

Product Instructions

Coliform Vial—5 mL

The Coliform Vial, 5 mL (CC-102) is a screening vial specific for coliform organisms. The vial has broad inclusivity and an assay time of 18 hours for most applications. The vial contains a peptone yeast extract base with lactose as a carbon source. The selective agents include bile salts, sodium lauryl sulfate, and other gram-positive inhibitors. Acidification of the medium due to the lactose utilization changes the pH. As coliforms metabolize, the pH indicator changes from a purple to a yellow color. The color change is read by optical sensors in the instrument.

In an AOAC Research Institute *Performance Tested Method Certificate* #010302 study, the Soleris® Coliform Vial was found to be an effective procedure for specification monitoring of total coliform count in the following sample types: pasteurized milk, ice cream, butter, yogurt, chocolate, raw eggs, pork sausage, ground beef, and raw chicken. Test duration is 18 hours with the exceptions of yogurt and queso, which require 24 hours.



CC-102 Vial uninoculated (left) and inoculated vial (right).

Materials Required

1. CC-102, Coliform Vial, 5.0 mL.
2. Soleris Next Generation Instrument
3. SNG Computer with Fusion Software

Dependent on Sample Tested

Vial Specifications

1. Sterile 1N to 5N sodium hydroxide (NaOH) and/or hydrochloric acid (HCl).
2. pH meter or pH paper.
3. Micropipettor and tips, 20–200 µL.

4. Micropipettor and tips, 100–1,000 µL.
5. Inoculating loops, 10 µL.
6. Butterfield’s Phosphate Buffer (BPB), 99 mL.
7. Buffered Peptone Water (BPW).
8. Tryptic Soy Broth (TSB), 90 mL.
 - a. If required, use a designated neutralization broth, such as D/E Neutralizer, TAT Broth, Modified Lethen Broth, etc.
9. Stomacher or equivalent.
10. Stomacher-type bags with mesh filter.
11. Balance: For weighing samples, minimum 100 g and 0.1 g capacity.

Optional Coliform Confirmation Materials (Choose One of the Following)

1. Neogen® Brilliant Green Bile (BGB) Broth tubes (or equivalent).
2. Neogen® Violet Red Bile Agar (VRBA), 500 g (or equivalent).

Vial Specifications

1. Vial pH is 6.7 ± 0.2.
2. Vial sample capacity up to 5.0 mL.

Sample Preparation

1. Add sample directly, or, if using dilute-to-specification, complete the dilution required.
 - a. For United States Pharmacopeia (USP) testing, perform 1:10 dilution by adding 10 g of sample in 90 mL of TSB or designated neutralization broth.
 - b. For all other testing, perform 1:10 dilution by adding 11 g of sample in 99 mL of BPB.
2. Check pH and adjust, if necessary, to 7.0 ± 1.0 .
3. If necessary, use BPB to create the dilutions to the appropriate specification.

Inoculation of Vial

1. Inoculate the vial with no more than 5.0 mL, and no less than 0.10 mL of the sample to be tested. If using dilute-to-specification, add the volume of the appropriate dilution required. Add Butterfield’s buffer to make up total volume of 10.0 mL.
2. Cap the vial and gently invert three times to mix sample. Keep cap tight.
3. Insert the vial into the Soleris instrument utilizing the applicable algorithm below. It is not recommended to adjust the parameters without consulting Neogen technical services at 517.372.9200 or visiting our website at neogen.com.

Algorithms Utilized (Yellow Test Type)

Food

Threshold	Skip	Shuteye	Temperature	Test Duration	Validation Scope
10	1	25	$35 \pm 2^\circ\text{C}$	18 hours ¹	AOAC PTM # 010302: Pasteurized milk, ice cream, butter, yogurt, chocolate, raw eggs, pork sausage, ground beef, and raw chicken. Validated in accordance with AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces ² : Broad food.

¹ Yogurt and queso require a test duration of 24 hours.

² U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM), Chapter 4 (Enumeration of *Escherichia coli* and the coliform bacteria) solid medium method referenced for food products.



Brilliant Green Bile (BGB) Broth negative result (left) and positive result (right)

Confirmation Step (Optional)

Test Method: Brilliant Green Bile (BGB) Broth

1. From a positive CC-102 Vial, invert to mix and inoculate 0.1 mL of the broth medium into a BGB Broth tube with the inverted Durham tube.
2. Incubate for 48 hours at 35°C . Gas production inside the Durham tube indicates a positive result.

Test Method: Violet Red Bile Agar (VRBA)

1. From a positive CC-102 Vial, invert to mix.
2. Using a $10 \mu\text{L}$ inoculating loop, streak from the Soleris vial to a VRBA plate.
3. Incubate for 18–24 hours at 35°C and examine for typical coliform colonies.

Disclaimers

Information provided is based on validation procedures that Neogen performed in Neogen laboratories. Deviation from procedures is possible, but should be discussed with Neogen technical services.

Samples may need to be pH adjusted for all vials.

Appearance of the vials should be inspected prior to use to ensure that the vial/contents are defect free.

If shuteye detections are observed, the threshold may need to be adjusted based on the product matrix. Certain product matrices may require parameter adjustments, including increased test duration. For more information contact Neogen technical services at 517.372.9200 or visit our website at neogen.com.

Some strains do not detect within the recommended test duration and will need an extended test duration. These organisms may have been strain-specific or described as being temperature sensitive.

Reference the Soleris Operating Manual for troubleshooting and instrument use information.

Safety Precautions

Use of this test should be restricted to individuals with appropriate laboratory training in microbiology as some coliform are potentially infectious. Reagents are for laboratory use only. Test samples and used Soleris vials may contain potentially infectious microorganism; follow appropriate laboratory procedures for the handling of microbial pathogens. (U.S. Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at: [www.cdc.gov/labs/pdf/CDC-Biosafety Microbiological Biomedical Laboratories-2020-P.pdf](http://www.cdc.gov/labs/pdf/CDC-Biosafety%20Microbiological%20Biomedical%20Laboratories-2020-P.pdf) (or most current version, found at cdc.gov). All pipetting transfers must be made using either a disposable pipette and pipetting aid or a micro pipettor with disposable tips. Culture media contains antimicrobial selective agents and dyes: wear appropriate PPE and avoid contact with skin and mucous membranes. Refer to the Safety Data Sheet available from Neogen for more information. Used enrichment cultures and agar media should be handled and disposed of as potentially infectious material. The preferred method for decontamination of contaminated material is autoclaving. Items that cannot be autoclaved may be decontaminated using a disinfectant solution, e.g., 10% household bleach, followed by rinsing with water. Follow local, regional, national and industry standards for disposal.

