Non-Fermenting Total Viable Count

Product Number: NF-TVC

NF-TVC Vial uninoculated (left) and inoculated vial (right).

Introduction

The Total Viable Count Vial, 9 mL (NF-TVC) is suitable for dilute-to-specification monitoring and sterility testing in a variety of matrices. The vial has broad inclusivity and an assay time of 24 hours or less for most applications. As organisms grow in the broth medium, the carbon dioxide (CO_2) produced diffuses through a membrane layer into a soft agar plug containing a dye indicator. The color change in the dye is read by the Soleris® instrument. The membrane layer also serves as a barrier, eliminating product interference with the reading frame.

In an AOAC Research Institute *Performance Tested Method* Certificate #071203 study, the Soleris NF-TVC vial was found to be an effective procedure for specification monitoring of total aerobic count in the following sample types: raw chicken, deli ham, lettuce, almonds, black pepper, cheesecake, ice cream mix, nonfat dry milk, cocoa powder, and dried cannabis flower. Test duration is 24 hours, except for cocoa powder and dried cannabis flower, which are 27 hours and 24–48 hours, respectively.

Materials Required

1. NF-TVC, Non-Fermenting Total Viable Count (NF-TVC) Vial.
2. Neogen® rapid microbiology instrument (product no. BSX-32, BSX-128, BLX-INS32, SNG-INS32). Containing one or four temperature-controlled (18–50°C ± 0.5°C) incubator drawers with 32 test locations per drawer. Each test location contains a light-emitting diode (LED) based optical sensor for measurement of changes in absorbance over time.
3. Soleris computer (product no. SNG-COMPUTER or equivalent).
4. Soleris Vial Rack (product no. VR-300, VR-200, or equivalent): Holds 32 vials.

Dependent on Sample Tested

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. Butterfield's Phosphate Buffer (BPB), 99 mL (BPB-99).

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6. Buffered Peptone Water (BPW) (product no. NCM0015 or equivalent).
7. ISO Buffered Peptone Water (product no. NCM0015 or equivalent)
8. Peptone Salt/Maximum Recovery Diluent (product no. NCM0085 or equivalent)
9. Tryptic soy broth (TSB), 90 mL (BLX-TSB90 or equivalent).
10. BPB, 90 mL (product no. 6654 or equivalent).
    a. If required, use a designated neutralization broth, such as D/E Neutralizer, TAT Broth, Modified Letheen Broth, etc.
11. Stomacher or equivalent.
12. Stomacher-type bags with mesh filter (product no. 6827 or equivalent).
13. Balance: For weighing samples, minimum 100 g ± 0.1 g capacity.

Vial Specifications

1. Vial pH is 7.3 ± 0.2.
2. Vial sample capacity up to 1.0 mL.

Vial Preparation

Remove NF-TVC vials from the refrigerator and allow them to equilibrate to room temperature.

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 cannabis testing, perform 1:10 dilution by adding 10 g of sample to 90 mL of TSB. Homogenize the sample thoroughly and decant the liquid. The liquid becomes the test sample.
    c. For all other tests, 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.

Sterility Testing

Preincubation for Sterility Testing

1. Preincubate the sample for 48–72 hours at 32–37°C as optimized for the customer.
2. Shake the container to thoroughly mix any organisms present in the product.
3. After the preincubation, test the sample by adding up to 1.0 mL of the sample to the vial.

Inoculation of Vial

1. Inoculate the vial with no more than 1.0 mL and no less than 0.10 mL of the sample to be tested. If using the dilute-to-specification method, add the volume of the appropriate dilution required.
2. Cap the vial and gently invert three times to mix the sample. Keep cap tight.
3. Insert the vial into the Soleris instrument utilizing the applicable algorithm below or as indicated by a trainer. The incubation temperature and test duration can be optimized within the listed ranges for different product types. It is not recommended to adjust 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	30	35 ± 2°C[1]	24 hours[2]	AOAC PTM # 071203: Raw chicken, deli ham, lettuce, almonds, black pepper, cheesecake, ice cream mix, nonfat dry milk, and cocoa powder.
			35 ± 2°C[1,3]	24 hours[2,4]	Validated in accordance with AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces[5]: Broad food and dairy.

Cannabis

Threshold	Skip	Shuteye	Temperature	Test Duration	Validation Scope
10	1	30	35 ± 2°C	24-48 hours	AOAC PTM # 071203: Dried cannabis flower [> 0.3% delta 9-tetrahydrocannabinol (THC)].
				24 hours[6]	Validated in accordance with USP <1223> Validation of Alternative Microbiological Methods[7]: Broad cannabis and cannabis-containing products.

Personal Care, Cosmetics, Nutraceuticals, and Dietary Supplements

Threshold	Skip	Shuteye	Temperature	Test Duration	Validation Scope
10	1	30	35 ± 2°C	24 hours	Validated in accordance with USP <1223> Validation of Alternative Microbiological Methods[7]: Broad personal care, cosmetic, nutraceutical, and dietary supplement products.

[1] 35 ± 2°C for non-dairy products or 32 ± 0.5°C for dairy products.
[2] Cocoa powder requires a test duration of 27 hours.
[3] 30°C for smoked salmon.
[4] Smoked salmon requires a test duration of 48 hours.
[5] AOAC Official Methods of Analysis 996.23, Standard Methods for the Examination of Dairy Products 6.020, or ISO 4833-1:2013 reference plate count methods, referenced for food, dairy, and smoked salmon, respectively.
[6] Dried cannabis flower, chocolate with THC, and distillate require a test duration of 48 hours.
[7] Compendial USP <2021> and <2022> methods referenced for cannabis, nutraceuticals, and dietary supplement products. USP <61> and <62> methods referenced for personal care and cosmetic products.

Caution

Products containing CO_2 releasing compounds (e.g., ascorbic acid, calcium carbonate, or calcium ascorbate) need to be carefully validated, as reactions with the vial chemistry may occur, causing false positive results.

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.

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 *Enterobacteriaceae* 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 BiomedicalLaboratories-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. Consult with your facility safety director for specific instructions.