Oxoid™ Salmonella Precis™ Method

Publication Number MAN0019556 Revision B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

1. Intended use

Oxoid™ *Brilliance*™ Salmonella Agar is a selective medium for the presumptive identification and differentiation of *Salmonella* species and has been certified EN ISO 16140-2:2016 by AFNOR Certification and AOAC Research Institute (AOAC-RI) as part of the Oxoid™ Salmonella Precis™ Method for the detection of *Salmonella* species.

The use of Thermo Scientific™ Oxoid™ Buffered Peptone Water (BPW) (with or without supplementation) or the Thermo Scientific™ Oxoid™ ONE Broth™ Salmonella Medium allows sufficient recovery of *Salmonella* species to be achieved with no need for a secondary enrichment broth. Simply inoculate the incubated broth onto Thermo Scientific™ Oxoid™ *Brilliance*™ Salmonella Agar.

2. Summary

The Oxoid™ Salmonella Precis™ Method combines the benefits of BPW or Oxoid™ ONE Broth™ Salmonella Medium, Oxoid™ *Brilliance*™ Salmonella Agar, and the Thermo Scientific™ Oxoid™ Salmonella Test Kit (latex test).

This method reduces the time to result over conventional culture methods. Both enrichment options are highly nutritious media for the recovery and growth of salmonellae while inhibiting competing organisms. The growth promoter in the Oxoid™ ONE Broth™ Salmonella Medium allows the recovery of stressed *Salmonella* cells, even when present in very low numbers. Oxoid™ *Brilliance*™ Salmonella Agar incorporates novel Inhibigen™ technology, which improves recovery of *Salmonella* by reducing background flora. Chromogens aid easy identification and differentiation by producing brightly colored colonies. The Oxoid™ Salmonella Test Kit (latex test) provides a quick and easy method for confirmation of *Salmonella* species from culture media. Isolated colonies can also be confirmed using an Oxoid™ Microbact GNB 24E Kit, or depending on the legislation territory, an appropriate EN ISO 16140-6:2019 or Official Method of Analysis of *AOAC International* (AOAC-OMA) validated confirmation method or the appropriate reference confirmation procedure (e.g. FDA/BAM Chapter 5, USDA/FSIS MLG 4.10, ISO 6579-1:2017).

3. Media composition

The following compositions are for typical formulae. Adjustments might be required to meet performance standards.

Table 1 Oxoid™ *Brilliance*™ Salmonella Agar

Reagents	Concentration
Salmonella Growth Mix	14 g/L
Chromogenic Mix	25 g/L
Cefsulodin	0.012 g/L
Novobiocin	0.005 g/L
Agar	15 g/L

Table 2 Oxoid™ Buffered Peptone Water (ISO)

Reagents	Concentration
Peptone	5 g/L
Sodium Chloride	5 g/L
Disodium Hydrogen Phosphate (anhydrous)	3.5 g/L
Potassium Dihydrogen Phosphate	1.5 g/L
pH 7.0±0.2 at 25°C	

Table 3 Oxoid™ ONE Broth-Salmonella Base

Reagents	Concentration
Peptone	5 g/L
Yeast Extract	5 g/L
Salt Buffer Mix	10 g/L
Growth Promoter Mix	5 g/L
pH 7.0±0.2 at 25°C[1]	

[1] pH of supplement medium

4. Materials required not supplied

- Inoculating loops, swabs, collection containers
- Incubators
- Quality control organisms

5. Before you begin

Note: Ready-to-use Oxoid™ Buffered Peptone Water (ISO) (Cat. No. BO1067S), Oxoid™ ONE Broth™ Salmonella Medium (Cat. No. BO1096S), and Oxoid™ *Brilliance*™ Salmonella Agar (Cat. No. PO5098A) can be used as well.

Prepare the Oxoid™ Buffered Peptone Water (ISO)

1. Suspend 4.5 g of Oxoid™ Buffered Peptone Water (ISO) in 225 mL of distilled water.
2. Mix well, distribute into final containers, then sterilize by autoclaving at 121°C for 15 minutes.

Prepare Oxoid™ Novobiocin Liquid

Resuspend the Oxoid™ Novobiocin Supplement (Cat. No. SR0249A, SR0181E, or equivalent) as directed. See recommendations in Table 4.

Table 4 Prepare Oxoid™ Novobiocin Supplement

Supplement			Reconstitution		Volume of supplement added to volume of BPW (ISO) to achieve 12 mg/L		
Cat. No.	Product name	mg/vial	Solvent	mL/vial	225 mL	1000 mL	3375 mL
SR0249A	Oxoid™ Novobiocin Supplement (Liquid format)	400	N/A	40	0.27 mL	1.2 mL	4.05 mL
SR0181E	Oxoid™ Novobiocin Supplement	10	Distilled sterile water	2	0.54 mL	2.4 mL	8.1 mL

Prepare the Oxoid™ Vancomycin Supplement

Resuspend the Oxoid™ Vancomycin Supplement (Cat. No. SR0247E or equivalent) as directed. See recommendation in Table 5.

Table 5 Prepare Oxoid™ Vancomycin Supplement

Supplement			Reconstitution		Volume of supplement added to volume of BPW (ISO) to achieve 6 mg/L		
Cat. No.	Product name	mg/vial	Solvent	mL/vial	225 mL	1000 mL	3375 mL
SR0247E	Oxoid™ Vancomycin Supplement	5	Distilled sterile water	2	0.54 mL	2.4 mL	8.1 mL

Prepare Oxoid™ ONE Broth™ Salmonella Medium

1. Suspend 5.6 g of Oxoid™ ONE Broth-Salmonella Base (Cat. No. CM1091) in 225 mL of distilled water.
2. Sterilize by autoclaving at 121°C for 15 minutes.
3. Cool to below 50°C, then add the content of 1 vial of Oxoid™ ONE Broth-Salmonella Supplement (Cat. No. SR0242E) resuspended as directed. See recommendation in Table 6.

Table 6 Prepare Oxoid™ ONE Broth-Salmonella Supplement

Supplement			Reconstitution		Volume of supplement added to volume of Oxoid™ ONE Broth™ Salmonella Medium		
Cat. No.	Product name	mg/vial	Solvent	mL/vial	225 mL	1000 mL	3375 mL
SR0242E	Oxoid™ ONE Broth-Salmonella Supplement	2.7	Distilled sterile water	2	2 mL	8.9 mL	30 mL

Prepare Oxoid™ *Brilliance*™ Salmonella Agar

1. Suspend 27 g of Oxoid™ *Brilliance*™ Salmonella Agar Base (Cat. No. CM1092) in 500 mL of distilled water.
2. Add the content of 1 vial of Oxoid™ Salmonella Selective Supplement (Cat. No. SR0194E) resuspended as directed.
3. Mix well, then sterilize by bringing to a boil with frequent agitation.
4. Cool to around 50°C, mix well, then pour into sterile Petri dishes.

6. Isolate *Salmonella* from food feed and environmental samples

Method certified EN ISO 16140-2:2016 by NF VALIDATION™ UNI 03/06 - 12/07

Comply with Good Laboratory Practices (refer to EN ISO 7218:2007 standard).

For preparation of initial suspensions, follow the instructions of EN ISO 6579-1:2017 standard and EN ISO 6887:2017 series.

1. Enrich the samples as follow.

Matrices	Media	Incubation
Food and feeding stuffs	Add up to 25 g or 25 mL of sample to 225 mL of Oxoid™ Buffered Peptone Water (ISO) supplemented with 12 mg/L of novobiocin solution. Or Add up to 25 g or 25 mL of sample to 225 mL of Oxoid™ ONE Broth™ Salmonella Medium supplemented with Oxoid™ ONE Broth-Salmonella Supplement.	34°C to 38°C for 20–26 hours for supplemented Oxoid™ Buffered Peptone Water (ISO) 42 ±1°C for 16–22 hours for the supplemented Oxoid™ ONE Broth™ Salmonella Medium
Environmental samples	• Add 25 g or 25 mL of sample or one wipe to 225 mL of Oxoid™ Buffered Peptone Water (ISO) or to 225 mL of Oxoid™ ONE Broth™ Salmonella Medium supplemented with Oxoid™ ONE Broth-Salmonella Supplement. • Add one swab to 10 mL of Oxoid™ Buffered Peptone Water (ISO) or to 10 mL of Oxoid™ ONE Broth™ Salmonella Medium supplemented with Oxoid™ ONE Broth-Salmonella Supplement. • Add one sponge to 100 mL of Oxoid™ Buffered Peptone Water (ISO) or to 100 mL of Oxoid™ ONE Broth™ Salmonella Medium supplemented with Oxoid™ ONE Broth-Salmonella Supplement.	34°C to 38°C for 20–26 hours for supplemented Oxoid™ Buffered Peptone Water (ISO) 42 ±1°C for 16–22 hours for the supplemented Oxoid™ ONE Broth™ Salmonella Medium
Bigger samples sizes of milk powders, infant formula, and infant cereals with or without probiotics	Add up to 375 g or 375 mL of sample to 3375 mL of Oxoid™ Buffered Peptone Water (ISO) supplemented with 6 mg/L of vancomycin solution.	34°C to 38°C for 18–24 hours
Bigger sample sizes of cocoa and chocolate products	Add up to 375 g of sample to 3375 mL of pre-warmed Oxoid™ Buffered Peptone Water (ISO) or use the recommendations of the EN ISO 6887-4:2017 standard.	34°C to 38°C for 22-28 hours if using pre-warmed Oxoid™ Buffered Peptone Water (ISO)

(continued)

Matrices	Media	Incubation
Bigger sample sizes of cocoa and chocolate products	Add up to 375 g of sample to 3375 mL of pre-warmed Oxoid™ Buffered Peptone Water (ISO) or use the recommendations of the EN ISO 6887-4:2017 standard.	34°C to 38°C for 20-26 hours if using the recommendations of the EN ISO 6887-4:2017 standard.
Bigger sample sizes of animal feed	Add up to 150 g or 150 mL of sample to 1350 mL of Oxoid™ Buffered Peptone Water (ISO) supplemented with 12 mg/L of novobiocin solution.	34°C to 38°C for 20-26 hours

Note: For solid sample, stomach for 30-60 seconds to mix the sample.
Samples containing hard particles or bone should be mixed thoroughly by hand.

2. Gently agitate the bag. Then, using a microbiological loop, inoculate a 10 µL loopful of the broth onto a plate of Oxoid™ *Brilliance*™ Salmonella Agar using a diminishing sweep technique to produce single colonies.

3. Incubate the plates at 34°C to 38°C for 24±2 hours.

Salmonella colonies grow as purple/pink colonies and non-target organisms are either inhibited or grow as blue or white colonies.

Purple colonies are presumptive positive for *Salmonella* (see “Example results” on page 10).

See “7. Confirmation of positive results” on page 10 to confirm the observed characteristic colonies.

Method certified by AOAC-RI

Comply with Good Laboratory Practice (refer to EN ISO 7218 standard).

For preparation of initial suspensions, follow the instructions of EN ISO 6579-2 standards.

1. Enrich the samples as follow.

Matrices	Media	Incubation
Raw ground beef	Add 25 g to 225 mL of Oxoid™ ONE Broth™ Salmonella Medium supplemented with Oxoid™ ONE Broth-Salmonella Supplement.	42°C for 18±2 hours
Raw ground chicken		
Lettuce		
Shrimp		
Shell eggs		

2. Gently agitate the bag. Then, using a microbiological loop, inoculate a 10 µL loopful of the broth onto a plate of Oxoid™ *Brilliance*™ Salmonella Agar using a diminishing sweep technique to produce single colonies.

3. Incubate the plates at 37°C for 24±2 hours.

Salmonella colonies grow as purple/pink colonies and non-target organisms are either inhibited or grow as blue or white colonies.

Purple colonies are presumptive positive for *Salmonella* (see "Example results" on page 10).

See "7. Confirmation of positive results" on page 10 to confirm the observed characteristic colonies.

Oxoid™ Salmonella Precis™ Method Workflow for usual sample sizes

Food, feeding stuffs, and production environmental samples

- 25 g or 25 mL sample + 225 mL Buffered Peptone Water (ISO) supplemented with 12 mg/L novobiocin
- Swab[1] + 10 mL Buffered Peptone Water (ISO)
- Sponge[1] + 100 mL Buffered Peptone Water (ISO)

or

- 25 g or 25 mL sample + 225 mL supplemented Oxoid™ ONE Broth™ Salmonella Medium
- Swab[1] + 10 mL supplemented Oxoid™ ONE Broth™ Salmonella Medium
- Sponge[1] + 100 supplemented Oxoid™ ONE Broth™ Salmonella Medium

▼

20–26 h at 34–38°C

▼

16–22 h at 42°C ± 1°C

▼

Possibility to store for 72 h at 5°C ± 3°C except for infant formula with probiotics (10 g sample size)

▼

Streaking 10 µl onto *Brilliance*™ Salmonella Agar

▼

24 h ± 2 h at 34°C to 38°C

▼

Typical colonies (purple/pink colonies)

▼

Oxoid™ Salmonella Test Kit (latex test)

▼

Biochemical gallery (e.g. Oxoid™ Microbact GNB 24E Kit)

or

Any appropriate EN ISO 16140-6:2019 validated method

or

EN ISO 6579-1:2017 confirmation procedure

or

Molecular hybridization as described in EN ISO 7218:2007 (e.g. SureTect™ Salmonella species PCR Assay)

[1] For sampling after cleaning process, premoisten:
- 1 swab + 1 mL broth universal neutralizing (+ 9 mL enrichment broth)
- 1 sponge + 10 mL broth universal neutralizing (+ 90 mL enrichment broth)
- 1 wipe + BPW + 10 % neutralizing agent (+ 225 mL enrichment broth)

Oxoid™ Salmonella Precis™ Method Workflow for large sample sizes

Bigger samples sizes of milk powders, infant formula, and infant cereals with or without probiotics	Bigger sample sizes of cocoa and chocolate products			Bigger sample sizes of animal feed
Add up to 375 g or 375 mL of sample to 3375 mL of Buffered Peptone Water (ISO) supplemented with 6 mg/L of vancomycin solution	Add up to 375 g of sample to 3375 mL of pre-warmed Oxoid™ Buffered Peptone Water (ISO)	**or**	Use the recommendations of the EN ISO 6887-4:2017 standard	Add up to 150 g or 150 mL of sample to 1350 mL of Oxoid™ Buffered Peptone Water (ISO) supplemented with 12 mg/L of novobiocin solution.
▼	▼		▼	▼
18–24 h at 34–38°C	22–28 h at 34–38°C		20–26 h at 34–38°C	20–26 h at 34–38°C
▼	▼		▼	▼

Possibility to store for 72 h at 5°C ± 3°C except for infant formula with probiotics (10 g sample size)

▼

Streaking 10 µl onto *Brilliance*™ Salmonella Agar

▼

24 h ± 2 h at 34°C to 38°C

▼

Typical colonies (purple/pink colonies)

▼

Oxoid™ Salmonella Test Kit (latex test)

▼

Biochemical gallery (e.g. Oxoid™ Microbact GNB 24E Kit)

or

Any appropriate EN ISO 16140-6:2019 validated method

or

EN ISO 6579-1:2017 confirmation procedure

or

Molecular hybridization as described in EN ISO 7218:2007 (e.g. SureTect™ Salmonella species PCR Assay)

Example results

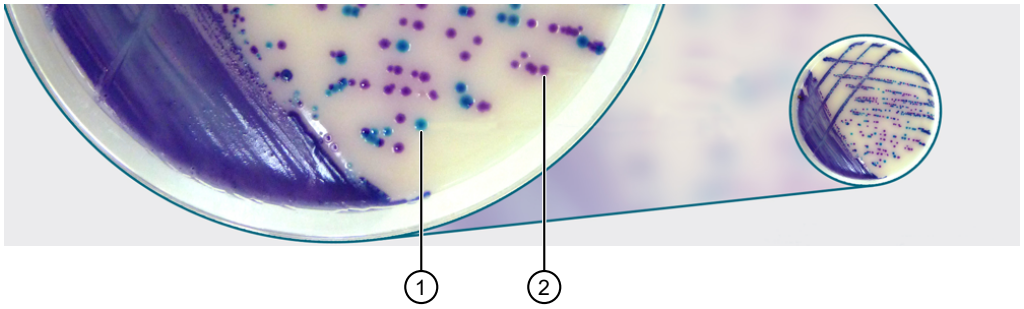

Figure 1 Example results—Mixed culture from a raw meat sample

① *Klebsiella* colony
② *Salmonella* colony

7. Confirmation of positive results

In the context of NF VALIDATION, all samples identified as positive by the *Salmonella* Precis method must be confirmed. Confirmation is performed from isolated characteristic colonies on Oxoid™ *Brilliance*™ Salmonella Agar and running one of these two options:

- **Option 1:** Oxoid™ Salmonella Test Kit (latex test) (Cat. No. DR1108A).
- **Option 2:** Microbact GNB 24E biochemical galleries [Oxoid™ Microbact GNB 24E Kit (Cat. No. MB1131A) or equivalent].

In the context of ISO general rules, it is as well possible to confirm the colonies with one of the following options:

- **Option 3:** Any appropriate EN ISO 16140-6:2019 validated method.
- **Option 4:** EN ISO 6579-1:2017 confirmation procedure.
- **Option 5:** Molecular hybridization as described in EN ISO 7218:2007 using for instance SureTect™ Salmonella species PCR Assay (Cat. No. PT0100A), RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (Cat. Nos. A33227 and A33227KF), and MicroSEQ™ Salmonella spp. Detection Kit (Cat. No. 4403930).

In the context of AOAC-RI, all samples identified as positive by the *Salmonella* Precis method must be confirmed. Confirmation is performed from isolated characteristic colonies on Oxoid™ *Brilliance*™ Salmonella Agar and running one of these three options:

- **Option 1:** Oxoid™ Salmonella Test Kit (latex test) (Cat. No. DR1108A).
- **Option 2:** Microbact GNB 24E biochemical galleries [Oxoid™ Microbact GNB 24E Kit (Cat. No. MB1131A) or equivalent].
- **Option 3:** Any appropriate AOAC-OMA validated method.
- **Option 4:** FDA/BAM Chapter 5 or USDA/FSIS MLG 4.10 or EN ISO 6579-1:2017 confirmation procedures.

In the event of discordant results (positive with the alternative method, non-confirmed by one of the means described above, and in particular for the latex test), the laboratory must follow the necessary steps to ensure the validity of the result obtained.

Limitations

It should be noted that, as with all chromogenic media, organisms with atypical enzyme patterns may give anomalous reactions on Oxoid™ *Brilliance*™ Salmonella Agar.

Note: A number of *S. typhi* and *S. paratyphi* spp. may fail to grow in the Oxoid™ ONE Broth™ Salmonella Medium enrichment. If testing for *S. typhi* or *S. paratyphi*, confirm absence of the organism using a secondary method for enrichment, followed by plating on Oxoid™ *Brilliance*™ Salmonella Agar.

Performance validation

Table 7 NF VALIDATION™ certification of the method

Certification	Expiration
BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140 UNI 03/06 – 12/07 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS http://nf-validation.afnor.org/en	The NF VALIDATION™ certificate can be obtained from our Technical Support team (Europe: email: microbiology.techsupport.uk@thermofisher.com telephone: +44 (0)1256 694238) or from AFNOR Certification (nf-validation.afnor.org/en). For more information about the validity of the NF VALIDATION™ certification, please refer to the certificate UNI 03/06-12/07 available at nf-validation.afnor.org/en or from our technical support team.

Table 8 *Performance Tested Methods*℠ Certification of the method

Certification	Expiration
PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 120802	The AOAC-RI certificate can be obtained from our Technical Support team (Europe: email: microbiology.techsupport.uk@thermofisher.com telephone: +44 (0)1256 694238) or from the AOAC website www.aoac.org. For more information about the validity of the AOAC-RI certification, please refer to the certificate PTM 120802.

References

AOAC INTERNATIONAL Guidelines Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, version 2012.

EN ISO 7218:2007. Microbiology of food and animal feeding stuffs -- General requirements and guidance for microbiological examinations

EN ISO 6579-1:2017. Microbiology of the food chain -- Horizontal method for the detection, enumeration and serotyping of *Salmonella* -- Part 1: Detection of *Salmonella* spp.

EN ISO 6887-1:2017. Microbiology of the food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 1: General rules for the preparation of the initial suspension and decimal dilutions.

EN ISO 6887-2:2017. Microbiology of the food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 2: Specific rules for the preparation of meat and meat products.

EN ISO 6887-3:2017. Microbiology of the food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 3: Specific rules for the preparation of fish and fishery products.

EN ISO 6887-4:2017. Microbiology of the food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 2: Specific rules for the preparation of miscellaneous products.

EN ISO 16140-2:2016. Microbiology of food and animal feed – Method validation – Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

EN ISO 16140-6:2016. Microbiology of food and animal feed – Method validation – Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.

FDA *Bacteriological Analytical Manual* (BAM), Chapter 5 - *Salmonella* spp.

USDA/FSIS *Microbiology Laboratory Guidebook*, Revision 4.10 - Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0019556

Revision	Date	Description
B.0	27 October 2021	• Added recommended resuspension methods for Oxoid™ Novobiocin Liquid, Oxoid™ Vancomycin Liquid, and Oxoid™ ONE Broth-Salmonella Supplement. • Added workflows for usual size samples and larger size samples.
A.0	12 April 2021	New document.