Microbiological Methods

Soleris® Enterobacteriaceae for the Detection of Enterobacteriaceae in Select Foods: AOAC Performance Tested MethodSM 121901

Susan Alles,1 Brooke Roman,1,* Gail Betts,2 Suzanne Jordan,2 Linda Everis,2 Carolyn Montei,1 Preetha Biswas,1 Mark Mozola,1 and Robert Donofrio1

1Neogen Corporation, 620 Lesher Pl, Lansing, MI 48912, USA, 2Campden BRI, Station Rd, Chipping Campden, Gloucestershire GL55 6LD, UK

*Corresponding author's email: broman@neogen.com.

Abstract

Background: Soleris® Enterobacteriaceae is a growth-based, automated method for detection of Enterobacteriaceae in food.

Objective: A study was conducted to validate the Soleris method for detection of Enterobacteriaceae in select foods (pasteurized milk, yogurt, mozzarella cheese, ice cream, dried milk, pasteurized liquid egg, frozen cooked chicken, deli ham, lettuce, and dry dog food) at a threshold of ≥ 10 CFU/g of product.

Methods: Inclusivity and exclusivity of the Soleris method were assessed by testing 55 and 38 target and non-target bacterial strains, respectively. Matrix testing was performed with one naturally contaminated and nine inoculated foods. Efficacy of the Soleris method was compared to that of the ISO 21528-2:2017 direct plating reference method using probability of detection analysis. Independent laboratory testing was conducted to verify method performance in two matrixes (yogurt and deli ham). Method robustness, stability, and lot-to-lot consistency of the Soleris reagents were also assessed.

Results: Inclusivity of the Soleris test was 91% and exclusivity was 100%. In matrix testing, there were no significant differences in the number of positive results obtained with the Soleris and reference methods for any of the matrixes examined. Overall, of 370 test portions, there were 176 positive results by the Soleris method and 177 positive results by the reference procedure.

Conclusions: Soleris Enterobacteriaceae is an effective method for detection of Enterobacteriaceae in the foods evaluated, with performance equivalent to that of the ISO 21528-2:2017 reference method.

Highlights: The Soleris method offers the advantages of labor savings and results within 18 h.

Enterobacteriaceae (EBAC) are a large family of Gram-negative bacteria including several genera containing well-established human pathogens such as Salmonella, Escherichia, Yersinia, Shigella, and Klebsiella. Food, nutraceutical, pharmaceutical, and cosmetic products are routinely monitored for the presence of EBAC to protect against adulterated products entering commerce.

Soleris Enterobacteriaceae is an automated, growth-based method for detection of EBAC in food. Growth of target organisms introduced from a sample homogenate or dilution into a test vial containing a selective medium is monitored by the Soleris instrument. When a threshold level is reached, the instrument signals the test result as positive. If no growth is detected within 18 h, the sample is reported as negative.

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This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
Soleris Enterobacteriaceae is a member of a large family of tests in the Soleris platform. Several Soleris methods have received AOAC Performance Tested Method certification, including methods for total viable count (2, 3), coliforms (4), Escherichia coli (5), and yeast and mold (6, 7). Here we report results of a study designed to validate the performance of the Soleris method for detection of EBAC in select foods at levels ≥ 10 CFU/g. Soleris method performance was compared to that of the ISO 21528-2:2017 reference method (1), which is based on a conventional colony count technique. The study was conducted in accordance with the current AOAC International Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (8).

Scope of Method

(a) **Target organisms.**—Enterobacteriaceae.

(b) **Matrixes.**—Pasteurized milk (whole milk, 3.25% milkfat by weight), yogurt (vanilla flavored probiotic yogurt), mozzarella cheese, ice cream (7% fat content, vanilla bean flavor), dried milk, pasteurized liquid egg, frozen cooked chicken, deli ham, lettuce (bagged shredded iceberg), dry dog food (main ingredients: beef, corn, barley, rice gluten meal).

(c) **Summary of validated performance claims.**—As determined by probability of detection (POD) analysis, Soleris® Enterobacteriaceae method performance is equivalent to that of the ISO 21528-2:2017 colony count reference method for detection of Enterobacteriaceae (1) at levels ≥ 10 CFU/g of product.

Definitions

(a) **Probability of Detection (POD).**—The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. Several POD measures can be calculated: PODₙ (reference method POD), PODₖ (confirmed candidate method POD), PODₐ (candidate method presumptive result POD), and PODₐC (candidate method confirmation result POD).

(b) **Difference of Probabilities of Detection (dPOD).**—Difference of probabilities of detection is the difference between any two POD values. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Principle

The Soleris vial is comprised of an upper portion containing a selective growth medium and a pH indicator, and a lower detection portion containing a matrix which excludes particulates but allows diffusion of gasses and small molecules. The vial contains a peptone yeast extract base with glucose as the carbon source. The selective agents include bile salts, sodium lauryl sulfate, and other Gram-positive inhibitors. The Soleris instrument is comprised of temperature-controlled chambers and optical sensors which monitor the color in the detection portion of the vial over time. An aliquot of a test sample homogenate or further dilution is introduced into the Soleris vial. The vial is capped and placed into the Soleris instrument programmed with specific test parameters including temperature and test duration. As EBAC grow and ferment glucose in the vial, the pH is reduced and the indicator color changes from purple to yellow. This change occurs in both the growth and detection portions of the vial. When a color change of a specific magnitude is detected, the instrument signals the test result as positive. If no change is detected within 18 h, the test result is reported as negative. Culture confirmation of Soleris results may be conducted by sampling from the upper chamber of the vial when the test is complete.

Materials and Methods

Test Kit Information

(a) **Test name.**—Soleris® Enterobacteriaceae Vial.

(b) **Cat. No.**—S2-EBAC9

(c) **Ordering information.**—In the United States.—Neogen Corp., 620 Lesher Pl, Lansing, MI 48912, Tel: 800-234-5333 or 517-372-9200, Fax: 517-372-2006, Website: www.neogen.com. Outside the United States.—Contact U.S. office for ordering or distributor information.

(d) **Soleris® Vial, Enterobacteriaceae, 9 mL.**—Sterile medium in plastic vial devices, box of 100, one test per vial, pH 6.7 ± 0.2, sample capacity 1 mL. Requires Soleris instrument or equivalent.

Supplies and Reagents

(a) **Soleris® 32 instrument (Product No. BSX32) or Soleris® 128 instrument (Product No. BSX128) or equivalent.**—Containing one or four temperature-controlled (18-60 ± 0.5°C) incubator drawers, respectively, 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.

(b) **Soleris computer system (Product No. BSC01).**—Includes vial rack.

(c) **Soleris operator’s manual (Product No. OM-710).**

(d) **Soleris® vial rack (Product No. VR-300 or equivalent).**—Holds 32 vials.

(e) **Soleris vial rack transfer mechanism (Product No. VRTM-200).**

(f) **Stomacher® or equivalent.**

(h) **Stomacher-type bags with mesh filter (Product No. 6827).**

(i) **Balance.**—For weighing samples, minimum 100 g ± ± 0.1 g capacity.

(j) **Micropipettor and tips.**—20–200 μL.

(k) **Micropipettor and tips.**—100–1000 μL.

(l) **Hydrochloric acid solution.**—1 N, sterile, for adjusting pH of sample.

(m) **Sodium hydroxide solution.**—1 N, sterile, for adjusting pH of sample.

(n) **Buffered peptone water (Product No. NCM0015 or equivalent).**

(o) **Violet red bile glucose agar (Product No. NCM0041A or equivalent).**—500 g (other sizes available).

Standard Reference Materials

Bacterial cultures used in this study were obtained from the following institutions: American Type Culture Collection (ATCC, Manassas, VA), Campden BRI (CRA, Chipping Campden, United Kingdom), National Collection of Type Cultures (NCTC, Porton...
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. All pipetting transfers must be made using either a disposable pipet and pipetting aid or micropipettor 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 Corp. for more information. Used Soleris vials should be handled and disposed of as potentially infectious material. The preferred method for disposal of contaminated materials, including used vials, sample homogenates, pipettes, etc., is autoclaving. Items that cannot be autoclaved may be decontaminated by using a disinfectant solution, e.g., 10% household bleach, followed by rinsing with water. Consult with your facility safety director for specific instructions.

Sample Preparation

(a) Combine 10 g sample and 90 mL sterile buffered peptone water in a stomacher-type bag, homogenize thoroughly.
(b) Check pH and adjust if necessary, to pH 7.0 ± 1.0.
(c) For testing at a threshold level of ≥ 10 CFU/g, the sample homogenate is used without further dilution. For testing at higher threshold levels, prepare the appropriate dilution in buffered peptone water.

Soleris Testing

Note: The Soleris system requires installation and operator training. Both are provided by Neogen Corp.

(a) In the Soleris software, select the test type and enter sample identification information into the sample position grid.
(b) Add 1.0 mL of the sample homogenate or dilution to a Soleris vial.
(c) Cap the vial and gently invert three times to mix. Keep the cap tight.
(d) Insert the vial into the Soleris instrument programmed with the following settings:
   (1) Test: S2-EBAC9
   (2) Threshold: 10
   (3) Skip: 1
   (4) Shuteye: 25
   (5) Duration: 18 h
   (6) Temperature: 36 ± 1°C
(e) Click Start Run. A detection curve will be generated in real time. The test will run for 18 h, but positive results may be reported at any time up to 18 h.

Interpretation of Results

(a) Negative criterion.—Tests producing no detection after 18 h are considered negative at the test threshold selected.
(b) Positive criterion.—Detection times within 18 h indicate a positive result at the test threshold selected.

Recommended Confirmation Procedure

Positive results may be confirmed by streaking the vial contents to violet red bile glucose agar and continuing with identification of presumptive Enterobacteriaceae colonies using standard methods (1).

Internal Validation Studies

Inclusivity Testing

(a) Methodology.—Inclusivity testing was conducted using 55 bacterial species of the family Enterobacteriaceae. Strains were grown in nutrient broth overnight at 37 ± 1°C and then diluted to approximately 100 CFU/mL. One mL was introduced to the Soleris vial and the test run on the Soleris instrument for 18 h at 36 ± 1°C. Strains were randomized, blind coded, and intermixed with exclusivity strains.
(b) Results.—Results are shown in Table 1. Fifty of the 55 strains (91%) produced a positive result within 18 h. The five organisms that showed no detection within 18 h were Butteria warmboldiae, one of two strains of Pantoea agglomerans, Serratia grimesii, Serratia proteamaculans, and Yersinia enterocolitica. Three of the five strains were detected outside of the 18 h test duration (see Table 1).

Exclusivity Testing

(a) Methodology.—Exclusivity testing was conducted using 38 strains of non-target Gram-negative and Gram-positive bacteria. Strains were grown in nutrient broth overnight at 37 ± 1°C and then diluted to approximately 1 × 10^5 CFU/mL. One mL was introduced to the Soleris vial and the test run in the Soleris instrument for 18 h at 36 ± 1°C.
(b) Results.—Results are shown in Table 2. Of the 38 strains tested, all produced no detection within 18 h for exclusivity of 100%.

Matrix Testing

(a) Methodology.—Performance of the Soleris EBAC method at a threshold level of ≥ 10 CFU/g was compared to that of the ISO 21528-2:2017 reference colony count method in testing of 9 food matrices. A tenth matrix was tested at a higher threshold level. The same amount from each test portion (1 mL of a 1:10 food sample homogenate, or 0.1 g) was used for both the Soleris and reference methods, therefore the two methods have the same theoretical detection limit. For the reference method, plate counts were scored for each test portion. For comparison to Soleris results at the ≥ 10 CFU/g threshold, plate counts ≥ 10 CFU/g were scored as positive and those < 10 CFU/g were scored as negative. The number of positive results obtained by the two methods was compared using POD analysis.

1) Sample preparation.—Food matrices and inoculation organisms are shown in Table 3. Levels shown in CFU/g reflect mean results of the reference method plate counts. Lettuce with naturally occurring EBAC was available, but all other matrices required inoculation. As the lettuce contained EBAC at a high level (approximately 5 × 10^5 cfu/g), the test threshold for this matrix was set at ≥ 100 000 CFU/g by making further dilutions of the sample homogenate. A liquid inoculum was used for all foods except dried milk which was...
Table 1. Inclusivity testing results for the Soleris EBAC method

| Organism                   | CRA strain no. | Other strain no. | Source                | Detection time, h | Result   |
|----------------------------|----------------|------------------|-----------------------|-------------------|----------|
| Buttiauxella warmboldiae    | 17112          | NA               | Rainwater             | 22.5              | Negative |
| Citrobacter amalonaticus    | 7458           | NA               | Beansprouts           | 8.8               | Positive |
| Citrobacter braakii         | 16279          | NA               | Industrial isolate    | 9.0               | Positive |
| Citrobacter diversus        | 7119           | NA               | Unknown               | 8.3               | Positive |
| Citrobacter freundii        | 3163           | NA               | Sausage               | 8.4               | Positive |
| Citrobacter gillenii        | NA             | NCTC 9054        | Unknown               | 10.4              | Positive |
| Citrobacter koseri          | 16279          | NCIMB 11446      | Unknown               | 8.6               | Positive |
| Citrobacter youngae         | 16923          | NCTC 13709       | Unknown               | 9.5               | Positive |
| Cronobacter sakazakii       | 16909          | NA               | Dried milk            | 8.4               | Positive |
| Enterobacter aerogenes      | 4232           | NA               | Sesame seeds          | 7.5               | Positive |
| Enterobacter amnigenus      | 7426           | NA               | Mushrooms             | 10.7              | Positive |
| Enterobacter asburiae       | NA             | NCTC 12123       | Unknown               | 8.0               | Positive |
| Enterobacter cloacae        | 7547           | NA               | Tomato salad          | 7.9               | Positive |
| Enterobacter dispar          | NA             | NCTC 8006        | Unknown               | 8.3               | Positive |
| Enterobacter gergoviae      | NA             | NCIMB 13304      | Unknown               | 9.6               | Positive |
| Enterobacter intermedia     | 17023          | NA               | Surface water         | 16.3              | Positive |
| Enterobacter intermedius    | NA             | NCTC 12125       | Unknown               | 16.8              | Positive |
| Enterobacter sakazakii      | 5172           | NA               | Unknown               | 8.1               | Positive |
| Enterobacter taylorae       | 7530           | NA               | Unknown               | 8.7               | Positive |
| Enterobacter xiangfangensis | NA             | NCIMB 14836      | Unknown               | 7.6               | Positive |
| Erwinia amylovorans         | 8037           | NA               | Industrial isolate    | 7.2               | Positive |
| Escherichia adcecarboxylata | 5501           | NA               | Skim milk powder      | 7.5               | Positive |
| Escherichia coli            | 16041          | NA               | Raw ground beef       | 7.5               | Positive |
| Escherichia fergusonii      | 7522           | NA               | Sausages              | 8.1               | Positive |
| Escherichia hermanii        | 7477           | NA               | Sesame seeds          | 10.4              | Positive |
| Escherichia vulneris        | 2005           | NA               | Vegetables            | 14.5              | Positive |
| Hafnia alvei                | 7480           | NA               | Prawn coleslaw        | 8.9               | Positive |
| Klebsiella aerogenes        | 8387           | NCTC 1317       | Unknown               | 9.3               | Positive |
| Klebsiella oxytoca          | 15926          | ATCC 13182      | Pharyngeal tonsil     | 9.0               | Positive |
| Klebsiella ozoenae          | 4273           | NA               | Industrial isolate    | 12.3              | Positive |
| Klebsiella pneumoniae       | 6650           | NCIMB 14469      | Industrial isolate    | 9.7               | Positive |
| Klebsiella rhinoscleromatis | 4272           | NA               | Unknown               | 14.9              | Positive |
| Klebsiella trevisanii       | NA             | NCIMB 8606      | Unknown               | 10.9              | Positive |
| Lederia ardecarboxyla       | 5121           | NA               | Oregano               | 8.0               | Positive |
| Methanobacter aracida       | NA             | NCIMB 14469      | Unknown               | 10.7              | Positive |
| Morganella morganii         | 5120           | NA               | Pork                  | 10.1              | Positive |
| Pantoea agglomerans         | 17030          | NCIMB 702072     | Pasteurized milk      | 19.3              | Negative |
| Pantoea agglomerans         | 5512           | NA               | Dried milk            | 7.2               | Positive |
| Proteus vulgaris            | 1581           | NA               | Unknown               | 12.8              | Positive |
| Providencia alcalfaciens    | 7469           | NA               | Chicken               | 14.6              | Positive |
| Providencia retgeri         | 8386           | NA               | Unknown               | 11.1              | Positive |
| Raoultella planticola       | 16820          | ATCC 43176      | Raw tuna              | 8.9               | Positive |
| Salmonella bongori          | 16379          | NA               | Unknown               | 8.6               | Positive |
| Salmonella enterica ssp. diarizonae | 16380 | NA               | Unknown               | 8.9               | Positive |
| Salmonella enterica ssp. arizonae | 16380 | NA               | Unknown               | 9.1               | Positive |
| Salmonella enterica ssp. enterica ser. Schwarzengrund | 1408 | NCTC 6756 | Unknown                | 8.5               | Positive |
| Salmonella enterica ssp. houtae | 1376 | NA               | Unknown               | 9.0               | Positive |
| Salmonella enterica ssp. enterica ser. Paratyphi B var. Java | 1378 | NA               | Unknown               | 8.3               | Positive |
| Serratia fonticola          | 4613           | NA               | Chicken               | 16.2              | Positive |
| Serratia grimesii           | 1521           | NA               | Unknown               | 20.4              | Negative |
| Serratia liquefaciens       | 1560           | NA               | Mince                 | 14.3              | Positive |
| Serratia proteamaculans     | 16463          | NCTC 11544      | Canine, Tennessee     | ND                | Negative |
| Shigella dysenteriae        | 4275           | NA               | Industrial isolate    | 9.7               | Positive |
| Shigella blattae            | 16931          | NA               | Cockroach             | 10.3              | Positive |
| Yersinia enterocolitica     | NA             | NCTC 10460      | Chinchilla            | ND                | Negative |

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*a Campden BRI, Chipping Campden, Gloucestershire, UK.
*b Detection times ≤ 18 h indicate a positive result.
*c NA – Not available.
*d National Collection of Type Cultures, Porton Down, Salisbury, UK.
*e National Collection of Industrial, Food, and Marine Bacteria, Aberdeen, Scotland, UK.
*f American Type Culture Collection, Manassas, VA, USA.
*g ND – No detection.
inoculated with a crushed, lyophilized cell pellet. For each food, bulk matrix was inoculated with the test organism (culture dilution or for milk powder blending of the inoculated powder with additional dried milk) at a level of approximately 10–50 CFU/g, a level intended to produce a fractionally positive data set. The bulk material was extensively mixed by hand to ensure homogeneity of the inoculum. From the inoculated fractional-level bulk matrix, 20 or 30 10 g test portions were prepared. For each matrix, 5 test portions at a higher level (expected to produce 100% positive results) were also prepared, as well as 5 uninoculated control test portions. Inoculated ice cream and frozen chicken test portions were held at –20°C for 14 days before testing. Dry dog food and dried milk were held at 15–25°C for 14 days. All other inoculated foods were held at 2–8°C for 48–72 h. The level of contamination for dried products after the 14-day hold was estimated by preparing a homogenate and plating on selective and nonselective media. Test portion homogenates were prepared by combining 10 g of food matrix with 90 mL buffered peptone water.

(2) ISO 21528-2:2017 reference method.—The reference method was performed as described. One mL of test portion homogenate was poured-plated to violet red bile glucose (VRBG) agar and incubated at 37±1°C for 24±2 h. Presumptive EBAC colonies were confirmed with oxidase and glucose fermentation tests. Colonies that were oxidase-negative and glucose-positive were considered EBAC.

(3) Soleris method.—One mL of test portion homogenate or further dilution was added to a Soleris vial.
test was performed using a temperature of 36 ± 1°C and a test duration of 18 h. All vials were sampled for confirmation at the end of the test, irrespective of result, by streaking to VRBG agar and continuing with confirmatory tests as described for the reference method.

(4) Data analysis.—The number of positive results from the Soleris presumptive and Soleris confirmed methods, by matrix and inoculation level, were compared using a paired POD test (8) at $P < 0.05$. The number of positive results from the Soleris confirmed and reference methods were compared using an unpaired POD test (8) at $P < 0.05$.

(b) Results.—Results for the Soleris presumptive and confirmed tests are shown in Table 3. Results for the Soleris confirmed and reference methods are shown in Table 4. At the fractional level, inoculation levels determined from the mean reference method plate counts ranged from 3 to 22 CFU/g. These levels are consistent with the fractional positive data sets obtained at the $> 10$ CFU/g test threshold level. Inoculation levels for the high-level test portions ranged from 12 to 218 CFU/g. The mean reference method plate count for naturally occurring EBAC in lettuce was $4.7 \times 10^9$ CFU/g.

Soleris presumptive and Soleris confirmed results were identical; there were no unconfirmed positive results by the Soleris test (Table 3). Comparing the Soleris and reference methods, out of 220 fractional-level results for the 10 matrices combined, there were 98 positive results by the Soleris method and 100 positive results by the reference plating method (Table 4).

### Table 3. Soleris Enterobacteriaceae results: Soleris presumptive vs. Soleris confirmed

| Matrix             | Strain                  | Mean Level, CFU/g | N$^b$ | x$^c$ | PODCP$^d$ | $95\%$ CI | X | PODCC$^e$ | $95\%$ CI | dPODCP$^f$ | $95\%$ CI |
|--------------------|-------------------------|------------------|-------|-------|-----------|-----------|---|-----------|-----------|-----------|-----------|
| Pasteurized milk   | Cronobacter sakazakii   | ATCC$^b$ 12868   | 8     | 0.40  | 0.22     | $0.61$    | 8 | 0.40      | $0.61$    | 0.47      | 0.47      |
| Yogurt             | Cronobacter sakazakii   | ATCC 29544       | 7     | 0.50  | 0.30     | $0.70$    | 10| 0.50      | $0.70$    | 0.13      | 0.13      |
| Yogurt$^i$         | Eschrichia chiaaeocarboxylata | CRA$^j$ 5501 | 9     | 0.50  | 0.30     | $0.70$    | 10| 0.50      | $0.70$    | 0.13      | 0.13      |
| Mozzarella cheese  | Klebsiella oxytoca      | ATCC 13118       | 13    | 0.65  | 0.43     | $0.82$    | 13| 0.65      | $0.82$    | 0.13      | 0.13      |
| Ice cream          | Citrobacter braakii     | ATCC 12012       | 22    | 0.65  | 0.43     | $0.82$    | 13| 0.65      | $0.82$    | 0.13      | 0.13      |
| Dried milk         | Enterobacter cloacae    | ATCC 35050       | 9     | 0.25  | 0.11     | $0.47$    | 5 | 0.25      | $0.47$    | 0.13      | 0.13      |
| Pasteurized liquid | Escherichia coli        | ATCC 25922       | 3     | 0.33  | 0.19     | $0.51$    | 10| 0.33      | $0.51$    | 0.09      | 0.09      |
| Frozen cooked      | Providencia alcalifaciens | ATCC 27970 | 4     | 0.35  | 0.18     | $0.57$    | 7 | 0.35      | $0.57$    | 0.13      | 0.13      |
| Deli ham           | Citrobacter freundii    | ATCC 8090        | 3     | 0.37  | 0.22     | $0.54$    | 11| 0.37      | $0.54$    | 0.09      | 0.09      |
| Deli ham$^i$       | Citrobacter freundii    | ATCC 8090        | 12    | 0.80  | 0.38     | 1         | 4 | 0.80      | 1         | 0.47      | 0.47      |
| Lettuce$^d$        | Naturally contaminated   | $4.7 \times 10^9$ | 20    | 0.70  | 0.48     | $0.85$    | 14| 0.70      | $0.85$    | 0.13      | 0.13      |
| Dry dog food       | Salmonella enterica ser. | Typhimurium | 7     | 0.35  | 0.18     | $0.57$    | 7 | 0.35      | $0.57$    | 0.13      | 0.13      |
|                   | ATCC 14028              | 42               | 5     | 0.50  | 0.57     | 1         | 5 | 0.57      | 1         | 0.47      | 0.47      |

$^a$From reference method plate counts.

$^b$N = Number of test portions.

$^c$x = Number of positive test portions.

$^d$PODCP = Candidate method presumptive positive outcomes divided by the total number of trials.

$^e$PODCC = Candidate method confirmed positive outcomes divided by the total number of trials.

$^f$dPODCP = Difference between the candidate method presumptive result and candidate method confirmed result POD values.

$^g$95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

$^h$American Type Culture Collection, Manassas, VA.

$^i$Trial performed by the independent laboratory.

$^j$Campden BRI, Chipping Campden, United Kingdom.

$^k$Tested at a cutoff of $\geq 1 \times 10^3$ CFU/g (1:100,000 dilution).
Using an unpaired POD test at \( P < 0.05 \), at the fractional level there were no significant differences in the number of positive results obtained by the Soleris and reference methods for any of the 10 matrices examined. At the high level, of 45 test portions (there were no high-level test portions for lettuce), there were 43 positives by each method, with no significant differences between methods for any matrix. There were no positive results on uninoculated test portions by either method.

### Robustness Testing

(a) **Methodology.**—The effect of modest perturbations introduced to Soleris operating parameters was studied in a robustness experiment. Variations were introduced simultaneously to three operating parameters (sample volume, temperature, and test duration) in a matrix of nine test conditions (Table 5). The ninth condition represents the standard conditions for the Soleris EBAC test. Test samples included an E. coli culture dilution at 1–5 CFU/vial (positive) and a Pseudomonas aeruginosa culture dilution at approximately 1 × 10^5 CFU/vial (negative). Ten replicate tests were performed for each sample type under each of the nine conditions. The number of positive results at each of the eight conditions containing variations to normal operating parameters were compared to the number of positive results at the standard condition for the Soleris EBAC test. Test samples included an E. coli culture dilution at 1–5 CFU/vial (positive) and a Pseudomonas aeruginosa culture dilution at approximately 1 × 10^5 CFU/vial (negative). Ten replicate tests were performed for each sample type under each of the nine conditions. The number of positive results at each of the eight conditions containing variations to normal operating parameters were compared to the number of positive results at the standard condition for the Soleris EBAC test.

(b) **Results.**—Results are shown in Table 5. For the negative sample, all Soleris tests were negative for all conditions. For the positive sample, the standard condition produced
80% positive results. The percentage of positive results for the conditions containing parameter deviations ranged from 70 to 100%. There were no conditions for which results were significantly different from those of the standard condition by POD analysis.

Stability and Lot-to-Lot Consistency Testing

(a) Methodology.—Real-time stability testing was conducted on three manufactured lots of Soleris EBAC vials. Mean detection times for 8 target bacteria were measured over a time period from date of manufacture to up to 13 months post-manufacture. Inoculum levels ranged from 10 to 200 CFU/vial. Duplicate tests were conducted for each organism at each time point.

(b) Results.—There was no evidence of change in mean detection time over the course of the study for any organism with any of the three lots of vials (data not shown). These results support the current expiration dating of 6 months from date of manufacture.

Independent Laboratory Study

(a) Methodology.—Performance of the Soleris EBAC method was verified in testing of two matrixes by the independent laboratory. Yogurt and deli ham were tested using procedures consistent with those employed in in-house testing.

(b) Results.—Soleris presumptive and confirmed results are shown in Table 3, while Soleris and reference method results are shown in Table 4. For yogurt, at the fractional level, there were 10 positive Soleris results, and all were confirmed by oxidase and glucose fermentation tests. There were 9 positive results by the reference method. This difference is not significant by unpaired POD analysis at $P < 0.05$. All high-level test portions were positive and all uninoculated control portions were negative by both methods. For deli ham, there were 15 Soleris positive results at the fractional level, and all were confirmed. There were also 15 positive results by the reference method. All high-level test portions were positive and all uninoculated control portions were negative by both methods. These results confirm the efficacy of the Soleris EBAC method for these two matrixes.

Discussion

Results of this validation study demonstrate that the Soleris EBAC method is an accurate and effective procedure for detection of EBAC in a variety of foods. Inclusivity was 91% for target bacteria tested and inclusivity was 100%.

Strains of five organisms (Buttiauxella warmboldiae, one of two strains of Pantoea agglomerans, Serratia grimesii, Serratia proteamaculans, and Yersinia enterocolitica) were not detected within 18 h by the Soleris test. In repeat testing, these strains were again not detected. An additional strain of Yersinia enterocolitica (ATCC 27729) was tested and produced a positive result, with a detection time of 17.4 h (data not shown). Eleven additional ATCC strains of Pantoea agglomerans were tested; nine were positive with detection times ranging from 8.6 to 16.3 h (data not shown). Results of the additional testing indicate that the original results were strain-specific and not necessarily indicative of the response of these organisms in the Soleris test. An additional strain of Serratia grimesii (ATCC 14460) was tested and again produced no detection within 18 h using the standard test parameters. This strain was also tested with the Soleris method using a temperature of 30°C rather than the normal 36°C. A positive result was obtained with a detection time of 15.0 h (data not shown). Temperature sensitivity may also explain the negative results obtained with Serratia proteamaculans and Buttiauxella warmboldiae; both of these organisms have been described as having optimal growth temperatures of 30°C or below in liquid media (9–11).

Considering the in-house and independent laboratory matrix testing data combined, there were 176 positive results by the Soleris method and 177 positive results by the ISO 21528-2:2017 reference plating method. In 12 matrix trials, there were no significant differences in results between the Soleris and reference methods as determined by POD analysis at $P < 0.05$.

Robustness testing established that the Soleris method can withstand modest variation to three critical test parameters simultaneously. Real-time stability testing results support expiration dating for the Soleris EBAC vials of 6 months from date of manufacture.

In this study, all matrixes except lettuce required inoculation with EBAC and all were tested at a positive/negative test threshold of $\geq 10$ CFU/g. Lettuce contained naturally occurring EBAC at a high level and was tested at a threshold of $\geq 1 \times 10^5$ CFU/g. Test thresholds for the Soleris method can be

| Condition | Volume homogenate, mL | Temp., °C | Test duration, h | % Positive results |
|-----------|-----------------------|------------|------------------|-------------------|
| 1         | 0.9                   | 35         | 16               | 0                 |
| 2         | 0.9                   | 35         | 20               | 0                 |
| 3         | 1.1                   | 35         | 16               | 0                 |
| 4         | 1.1                   | 35         | 20               | 0                 |
| 5         | 0.9                   | 37         | 16               | 0                 |
| 6         | 0.9                   | 37         | 20               | 0                 |
| 7         | 1.1                   | 37         | 16               | 0                 |
| 8         | 1.1                   | 37         | 20               | 0                 |
| 9         | 1.0                   | 36         | 18               | 0                 |

*Ten replicates tested.

P<sub>0.05</sub>

% Positive results

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Table 5. Results of robustness testing for the Soleris EBAC method
adjusted to any level to match product specifications for EBAC. In addition to this flexibility, the Soleris method offers labor savings and decreased analysis time in comparison to the reference plating method. Soleris results are available within 18 h, while the reference method requires 22 h to produce negative results, and a minimum of an additional 44 h to produce a confirmed positive result.

Conclusions

Based on results of the validation study reported herein, it is recommended that the Soleris Enterobacteriaceae test be granted AOAC Performance Tested Method status for detection of Enterobacteriaceae in pasteurized milk, yogurt, mozzarella cheese, ice cream, dried milk, pasteurized liquid egg, frozen cooked chicken, deli ham, lettuce, and dry dog food.

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Separating Company

Neogen Corporation
620 Lesher Pl.
Lansing, MI 48912

Independent Laboratory

Campden BRI
Station Rd., Chipping Campden
Gloucestershire GL55 6LD
United Kingdom

Reviewers

Yvonne Salfinger
2935 Parrish Drive, Tallahassee, FL 32309

Michael Brodsky
Brodsky Consultants, 73 Donnamora Crescent, Thornhill, Ontario L3T 4K6, Canada

Wayne Ziemer
1301 Kristen Ln, Loganville, GA

Certification Information

The method was independently tested, evaluated, and certified by the AOAC Research Institute as a Performance Tested Method®. See https://www.aoac.org/scientific-solutions/research-institute-ptm/ for information on certification

References

1. British Standards Institution (2017) Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of Enterobacteriaceae, Part 2: Colony-Count Technique (EN ISO 21528-2:2017)
2. Mozola, M., Gray, R.L., Feldpausch, J., Alles, S., McDougal, S., Monteı, C., Sarver, R., Steiner, B., Cooper, C., & Rice, J. (2013) J. AOAC Int. 96, 399–403
3. Monteı, C., McDougal, S., Mozola, M., & Rice, J. (2014) J. AOAC Int. 97, 155–158
4. Firstenberg-Eden, R., Fotı, D., McDougal, S., & Beck, S. (2004) J. Food Prot. 67, 2760–2766
5. Fotı, D., Romano, L., Alles, S., & Mozola, M. (2012) J. AOAC Int. 95, 786–794
6. Pereault, M., Alles, S., Caballero, O., Sarver, R., McDougal, S., Mozola, M., & Rice, J. (2014) J. AOAC Int. 97, 1084–1091
7. Alles, S., McDougal, S., Caballero, O., Mozola, M., & Rice, J. (2015) J. AOAC Int. 98, 1286–1289
8. Official Methods of Analysis (2019) 21st Ed., Appendix J, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/methods/search.asp?string=b (accessed 2019)
9. National Collection of Type Cultures https://www.phe-collections.org.uk/products/bacteria/detail.jsp?refId=NCTC+nctc (accessed 2019)
10. American Type Culture Collection https://www.atcc.org/products/all/51608.aspx#culturemethod (accessed 2019)
11. American Type Culture Collection https://www.atcc.org/products/all/35477.aspx#culturemethod (accessed 2019)