

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.

The term of validity is 07 October 2023.

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 validated for test portions of 50 g to 375 g for milk powders (including infant milk powders with and without probiotics) and from 50 g to 125 g for flours and croquettes used in animal feedstuffs.



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METHODES 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 ± 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 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® Agar**

Salmonella give rise to magenta colonies.

The selective agents insure the inhibition of Gram positive and some 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® 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.

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 ± 0.2.

IRIS Salmonella® Supplement (Qsp 225 mL)

For 1 tablet:

- Selective system	0.1 g
- Coloring agent.....	0.005 g

IRIS Salmonella® Supplement (Qsp 90 mL)

For 1 tablet:

- Selective system	0.04 g
- Coloring agent.....	0.002 g

IRIS Salmonella® Liquid Supplement

For 1 vial of 50 mL:

- Selective system	2 g
- Coloring agent.....	0.1 g

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 ± 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 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.
- 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:**
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.
- Dispense the appropriate amount 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.

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.

✓ **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® 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

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

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
Salmonella spp. (including <i>Salmonella</i> Typhi, Paratyphi, lactose-positive, saccharose-positive, immobile, monophasic, Dublin, <i>bongori</i>)	Pink to Magenta
<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 (Oxoid) from an isolated magenta colony.
- Option 3: Use of any other method certified NF VALIDATION, of different principle. The validated protocol of the second method will have to be respected as a whole, that is to say that all the steps preceding the intermediate step of which one leaves for the confirmation must be common to the two methods. The two validated methods (one used in detection and the other in confirmation) must therefore have a common core.

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, 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.

IRIS Salmonella® Agar:

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

Dehydrated **IRIS Salmonella® 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

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

IRIS Salmonella® Agar:

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

Latex Agglutination test:

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

11 BIBLIOGRAPHY

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

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*[®] Agar

Detection of *Salmonella*

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

