

Neogen's Soleris[®] Nonfermenting Total Viable Count Granted PTM Status



Neogen Corp.
Soleris Nonfermenting Total Viable Count (NF-TVC)
PTM Status: July 18, 2012
Certificate No.: 071203

Neogen Corp. developed the Soleris Nonfermenting Total Viable Count (NF-TVC) test vial (Figure 1) for use with the company's Soleris optical microbial detection system to detect the growth of heterotrophic bacterial microorganisms (e.g., *Pseudomonas* spp.) in as little as 24 hours. Standard methods for detection or enumeration of total aerobic bacteria, yeasts, and molds in foods are based on dilution plating, filtration, or most probable number (MPN) approaches. Examples of dilution plating methods include AOAC Official MethodSM 966.23 and the standard plate count method from *Standard Methods for the Examination of Dairy Products*. These methods require 48 hours to obtain results. The Soleris method, through sensitive determination of metabolic activity during microbial growth, produces results within 24 hours for most foods.

The purpose of this study was to validate the Soleris NF-TVC automated growth-based method for semi-quantitative detection of mesophilic, aerobic microorganisms in a variety of food products. A probability of detection (POD) statistical model was used to compare Soleris results at multiple test thresholds (dilutions) with aerobic plate counts determined using refer-



Figure 1. Soleris Nonfermenting Total Viable Count (NF-TVC) test vial.

ence dilution plating procedures. Nine naturally contaminated food products were tested, with Soleris testing performed at three or four threshold levels for each food. Using the POD model, all Soleris test results were in statistical agreement with the reference plating procedures with the exception of a single threshold level in two trials with black pepper, and a single threshold level in the independent laboratory trial with cheesecake. Results of the internal and independent laboratory validation

studies showed that the Soleris NF-TVC method can be used as an accurate alternative to conventional dilution plating procedures, while saving 24 hours or more in analysis time.

Principle of the Assay

Soleris NF-TVC is a growth-based, automated method with an optical endpoint. A dilution of the test sample homogenate is inoculated directly into the test vial. The prepared vial is then placed in the Soleris instrument set at the appropriate incubation temperature. As microorganisms grow and metabolize, carbon dioxide is produced which diffuses from the growth medium through a gas-permeable layer and into the indicator portion of the Soleris vial. Reaction of carbon dioxide with the indicator chemicals results in a color change over time. The color is monitored by the instrument and a change in color of a certain magnitude, determined by the Soleris software, is indicative of a positive test result. The Soleris instrument contains temperature-controlled incubation chambers and photodiode-based optical detection devices for measurement of color changes in the bottom indicator portion of the vial.

The Soleris NF-TVC vial represents an improvement over the original Soleris TVC vial. The indicator system has been changed to utilize detection of carbon dioxide rather than detection of acid production, expanding the inclusivity of the vial to include nonfermenting organisms.

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Table 1. Comparative testing results and probability of detection calculations for the Soleris NF-TVC method

Food type	Reference plate count (CFU/g) ^a	Soleris test threshold (CFU/g) ^b	CFU/vial ^c	Predicted POD ^d	Soleris result		Observed POD ^e			Interpretation ^f
					No. vials positive	No. vials tested	LCI	POD	UCI	
Raw chicken	8.7 x 10 ⁶	1,000,000	8.7	0.999	20	20	0.832	1	1	Pass
		10,000,000	0.87	0.581	9	20	0.258	0.450	0.678	Pass
		100,000,000	0.087	0.083	1	20	0.009	0.050	0.236	Pass
Raw chicken ^g	6.5 x 10 ³	100	65	1	20	20	0.832	1	1	Pass
		1,000	6.5	0.998	20	20	0.832	1	1	Pass
		10,000	0.65	0.478	10	20	0.299	0.500	0.701	Pass
Deli ham	1.3 x 10 ³	100	13	1	20	20	0.832	1	1	Pass
		1,000	1.3	0.727	12	20	0.387	0.600	0.781	Pass
		10,000	0.13	0.122	0	20	0	0	0.168	Pass
Lettuce	1.8 x 10 ⁴	1,000	18	1	20	20	0.832	1	1	Pass
		10,000	1.8	0.835	16	20	0.584	0.800	0.919	Pass
		100,000	0.18	0.165	1	20	0.009	0.050	0.236	Pass
Almonds	2.0 x 10 ²	10	20	1	20	20	0.832	1	1	Pass
		100	2.0	0.865	16	20	0.584	0.800	0.919	Pass
		1,000	0.2	0.181	2	20	0.028	0.100	0.301	Pass
Black pepper	2.9 x 10 ³	1,000	2.9	0.945	20	20	0.832	1	1	Pass
		10,000	0.29	0.252	9	20	0.258	0.450	0.658	Fail
		100,000	0.029	0.029	0	20	0	0	0.168	Pass
Black pepper	1.3 x 10 ³	100	13	1	20	20	0.832	1	1	Pass
		1,000	1.3	0.727	9	20	0.258	0.450	0.658	Fail
		10,000	0.13	0.122	4	20	0.081	0.200	0.416	Pass
		100,000	0.013	0.013	1	20	0.009	0.050	0.236	Pass
Cheesecake	1.2 x 10 ⁵	10,000	12	1	20	20	0.832	1	1	Pass
		100,000	1.2	0.699	15	20	0.531	0.750	0.888	Pass
		1,000,000	0.12	0.113	2	20	0.028	0.100	0.301	Pass
		10,000,000	0.012	0.012	0	20	0	0	0.168	Pass
Cheesecake ^g	1.1 x 10 ⁷	1,000,000	11	1	20	20	0.832	1	1	Pass
		10,000,000	1.1	0.667	15	20	0.531	0.750	0.888	Pass
		100,000,000	0.11	0.104	14	20	0.481	0.700	0.854	Fail
Ice cream mix	3.6 x 10 ⁴	1,000	36	1	20	20	0.832	1	1	Pass
		10,000	3.6	0.973	19	20	0.764	0.950	0.991	Pass
		100,000	0.36	0.302	5	20	0.112	0.250	0.469	Pass
Nonfat dry milk	2.9 x 10 ²	10	29	1	20	20	0.832	1	1	Pass
		100	2.9	0.945	19	20	0.764	0.950	0.991	Pass
		1,000	0.29	0.252	2	20	0.028	0.100	0.301	Pass
		10,000	0.029	0.029	1	20	0.009	0.050	0.236	Pass
Cocoa powder ^h	1.8 x 10 ¹	10	1.8	0.835	17	20	0.640	0.850	0.948	Pass
		100	0.18	0.165	6	20	0.145	0.300	0.519	Pass
		1,000	0.018	0.018	1	20	0.009	0.050	0.236	Pass

^a Direct plating methods: AOAC OMA 966.23 (nondairy products), Standard Methods for the Examination of Dairy Products method 6.020 (dairy products).
^b Dilute-to-specification approach. Positive result expected if organism concentration in test dilution is greater than Soleris test threshold.
^c CFU per Soleris vial based on reference plate count.
^d Probability of detection (predicted); based on reference plate count (POD = 1 - e^{-x}).
^e Probability of detection (observed); fraction of Soleris results positive. LCI and UCI are 95% lower and upper confidence intervals, respectively.
^f Test for equivalence of reference plate count and Soleris results. For "Pass," POD (pred.) must lie with POD (obs.) LCI-UCI range.
^g Trial performed by independent laboratory.
^h Soleris vials were incubated for 27 hours.

The Soleris NF-TVC test can be used in a “dilute-to-specification” or threshold manner in which the result is positive or negative around a desired cutoff (in CFU/g) determined by the dilution and volume of sample homogenate added to the vial. It is assumed that 1 CFU introduced into the Soleris vial will lead to a positive result. Alternatively, the test can be used in a fully quantitative manner by building product-specific calibration curves relating initial analyte concentration to Soleris detection time.

Validation Studies

Scheduled for publication in the *Journal of AOAC INTERNATIONAL*, the Soleris NF-TVC validation study consisted of in-house and independent laboratory validations. All independent laboratory experiments were conducted by Q Laboratories (Cincinnati, Ohio, USA).

The Soleris NF-TVC method was compared to AOAC *Official Method of Analysis*SM 966.23 (aerobic plate count) for the following foods: raw chicken, deli ham, lettuce, almonds, black pepper, cheesecake, and cocoa powder. The Soleris method was compared to method 6.020 of *Standard Methods for the Examination of Dairy Products* (standard plate count) for ice cream mix and nonfat dry milk. All foods were naturally contaminated and were tested without inoculation.

Results are reported in Table 1. Reference method aerobic plate counts ranged from a low of 1.8×10^1 CFU/g for cocoa powder to a high of 8.7×10^6 CFU/g for raw chicken. All trials produced fractional positive results at one of more test threshold levels with the Soleris method. Based on the POD analysis, there were no statistically significant differences in results between the Soleris and reference methods for eight of the nine foods at any test threshold.

In the initial trial with black pepper, POD (pred.) was slightly outside of the POD (obs.) lower confidence limit at the $>10,000$ CFU/g test threshold (0.252 vs 0.258). Results at both the >1000 and $>100,000$ CFU/g test thresholds passed the statistical test. For cocoa powder, initial experiments indicated that Soleris method sensitiv-

ity was inadequate with a 24-hour test duration. Therefore a test duration of 27 hours was used, with good results.

Independent Laboratory Validation

Soleris for NF-TVC was submitted to Q Laboratories for independent analysis. The laboratory

performed two trials, one with raw chicken and one with cheesecake. Test protocols and data analysis methods were identical to those used for the internal validation studies.

Results are shown in Table 1. The aerobic plate count of the raw chicken sample was 6.5×10^3 CFU/g. Fractional positive Soleris results were obtained at one level, and POD (pred.) was within the confidence interval of POD (obs.) for all three test thresholds. The aerobic plate count of the cheesecake sample was 1.1×10^7 CFU/g. The data passed the statistical test at both the $>1,000,000$ and $>10,000,000$ CFU/g test thresholds (inputs of 11 and 1.1 CFU/vial based on the reference method plate count), but failed at the $>100,000,000$ CFU/g test threshold (0.11 CFU/vial) with POD (pred.) of 0.104 vs POD (obs.) confidence interval of 0.481–0.854. Overall, results of the independent laboratory trials are consistent with those of the internal studies.

Discussion

Results of the internal and independent laboratory studies clearly indicate that the Soleris NF-TVC method can be used as an accurate alternative to reference dilution plating procedures for semi-quantitative determination of mesophilic aerobic counts in a wide variety of food products. With few exceptions, Soleris method results were statistically equivalent to those of the reference plating procedures as determined using a POD model. Use of the Soleris NF-TVC method in a “dilute-to-specification” mode allows users to match test thresholds with product

The Soleris NF-TVC test can be used in a “dilute-to-specification” or threshold manner in which the result is positive or negative around a desired cutoff (in CFU/g) determined by the dilution and volume of sample homogenate added to the vial.

release specifications. Compared to dilution plating procedures, the Soleris method offers the advantages of minimal labor and reduced analysis time; results are obtained in 24 hours or less compared to the 48 hours required by the conventional plating procedures.

Based on results of studies, the Soleris NF-TVC method was granted *Performance Tested Method*SM status for detection of mesophilic aerobic bacteria, yeasts, and molds in raw chicken, deli ham, lettuce, almonds, black pepper, cheesecake, ice cream mix, nonfat dry milk, and cocoa powder. Results, as well as complete sample preparation and test procedures, will be presented in the validation study report that will appear in *J. AOAC Int.* ■

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Mention of trade names or commercial products is for identification only and does not constitute preference over similar ones not mentioned. If you are interested in submitting an article regarding a test kit that has been granted Performance Tested MethodSM status, contact Deborah McKenzie, senior director, standards development, at dmckenzie@aoac.org.

ORDERING INFORMATION

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