soleris

Enterobacteriaceae Vial

Product Number: S2-EBAC9

S2-EBAC9 Vial uninoculated (left) and inoculated vial (right).

Introduction

The *Enterobacteriaceae* Vial, 9 mL (S2-EBAC9) is a screening vial specific for organisms in the family *Enterobacteriaceae*. The vial has broad inclusivity and an assay time of 18 hours for most applications. The vial contains a peptone yeast extract base with glucose 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 glucose utilization changes the pH. As *Enterobacteriaceae* 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 #121901 study, the Soleris® for *Enterobacteriaceae* was found to be an effective method for detection of *Enterobacteriaceae* in the following sample types: pasteurized milk, yogurt, mozzarella cheese, ice cream, dried milk, pasteurized liquid egg, frozen cooked chicken, deli ham, lettuce, dry dog food, and dried cannabis flower [>0.3% delta 9-tetrahydrocannabinol (THC)]. Test duration is 18 hours, with the exception of dried cannabis flower, which is 20–24 hours.

The S2-EBAC9 vial has been awarded MicroVal certification (#2018LR83) for the detection of >10 cfu per gram *Enterobacteriaceae* in a broad range of foods. The ISO 16140-2:2016 validation concluded that the S2-EBAC9 detection method is selective and specific. Test duration for all foods is 18 hours.

Materials Required

1. S2-EBAC9, *Enterobacteriaceae* Vial (product no. S2-EBAC9).
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.

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 121901

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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. Inoculating loops, 10 µL.
6. Butterfield's Phosphate Buffer (BPB), 99 mL (BPB-99).
7. Buffered Peptone Water (BPW) (product no. NCM0015 or equivalent).
8. Tryptic Soy Broth (TSB), 90 mL (BLX-TSB90 or equivalent).
9. 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.
10. Stomacher or equivalent.
11. Stomacher-type bags with mesh filter (product no. 6827 or equivalent).
12. Balance: For weighing samples, minimum 100 g ± 0.1 g capacity.
13. Violet Red Bile Glucose Agar (VRBGA) (product no. NCM0041A or equivalent).

Vial Specifications

1. Vial pH is 6.6+/-0.3.
2. Vial sample capacity up to 1.0 mL.

Sample Preparation

1. For United States Pharmacopeia (USP) testing, perform 1:10 dilution by adding 10 g of sample in 90 mL of TSB (See Neogen rapid microbiology system Validation Book, Introduction, p.5) or designated neutralization broth.
2. For food testing, if liquid sample add the sample directly or prepare a 1:10 dilution by adding 10 g of sample to 90 mL of sterile BPW. Vial sample capacity up to 1 mL.
3. For cannabis testing, perform 1:10 dilution by adding 10 g of sample to 90 mL of TSB. Massage/homogenize the sample by hand thoroughly and decant the liquid. Squeeze remaining sample to remove as much TSB as possible. The liquid becomes the test sample.
4. For all other testing, add the sample directly or prepare a 1:10 dilution by adding 11 g of sample to 99 mL of sterile BPB. Vial sample capacity up to 1 mL.
5. Check pH and adjust, if necessary, to 7.0 ± 1.0, except for testing cannabis flower.
6. If using the dilute-to-specification method, complete the dilution required using TSB, BPW, or BPB.

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 dilute-to-specification method, add the volume of the appropriate dilution required.
   a. For AOAC testing, use 1.0 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 or as indicated by a trainer. The incubation temperature and test duration can be optimized if required. It is not recommended to adjust parameters without consulting Neogen technical services at 517.372.9200 or visiting our website at neogen.com.
4. A detection curve will be generated in real time. The Soleris software will indicate a positive or negative test result. Positive results will generally be reported in less than the designated test duration.

Confirmation Step (Optional)

Test Method: Violet Red Bile Agar (VRBA)

1. From a positive S2-EBAC9 Vial, invert to mix.
2. Using a 10 µL inoculating loop, streak from the Soleris vial to a VRBGA plate.
3. Incubate for 24 hours at 37 ± 1°C and examine for typical *Enterobacteriaceae* colonies and continue with confirmation following ISO 21528–2:2017 procedures.

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Algorithms Utilized (Yellow Test Type)

Food

Threshold	Skip	Shuteye	Temperature	Test Duration	Validation Scope
10	1	25	36 ± 1°C	18 hours	AOAC PTM # 121901: Pasteurized milk, yogurt, mozzarella cheese, ice cream, dried milk, pasteurized liquid egg, frozen cooked chicken, deli ham, lettuce, and dry dog food.
					Validated in accordance with AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces[1]: Broad food and environmental surfaces.
			37°C	18 hours	MicroVal # 2018LR83: Broad food.

Cannabis

Threshold	Skip	Shuteye	Temperature	Test Duration	Validation Scope
10	1	25	35 ± 0.5°C	20–24 hours	AOAC PTM # 121901: Dried cannabis flower [>0.3% delta 9-tetrahydrocannabinol (THC)].
				18 hours[2]	Validated in accordance with USP <1223> Validation of Alternative Microbiological Methods[1]: Broad cannabis and cannabis-containing products.

Personal Care, Cosmetics, Nutraceuticals, and Dietary Supplements

Threshold	Skip	Shuteye	Temperature	Test Duration	Validation Scope
10	1	25	35 ± 0.5°C	18 hours	Validated in accordance with USP <1223> Validation of Alternative Microbiological Methods[1]: Broad personal care, cosmetic, nutraceutical, and dietary supplement products.

[1] ISO 21528–2:2017 direct plating method referenced for food products and environmental samples, and modified for evaluation with cannabis, personal care products, cosmetics, nutraceuticals, and dietary supplements.

[2] Dried cannabis flower requires a test duration of 20–24 hours.

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.

The following organisms produced negative results in inclusivity testing: *Buttiauxella warmboldiae, Serratia grimesii, Kluyvera ascorbata, Pantoea agglomerans, Proteus penneri, Serratia proteamaculans, Proteus myxofaciens, Serratia grimesii,* and *Yersinia enterocolitica*. In some cases (e.g., *Pantoea agglomerans*), these reactions have proven to be strain specific, while in other cases the organisms have been described as being temperature sensitive.

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.

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