TECHNICAL DATA SHEET

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 medium 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 esterasic activity on other medium (*Salmonella* bongori, *Salmonella* Dublin and Atento, certain strains of *S. enterica*, *S. houtenae* and *S. diarizonae* subspecies).

The method is NF VALIDATION certified, with the IRIS *Salmonella*® supplement, according to the NF EN ISO 16140-2 validation protocol of 2016 for the following categories:

- All food products (from 0 to 25 g)
- All animal feed products (from 0 to 25 g), animal meals and kibbles (from 50 g to 125 g)
- All samples from the industrial production environment
- Milk powders (including infant milk powders with and without probiotics) from 50 to 375 g

The method is also certified NF VALIDATION with the CSD supplement according to the validation protocol NF EN ISO 16140-2 of 2016 for the following categories:
- Infant milk powders, with and without probiotics; ingredients for test intakes up to 50 g, with a 1/10th dilution
- Infant milk powders, with and without probiotics for test intakes of 50-375 g, at 1/4 dilution
- Samples from the production environment

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

Refer to the certificate available on the NF VALIDATION website for the end date of validity of the method.
The reference method used for the validation is the standard NF EN ISO 6579-1 of 2017.

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

The **CSD supplement allows** the common **enrichment** of ***Salmonella*** and ***Cronobacter*** in infant milk powders with and without probiotics, ingredients, and environmental products.
Refer to the CSD method, BKR 23/12-12/20, certified NF VALIDATION, for the detection of *Cronobacter spp.*

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 performed by adding the IRIS *Salmonella*® selective supplement to the broth previously mixed with the sample to be analyzed. After this addition, the enrichment broth turns green.
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.
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

The composition can be adjusted in order to obtain optimal performance.

Salmonella Enrichment
For 1 liter of medium:

- Peptone ..... 10.00 g
- Sodium chloride ..... 5.00 g
- Phosphate buffer ..... 5.06 g

pH of the ready-to-use medium 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 medium:

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

pH of the ready-to-use medium at 25 °C: 7.0 ± 0.2.

IRIS *Salmonella*® Agar
For 1 liter of medium:

- 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 medium at 25°C: 7.0 ± 0.2.

4 PREPARATION

Preparation of dehydrated medium *Salmonella* Enrichment:

- Dissolve 20.0 g of dehydrated medium (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 medium (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 medium:

- Dissolve 60.7 g of dehydrated medium (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 all food products (test samples up to 25 g)

Within the framework of the NF VALIDATION mark, test samples exceeding 25 g have not been tested.

- Aseptically introduce (x) g of sample to be analyzed into 9 (x) mL of *Salmonella* Enrichment.
- Introduce IRIS *Salmonella*® Liquid Supplement (BS078) at a rate of 0.1 mL per gram of sample (i.e. 2.5 mL per 25 g).
- Or introduce IRIS *Salmonella*® supplement (BS077) at a rate of 1 tablet per 25 g of sample
- Homogenize or stomacher if necessary.
- Incubate the broth at 41.5 ± 1.0 °C for 16 to 24 hours.
- Isolate 10 µL of the enrichment obtained on 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

NF VALIDATION certified protocols for animal feed

Protocol for test samples up to 25 g

- Aseptically introduce (x) g of sample to be analyzed into 9 (x) mL of *Salmonella* Enrichment.
- Introduce IRIS *Salmonella*® Liquid Supplement (BS078) at a rate of 0.1 mL per gram of sample (i.e. 2.5 mL per 25 g).
- Or introduce IRIS *Salmonella*® supplement (BS077) at a rate of 1 tablet per 25 g of sample
- Homogenize or stomacher if necessary.
- Incubate the broth at 41.5 ± 1.0 °C for **16 to 24 hours**.
- Isolate 10 µL of the enrichment obtained on 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

Protocol for flours or kibbles of animal feed, test samples ranging from 50 to 125 g

- Aseptically introduce (x) g of sample to be analyzed 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).
- Or introduce IRIS *Salmonella*® Liquid Supplement Concentrate (BS101, BS102) at a rate of 3 mL per 125 g of sample.
  Or introduce the IRIS *Salmonella*® supplement (BS077) at a rate of 2 tablets per 50 g of sample to 5 tablets per 125 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

NF VALIDATION certified protocol for infant milk powders, with and without probiotics

Protocol for test samples up to 25 g

- Aseptically introduce (x) g of sample to be analyzed into 9 (x) mL of *Salmonella* Enrichment.
- Introduce IRIS *Salmonella*® Liquid Supplement (BS078) at a rate of 0.1 mL per gram of sample (i.e. 2.5 mL per 25 g).
- Or introduce IRIS *Salmonella*® Supplement (BS077) at a rate of 1 tablet per 25 g of sample.
- Homogenize or stomacher if necessary.
- Incubate the broth at 41.5 ± 1.0 °C for **16 to 24 hours**.
- Isolate 10 µL of the resulting enrichment on IRIS *Salmonella*® Agar.
- Incubate at 37 ± 1 °C for 24 hours ± 3 hours.

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

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

Protocol for test samples including ingredients, up to 50 g

- Aseptically introduce (x) g of sample to be analyzed into 9 (x) mL of *Salmonella* Enrichment.
- Introduce the CSD supplement at a rate of 0.1 mL of BS095 liquid supplement per gram of sample (i.e. 2.5 mL for 25 g),
- Or introduce the CSD supplement (BS100) at a rate of 1 tablet per 25 g of sample and 2 tablets per 50 g of sample.
- Homogenize or stomacher if necessary.
- Incubate the broth at 41.5 ± 1.0 °C for **16 to 22 hours**.
- Isolate 10 µL of the enrichment obtained on IRIS *Salmonella* agar.
- Incubate at 37 ± 1 °C for 24 hours ± 3 hours.

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

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

NF VALIDATION certified protocol for infant milk powders, with and without probiotics - continued

Protocol for test samples ranging from 50 to 375 g, 1:10 dilution

- Aseptically introduce (x) g of sample to be analyzed in 9 (x) mL of *Salmonella* Enrichment pre-warmed to 41.5°C.
- Introduce the IRIS *Salmonella*® liquid supplement (BS078) at a rate of 0.1 mL per gram of sample (i.e. 37.5 mL for 375 g of sample),
  Or introduce IRIS *Salmonella*® Liquid Concentrate Supplement (BS101, BS102) at a rate of 9 mL per 375 g of sample (3 mL per 125 g; etc...),
  Or introduce IRIS *Salmonella*® supplement (BS077) at a rate of 2 tablets per 50 g of sample to 15 tablets per 375 g of sample.
- Homogenize or stomacher if necessary.
- Incubate broth at 41.5 ± 1.0 °C for **18 to 24 hours**.
- Isolate 10 µL of the resulting enrichment on IRIS *Salmonella*® Agar.
- Incubate at 37 ± 1 °C for 24 hours ± 3 hours.

✓ **Enrichment:**
At 1:4 dilution,
18-24 h at 41.5 °C

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

Protocol for test samples, including ingredients, ranging from 50 g to 375 g, 1:4 dilution

- Aseptically introduce (x) g of sample to be analyzed into 3 (x) mL of pre-warmed *Salmonella* Enrichment.
- Introduce CSD® Supplement (BS095) at a rate of 0.1 mL per gram of sample (i.e. 37.5 mL for 375 g).
  Or add CSD® supplement (BS100) at a rate of 2 tablets per 50 g of sample to 15 tablets per 375 g of sample,
- Homogenize or stomacher if necessary.
- Incubate the broth at 41.5 ± 1.0 °C for **18 to 24 hours.**
- Isolate 10 µL of the resulting enrichment on IRIS Agar.
- Incubate at 37 ± 1 °C for 24 hours ± 3 hours.

✓ **Enrichment:**
At 1:4 dilution,
18-24 h at 41.5 °C

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

NF VALIDATION certified protocols for environmental products

Protocol for test samples up to 25 g

- Aseptically introduce (x) g of sample to be analyzed into 9 (x) mL of Salmonella Enrichment.
- Introduce IRIS Salmonella® Liquid Supplement (BS078) at a rate of 0.1 mL per gram of sample (i.e. 2.5 mL per 25 g),
  Or introduce IRIS Salmonella® supplement at a rate of 1 tablet per 10 g of sample (BS093) or 1 tablet per 25 g of sample (BS077).
- Homogenize or stomacher if necessary.
- Incubate the broth at 41.5 ± 1.0 °C for **16 to 24 hours.**
- Isolate 10 µL of the resulting enrichment on IRIS *Salmonella*® Agar.
- Incubate at 37 ± 1 °C for 24 hours ± 3 hours.

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

✓ **Detection :**
Isolation 10 µL,
24 h à 37 °C

Protocol for test samples including ingredients, up to 50 g

- Aseptically introduce (x) g of sample to be analyzed into 9 (x) mL of Salmonella Enrichment.
- Introduce DSC supplement at a rate of 0.1 mL of BS095 liquid supplement per gram of sample (i.e. 2.5 mL per 25 g),
  Or introduce CSD supplement at a rate of 1 to 5 tablets Qs 10 g (BS099) or 1 to 2 tablets Qs 25 g (BS100).
- Homogenize or stomacher if necessary.
- Incubate the broth at 41.5 ± 1.0 °C for **16 to 22 hours.**
- Isolate 10 µL of the resulting enrichment on IRIS *Salmonella*® agar.
- Incubate at 37 ± 1 °C for 24 hours ± 3 hours.

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

✓ **Detection :**
Isolation 10 µL,
24 h à 37 °C

Notes :
For surface samples after cleaning, which may contain disinfectant residues, it is recommended to use swabs, sponges or wipes already soaked in neutralizing solution, or to use 10% universal neutralizers and 90% Salmonella Enrichment, before adding the CSD supplement.

Notes concerning all NF VALIDATION certified protocols

- Refer to the various parts of EN ISO 6887 :

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

- IRIS *Salmonella* Enrichment Broth, after incubation, can be stored for up to 3 days at 2-8°C before subculturing on IRIS *Salmonella*® Agar (except for animal feed).

- Similarly, IRIS agar, after incubation, can be stored up to 3 days at 2-8°C before reading and possible confirmations.

6 RESULTS

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

Microorganisms	Characteristic colonies
***Salmonella* spp.** (including *Salmonella* Typhi, Paratyphi, lactose-positive, saccharose-positive, immobile, monophasic, Dublin, *bongori*)	**Pink to Magenta**
Escherichia coli	Uncolored
Enterobacter spp., *Klebsiella* spp.	Blue-green to violet
Proteus spp.	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 EN 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
Salmonella Typhimurium	WDCM 00031	Good, magenta colonies
Salmonella Enteritidis	WDCM 00030	Good, magenta colonies
Enterobacter aerogenes	WDCM 00175	Good, blue colonies
Escherichia coli	WDCM 00013	Partially inhibited, uncolored colonies
Staphylococcus aureus	WDCM 00034	Inhibited
Pseudomonas aeruginosa	WDCM 00025	Inhibited

9 STORAGE / SHELF LIFE

***Salmonella* Enrichment, *Salmonella* Enrichment double-strength buffered:**
Dehydrated medium: 2-30 °C.
Ready-to-use medium in vials or flexible bags: 2-25 °C.

***Salmonella* Enrichment with Tween:**
Ready-to-use medium in vials or flexible bags: 2-25 °C.

IRIS *Salmonella*® Supplement
Liquid supplement: 2-8 °C.
Tablets: 2-8 °C.

CSD® Supplement
Liquid supplemen: 2-8 °C.
Tablets : 2-8 °C.

IRIS *Salmonella*® Agar:
Pre-poured medium 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

***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*® concentrate Supplement :
50 tubes of 9 mL BS10108
10 bottles of 90 mL BS10208

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 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

IRIS *Salmonella*® is a registered trademark of BIOKAR DIAGNOSTICS (division 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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ANNEX 1 : PHOTO SUPPORT

IRIS *Salmonella*® Agar

Detection of *Salmonella*

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