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 ± 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 ± 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

**Notes**

- The IRIS agar, after incubation, can be stored for up to 3 days at 2-8 °C before reading and possible confirmation.
- Within the framework of the NF VALIDATION mark, only test intakes between 50 g and 125 g have been tested for flours or croquettes in animal feed.

**NF VALIDATION certified protocol for infant milk powders, with and without probiotics, ingredients and environmental products, up to 50 g :**

- Aseptically introduce (x) g of test material into 9 (x) mL of Salmonella Enrichment.
- Introduce the CSD supplement at a rate of 0.1 mL of liquid supplement BS095 per gram of sample (i.e. 2.5 mL per 25 g).
- Homogenize or stomacher if necessary.
- Incubate broth at 41.5 ± 1.0 °C for **16 to 22 hours**.
- Isolate 10 µL from the enrichment obtained on IRIS Salmonella agar.
- Incubate at 37 ± 1 °C for 24 ± 3 hours.

✓ **Enrichissement:**  
1 :10 dilution,  
16-22 h at 41.5 °C

✓ **Detection :**  
Isolement 10 µL,  
24 h at 37 °C

## NF VALIDATION certified protocol for infant milk powders, with and without probiotics and ingredients, from 50 g to 375 g :

- Aseptically introduce (x) g of test material into 3 (x) mL of pre-warmed Salmonella Enrichment.
- Introduce the CSD supplement (BS095) at a rate of 0.1 mL per gram of sample (i.e. 37.5 mL per 375 g).
- Homogenize or stomacher if necessary.
- Incubate the broth at 41.5 ± 1.0 °C for **18 to 24 hours**.
- Isolate 10 µL from the enrichment obtained on IRIS Agar
- Incubate at 37 ± 1 °C for 24 ± 3 hours.

✓ **Enrichissement:**  
At 1/4,  
18-24 h at 41.5 °C

✓ **Detection :**  
Isolement 10 µL,  
24 h at 37 °C

### Notes concerning NF VALIDATION certified protocols

- One CSD Qsp 10 g tablet (BS09908) may be added to Salmonella Enrichment for 10 g test intakes.
- One CSD Qsp 25 g tablet (BS10008) can be added to Salmonella Enrichment for 25 g test intakes.
- Several tablets can be added to the broth in accordance with the test sample (e.g. 3 BS100 tablets for a 75g test sample).

Refer to the different parts of the ISO 6887 standard:

- Use Salmonella Enrichment with Tween for the mother suspension and enrichment of matrices with more than 20 % fat content
- Use Salmonella Double Buffered Enrichment or Salmonella Enrichment for acid and acidifying matrices, or milk with probiotics.
- Add 0.1 g/L of α amylase for infant cereals.

For surface samples after cleaning, which may contain residues of disinfectants, it is recommended to use 10% universal neutralisers and 90% Salmonella Enrichment before adding the CSD supplement. It is also possible to use swabs, sponges or cloths already soaked in neutralising solution.

- The enrichment broth, after incubation, can be stored for up to 3 days at 2-8°C before transfer to IRIS Salmonella Agar.
- IRIS Salmonella Agar, after incubation, can be stored for up to 3 days at 2-8°C before reading and possible confirmation.

## 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 spp.</i> , <i>Klebsiella spp.</i>	Blue-green to violet
<i>Proteus spp.</i>	Uncolored to brownish
Gram positive	Inhibited

See ANNEX 1: PHOTO SUPPORT.

## 7 CONFIRMATION

All presumed positive results must be confirmed in one of the following ways:

### Validated method or standardised ISO 16140-6

As IRIS Salmonella Agar is based on the detection of Salmonella C8 esterase activity, the following methods can be used:

- Implementation of the classical tests described in the CEN or ISO standard methods (including the purification step), starting from a magenta colony isolated on IRIS Salmonella.

- Implementation of methods certified according to ISO 16140-6 using characteristic colonies isolated on IRIS Salmonella.

### **Methods certified NF VALIDATION**

Within the framework of the NF VALIDATION mark, all positive results must be confirmed in one of the following ways:

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- Option 1: Implementation of the classical tests described in the CEN or ISO standard methods (including the purification step), starting from a magenta colony isolated on IRIS Salmonella® Agar.
- Option 2: Implementation of CONFIRM' Salmonella or Salmonella Latex Test (Thermo Fisher) from an isolated magenta colony.
- Option 3: Use of any other NF VALIDATION certified method, of a different principle. The validated protocol of the second method will have to be respected as a whole, i.e. all the steps prior to the intermediate step from which the confirmation starts again must be common to both methods. The two validated methods (one used in detection and the other in confirmation) must therefore have a common core.

In the event of conflicting results (presumptive positives by the alternative method, not confirmed by one of the options described above, and in particular by the latex test(s)), the laboratory shall implement sufficient means to ensure the validity of the result returned. It is possible, for example, to carry out biochemical tests or to use nucleic probes as described in 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, Salmonella Enrichment double-strength buffered:**

Dehydrated media: 2-30 °C.

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

### **Salmonella Enrichment with Tween:**

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

### **IRIS Salmonella® Supplement**

Liquid supplement: 2-8 °C.

Tablets: 2-8 °C.

### **CSD® Supplement**

Liquid supplement: 2-8 °C.

Tablets : 2-8 °C.

### **IRIS Salmonella® Agar:**

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

Dehydrated **IRIS Salmonella® Agar**: 2-8 °C.

### **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® 80 (10 g/L):**

3 x 3 L flexible bag .....	BM16308
2 x 5 L flexible bag .....	BM19808
10 x 225 mL bottles.....	BM21608

### **Salmonella Enrichment double-strength buffered:**

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

### **IRIS Salmonella® Supplement:**

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

### **Supplément CSD :**

10 vials of 100 mL.....	BS09508
Tablets Qsp 10 g .....	BS09908
Tablets Qsp 25 g.....	BS10008

### **IRIS Salmonella® Agar:**

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

### **Latex Agglutination test:**

CONFIRM' Salmonella.....	BT01108
Oxoïd Salmonella Latex Test.....	

## 11 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 spp. - Part 1: Horizontal method for the detection of Salmonella spp.

NF EN ISO 16140-2. September 2016. Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (commercial) methods to a reference method - Food microbiology

ISO 16140-6: 2019. Microbiology of the food chain - Method validation - Part 6: Protocol for the validation of alternative (commercial) methods for microbiological confirmation and typing.

NF EN ISO 7218. October 2007. Food Microbiology. General requirements and recommendations. Amended in December 2013 by amendment A1.

ISO 6887. Microbiology of the food chain. Preparation of samples, stock suspension and decimal dilutions for microbiological examination. Parts 1 to 6.

## 12 ADDITIONAL INFORMATION

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**IRIS Salmonella®** 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.

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## IRIS *Salmonella*<sup>®</sup> Agar

Detection of *Salmonella*

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

