# Oxoid™ Cronobacter Precis™ Method

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**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**.

## Summary

*Cronobacter* (formerly *Enterobacter sakazakii*) are Gram-negative rod-shaped members of the Family *Enterobacteriaceae* that have been implicated in outbreaks of disease in premature infants (neonates), causing sepsis, meningitis, and necrotizing enterocolitis. Neurological damage can be permanent, and the death rate is reported to be as high as 40–80%.

*Cronobacter* spp. have been isolated at low levels from powdered infant formulae, and the organisms' high tolerance to desiccation provides a competitive advantage in the dry environments of milk powder factories, increasing the risk of product contamination.

The protocol of the primary enrichment is not clearly described in the ISO 22964:2017 standard for the detection of *Cronobacter* spp. For instance, is there a need to supplement the primary enrichment with antibiotics for infant formula with probiotics or with α-amylase for infant cereals with starch as described in the ISO 6887-4:2017? Additionally, a selective sub-culture is required, extending the time-to-result for 24 hours.

The Oxoid™ Cronobacter Precis™ Method offers an alternative to the ISO 22964:2017 workflow by requiring only one single enrichment prior to streaking on Oxoid™ *Brilliance*™ CCI Agar Base (*Brilliance*™ CCI). This simplified and fast workflow has been validated according to the ISO 16140-2:2016 standard by MicroVal™.

## Intended use

Oxoid™ *Brilliance*™ CCI Agar Base (*Brilliance*™ CCI) is an optimization of the original *Brilliance*™ Enterobacter Sakazakii Agar (DFI), developed by Iversen et al. in 2004. The concentration of the X-α-glucoside chromogen was increased to improve the blue-green coloration of *Cronobacter* strains with weak glucosidase activity. Sodium thiosulphate and ammonium iron (III) citrate are included to differentiate hydrogen sulfide-producing *Enterobacteriaceae*, which are colored grey/brown, and sodium deoxycholate is added to inhibit the growth of Gram positive competitors.

Oxoid™ Cronobacter Precis™ Method offers a fast screening of the samples after an overnight enrichment and a selective plating onto Oxoid™ *Brilliance*™ CCI Agar Base (*Brilliance*™ CCI).

This workflow is meant for use in laboratories undertaking microbiological analysis.

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**For testing of Food and Environmental samples only.**

# Method

The Oxoid™ Cronobacter Precis™ Method combines the benefits of the Oxoid™ Buffered Peptone Water (ISO) (BPW), Oxoid™ *Brilliance*™ CCI Agar Base (*Brilliance*™ CCI), and Oxoid™ Microbact GNB 24E Kit.

This method reduces the time to result over conventional culture methods. The prescribed Oxoid™ BPW is highly nutritious, enabling the fast recovery and growth of *Cronobacter*, including stressed cells.

Oxoid™ *Brilliance*™ CCI improves recovery of Cronobacter by reducing background flora. Chromogens aid easy identification and differentiation by producing brightly colored colonies.

The Oxoid™ Microbact GNB 24E Kit or the SureTect™ Cronobacter species PCR Assay ensure a rapid and simple confirmation for isolated characteristic colonies from the Oxoid™ *Brilliance*™ CCI plate.

Depending on the legislation territory, it is also possible to use any appropriate EN ISO 16140-6:2019 or Official Method of analysis of AOAC International (AOAC-OMA) validated confirmation method or any appropriate reference confirmation procedure.

# Media composition

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

**Table 1 Oxoid™ Buffered Peptone Water (ISO) (Cat. No. CM1049, CM1211, or equivalent)**

| Reagents | Concentration |
|---|---|
| Peptone | 10 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 2 Oxoid™ *Brilliance*™ CCI Base (Cat. No. CM1122B)**

| Reagents | Concentration |
|---|---|
| Yeast Extract | 3.0 g/L |
| Sodium Chloride | 5.0 g/L |
| Tryptone | 7.0 g/L |
| Sodium Thiosulphate | 1.0 g/L |
| Sodium Deoxycholate | 0.25 g/L |
| Ferric Ammonium Citrate | 1.0 g/L |
| 5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside | 0.15 g/L |

**Table 2 Oxoid Brilliance CCI Base (Cat. No. CM1122B)** *(continued)*

| Reagents | Concentration |
|---|---|
| Agar | 13.2 g/L |
| pH 7.3±0.2 at 25°C | |

## Materials required (not supplied)

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

# Prepare the materials

**Note:** Ready-to-use Oxoid™ Buffered Peptone Water (ISO) (Cat. No. BO1067S) 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 the Novobiocin Selective Supplement

Resuspend or suspend the Oxoid™ Novobiocin Selective Supplement or Oxoid™ PrecisBlue Supplement (Cat. No. SR0181E, SR0249A, SR0259A or equivalent) as directed in Table 3.

**Table 3 Prepare the Oxoid™ Novobiocin Selective Supplement or Oxoid™ PrecisBlue 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 | 1350 mL | 3375 mL |
| SR0259A | Oxoid™ PrecisBlue Selective Supplement (Liquid format) | 216 | N/A | 40 | 0.25 mL | N/A | 1.5 mL | 1.04 mL |
| SR0249A | Oxoid™ Novobiocin Selective Supplement (Liquid format) | 400 | N/A | 40 | 0.14 mL | 0.6 mL | N/A | 2.0 mL |
| SR0181E | Oxoid™ Novobiocin Supplement | 10 | Distilled sterile water | 2 | 0.27 mL | 1.2 mL | N/A | 4.1 mL |

## Prepare the Oxoid™ Vancomycin Supplement

Resuspend the Oxoid™ Vancomycin Supplement (Cat. No. SR0247E or equivalent) as directed. See recommendation in Table 4.

**Table 4 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 the Oxoid™ *Brilliance*™ CCI Agar Base (*Brilliance*™ CCI)

1. Suspend 30.6 g of Oxoid™ *Brilliance*™ CCI Agar Base (*Brilliance*™ CCI) in 1 L of distilled water.
2. Bring to a boil to dissolve completely.
3. Sterilize by autoclaving at 121°C for 15 minutes.
4. Cool to approximately 50°C.
5. Mix well and pour into sterile Petri dishes.

# Isolate *Cronobacter* from infant formula, infant cereals, and related ingredients and from environmental samples

## Method certified EN ISO 16140-2:2016 by MicroVal™

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

1. Homogenize the sample for 30–60 seconds with a homogenizer.

2. Enrich the samples as follows:

| Matrices | Media | Incubation |
|---|---|---|
| 10 g infant formula with or without probiotics[1] | 1:10 ratio of sample to media. For example, add up to 10 g of sample to 90 mL of Oxoid™ Buffered Peptone Water (ISO) | 34ºC to 38°C for 16–22 hours |
| Up to 375 g infant formula with and without probiotics, infant cereals with and without probiotics, and related ingredients[1] | 1:10 ratio of sample to media. For example, add up to 375 g of sample to 3,375 mL of Oxoid™ Buffered Peptone Water (ISO) supplemented with 6 mg/L of vancomycin solution | 34ºC to 38°C for 20–26 hours |
| | 1:6 ratio of sample to media. For example, 375 g of sample and 1,875 mL of pre-warmed Buffered Peptone Water (ISO) (supplemented with 6 mg/L novobiocin/PrecisBlue for probiotics only) | 34ºC to 38°C for 18–24 hours |
| Environmental samples | 1:10 ratio of sample to media<br>• Add up to 25 g of sample to 225 mL of Oxoid™ Buffered Peptone Water (ISO) supplemented with 6 mg/L of vancomycin solution<br>• Add one swab to 10 mL of Oxoid™ Buffered Peptone Water (ISO) supplemented with 6 mg/L of vancomycin solution<br>• Add one sponge to 100 mL of Oxoid™ Buffered Peptone Water (ISO) supplemented with 6 mg/L of vancomycin solution | 34ºC to 38°C for 18–24 hours |
| | 1:10 ratio of sample to media<br>• Add up to 25 g of sample to 225 mL of Oxoid™ Buffered Peptone Water (ISO)<br>• Add one swab to 10 mL of Oxoid™ Buffered Peptone Water (ISO)<br>• Add one sponge to 100 mL of Oxoid™ Buffered Peptone Water (ISO) | 34ºC to 38°C for 20–26 hours |

[1] The enrichment conditions are harmonized with Oxoid™ Salmonella Precis™, SureTect™ Salmonella species PCR Assay, and SureTect™ Cronobacter species PCR Assay, with no addition of α-amylase required.

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

   **Note:** It is possible to store the enrichment broth after incubation for 72 hours at 5°C±3°C before streaking onto *Brilliance*™ CCI except when testing 10 g infant formula with or without probiotics enriched in non-supplemented Oxoid™ Buffered Peptone Water.

4. Incubate the plates at 37±1ºC or 41.5±1ºC for 24±2 hours.

   **Note:** It is possible to store the plates for 72 hours at 5°C±3°C before confirmation.

*Cronobacter* colonies grow as 1–3 mm blue/green colonies, and non-target organisms are either inhibited or grow as straw or white colonies.

Blue colonies are presumptive positive for *Cronobacter* (see Figure 1).

See Confirm positive results on page 9 to confirm the observed characteristic colonies.

## Oxoid™ Cronobacter Precis™ Method Workflow

| Infant formula with and without probiotics[1] | Infant formula with or without probiotics, infant cereals with or without probiotics, and related ingredients[2] | | Production environmental samples[3] | Production environmental samples |
|---|---|---|---|---|
| 10 g sample | 375 g sample | 375 g sample | 25 g sample | 25 g sample |
| 10 g + 90 mL BPW | 375 g + 3,375 mL BPW + vancomycin (6 mg/L) | 375 g + 1,875 mL prewarmed BPW (with 6 mg/L novobiocin/PrecisBlue for probiotics) | • 25 g + 225 mL BPW + vancomycin (6 mg/L)<br>• Swab + 10 mL BPW + vancomycin (6 mg/L)<br>• Sponge + 100 mL BPW + vancomycin (6 mg/L) | • 25 g + 225 mL BPW<br>• Swab + 10 mL BPW<br>• Sponge + 100 mL BPW |
| ▼ | ▼ | ▼ | ▼ | ▼ |
| 34–38°C for 16–22 h | 34–38°C for 20–26 h | 34–38°C for 18–24 h | 34–38°C for 18–24 h | 34–38°C for 20–26 h |

▼

*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*™ CCI Agar

▼

24 h±2 h at 37±1°C or 41.5°C±1°C

▼

*Possibility to store for 72 h at 5°C±3°C*

Typical colonies (blue green), confirmed by one of the following:

- **Option 1:** Biochemical galleries [Oxoid™ Microbact GNB 24E Kit or equivalent]
- **Option 2:** Tests of the ISO 22964 method
- **Option 3:** Molecular hybridization test as described in EN ISO 7218:2007 (e.g. SureTect™ Cronobacter species PCR Assay)
- **Option 4:** Appropriate ISO 16140-6:2019 validated confirmation method

[1] No addition of α-amylase required.
[2] No addition of α-amylase required. It is possible to test smaller sample sizes by adjusting the volume of BPW + vancomycin (6 mg/L).
[3] For sampling after cleaning process, premoisten:

- 1 swab + 1 mL broth universal neutralizing (+ 9 mL BPW)
- 1 sponge + 10 mL broth universal neutralizing (+ 90 mL BPW)

- 1 wipe + BPW + 10% neutralizing agent (+ 225 mL BPW)

## Example results

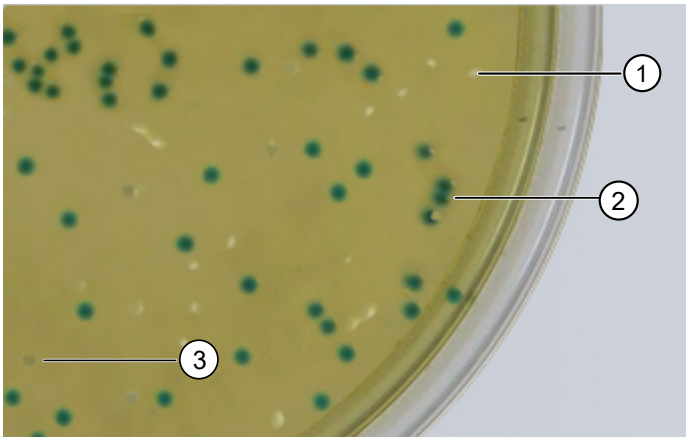


**Figure 1 Example results—Mixed culture**

① *E. coli* colony
② *Cronobacter spp.* colony
③ *Enterobacter cloacae* colony

# Confirm positive results

Confirmation is performed from isolated characteristic colonies on Oxoid™ *Brilliance*™ CCI Agar Base (*Brilliance*™ CCI) and running one of these options:

- Option 1: Biochemical galleries [Oxoid™ Microbact GNB 24E Kit (Cat. No. MB1131A) or equivalent].
- Option 2: EN ISO 22964:2017 confirmation procedure.
- Option 3: Any appropriate EN ISO 16140-6:2019 validated method.
- Option 4: Molecular hybridization as described in EN ISO 7218:2007 using for instance the SureTect™ Cronobacter species PCR Assay (Cat. No. PT1060A or A56845) that is validated according to the ISO 16140-2:2016 standard (NF Validation certificate UNI 3/11-12/15).

In the event of discordant results (positive with the Oxoid™ Cronobacter Precis™ Method or non-confirmed by one of the means described above), the laboratory must follow the necessary steps to ensure the validity of the result obtained.

# Performance validation

**Table 5 MicroVal™ certification of the method**

| Certification | Scope and expiration |
|---|---|
| MICROVAL® NEN<br>MicroVal™ certificate 2020LR93<br>http://www.microval.org | The MicroVal™ certificate can be obtained from our technical support team.<br>• email: microbiology.techsupport.uk@thermofisher.com<br>• telephone: +44 (0)1256 694238)<br>• or from the MicroVal™ website: http://www.microval.org<br>For more information about the validity of the MicroVal™ certification, see the certificate 2020LR93 available at http://www.microval.org or obtain from our technical support team. |

# References


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Mullane N. R., Iversen C., Healy B., Walsh C., Whyte P., Wall P. G., et al, 2007. *Enterobacter sakazakii* an emerging bacterial pathogen with implications for infant health. *Minerva Pediatr.* 59:137–148.

Lai K.K., 2001 *Enterobacter sakazakii* infections among neonates, infants, children, and adults: case reports and a review of the literature. *Medicine* (Baltimore) 80:113–122.

EN ISO 22964:2017. Microbiology of the food chain – Horizontal method for the detection of *Cronobacter* spp.

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

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.

**Revision history: MAN0025440 C00 (English)**

| Revision | Date | Description |
|---|---|---|
| C00 | 8 April 2024 | A new Novobiocin selective supplement was added, Oxoid™ PrecisBlue Selective Supplement. |
| B.0 | 14 December 2023 | • Matrices were added for infant formula with and without probiotics, infant cereals with and without probiotics, and related ingredients.<br>• Matrices were added for environmental samples in Oxoid™ Buffered Peptone Water (ISO) not supplemented with vancomycin solution.<br>• Instructions were added for how to prepare Novobiocin. |
| A.0 | 28 September 2021 | New document for MicroVal™ validation study of Oxoid™ Cronobacter Precis™ Method. |

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