

## GENERAL REFEREE REPORTS

## Methods Committee on Microbiology

### Food Microbiology, Nondairy

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#### Recommendations

(1) **998.09**, *3M/TECRA ULTIMA*, *Colorimetric Polyclonal Enzyme Immunoassay Screening Method, with Rappaport-Vassiliadis (R10) Broth and/or Tetrathionate Broth, for the Detection of Salmonella in All Foods*.—Study Director DeAnn Benesh, 3M Microbiology, 3M Center, Bldg 260-6B-01, St. Paul, MN 55514-1000, Tel: 651-736-3594, Fax: 651-733-1804, E-mail: dbenesh1@mmm.com. This method was adopted First Action in 1998, Revised First Action in 1999, and Final Action in 2001. It was further modified by allowing for the direct analysis of RV [R10] medium in the visual immunoassay (VIA) without subsequent post-enrichment in M broth. The method was adopted Revised First Action in 2003. No adverse comments have been received; therefore, the Study Director recommends that the First Action Method be adopted Final Action. The General Referee concurs. Continue study.

(2) **999.09**, *VIP for Salmonella, Detection of Motile and Nonmotile Salmonella in All Foods*.—Study Director, Philip T. Feldsine, BioControl Systems, Inc., 12822 SE 32nd St, Bellevue, WA 98005, Tel: 425-603-1123, Fax: 425-603-0070, E-mail: ptf@biocontrolsys.com. This method was adopted First Action in 1999 with Final Action in 2002. The device housing was modified and the method was adopted Revised First Action in 2008. Continue study.

(3) **2007.02**, *Salmonella spp. in Select Foods, GeneQuence<sup>®</sup> Salmonella DNA Hybridization with 24 H Enrichment*.—Study Director Mark Mozola, Neogen Corp., 620 Leshler Pl, Lansing, MI 48912, Tel: 517-372-9200, Fax: 517-372-0108, E-mail: mmozola@neogen.com. This method was adopted First Action in 2007. Continue study.

(4) **996.14**, *Assurance Polyclonal Enzyme Immunoassay (EIA) for the Detection of Listeria monocytogenes in Selected Foods and from Environmental Surfaces*.—Study Director Philip T. Feldsine. This method was adopted First Action in 1996, was modified, and adopted Revised First Action in 1999 with Final Action in 2001. It was modified again and was adopted Revised First Action in 2001 with Final Action in 2005. In the latest modification, the antibody/conjugate chemistry was modified so that 2 assay steps could be combined into a single step. This modification was adopted Revised First Action in 2008. Continue study.

(5) **997.03**, *Visual Immunoprecipitate Assay (VIP) for the Detection of Listeria monocytogenes and Related Listeria Species in Selected Foods and from Environmental Surfaces*.—Study Director Philip T. Feldsine. This method was adopted First Action in 1997 with Final Action in 2001. It was modified and adopted Revised First Action in 2001 with Final Action in 2005. For the latest approved modification, the device housing was modified and it was adopted Revised First Action in 2008. A minor modification study protocol, to change the color indicators in the VIP device, has been approved by the Methods Committee and the study is in progress. Continue study.

(6) **2004.02**, *VIDAS Listeria monocytogenes II (LMO2) Immunoassay for the Detection of Listeria monocytogenes in Foods*.—Study Director Ronald L. Johnson, bioMérieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042-2320, Tel: 314-731-7343, Fax: 314-731-8678, E-mail: ron.johnson@na.biomerieux.com. This method was adopted First Action in 2004 and Final Action in 2007. This method was adopted Final Action; therefore, it is recommended that this topic be discontinued.

(7) **995.22**, *Modified 2/6/01, Listeria spp., 3M TECRA Visual Immunoassay for Environmental Surfaces*.—Study Director DeAnn Benesh. This method was approved First Action in 1995 and Final Action in 1998 for the detection of *Listeria* spp. in dairy foods, seafoods, poultry, meats (except raw ground chuck), and leafy vegetables. 3M Microbiology planned to extend the applicability of the method to detect *Listeria* spp. on environmental surfaces. The precollaborative study has been completed, and the protocol for the collaborative study has been approved by the Methods Committee. The Study Director indicates that this method will not be validated through collaborative study; therefore, the General Referee recommends that this topic be discontinued.

(8) **H17**, *Listeria in Selected Foods by TECRA Unique Listeria Method (3M TECRA Listeria IC)*.—Study Director DeAnn Benesh. The precollaborative and collaborative study protocols have received approval from the Methods Committee. The Study Director indicates that this method will not be validated through collaborative study; therefore, the General Referee recommends that this topic be discontinued.

(9) **2004.06** (formerly **996.06**, *Modified; Hg135*), *VIDAS Listeria (LIS) Immunoassay for the Detection of Listeria Species in Foods Using Demi-Fraser and Fraser Enrichment Broths*.—Study Director Ronald L. Johnson. This method was adopted Final Action in 2007; therefore, it is recommended that this topic be discontinued.

(10) **OMA-2008-Feb-007**, *VIDAS Listeria species Xpress (LSX) for the Detection of Listeria Species in Foods*.—Study Director Ron Johnson. A collaborative study protocol has been submitted to the Methods Committee. Continue study.

(11) **2005.04** *Escherichia coli* O157:H7 in Selected Foods, Assurance GDS™ for *E. coli* O157:H7.—Study Director Philip T. Feldsine. This method was adopted First Action in 2005 and Final Action in 2007. It is recommended that this topic be discontinued.

(12) **2005.05**, *Shigatoxin genes, from E. coli* O157:H7, in Selected Foods, Assurance GDS™ for *Shigatoxin genes*.—Study Director Philip T. Feldsine. This method was adopted First Action in 2005 and Final Action in 2007. It is recommended that this topic be discontinued.

(13) **996.10**, Modified 9/21/00, Assurance Enzyme Immunoassay for the Detection of *Escherichia coli* O157:H7 in Beef.—Study Director Philip T. Feldsine. This method was adopted First Action in 1996 and Final Action in 1998. A method applicability modification was submitted to revise the enrichment protocol to allow for an 8 h enrichment for raw and cooked beef products only. This modification was adopted revised First Action in 2002 and Final Action in 2005. The method was further modified by changing the enrichment formulation. This modification was adopted revised First Action in 2005. The Study Director reports no adverse comments; therefore, the Study Director recommends that this method be adopted Final Action. The General Referee concurs. Continue study.

(14) **996.09**, Visual Immunoprecipitate Assay for the Analysis of Beef for *Escherichia coli* O157:H7.—Study Director Philip T. Feldsine. This method was adopted First Action in 1996 and Final Action in 1998. A method applicability modification was submitted to revise the enrichment protocol to allow for an 8 h enrichment for raw and cooked beef products only. This modification was adopted revised First Action in 2002 and Final Action in 2005. The method was further modified by changing the enrichment formulation. This modification was adopted revised First Action in 2005 and Final Action in 2008. It was further modified and received Revised First Action in 2008 for changes in the device housing. Continue study.

(15) **2000.13**, Reveal for *E. coli* O157:H7 8-Hour Test in Selected Foods and Environmental Swabs.—Study Director Mark Mozola. Method was adopted First Action in 2000 and Final Action in 2005. A minor modification protocol has been approved by the Methods Committee. Continue study.

(16) **2000.14**, Reveal for *E. coli* O157:H7 Test in Selected Foods and Environmental Swabs, 20 Hour Method.—Study Director Mark Mozola. Method was adopted First Action in 2000 and Final Action in 2005. A minor modification protocol has been approved by the Methods Committee. Continue study.

(17) *H71, Staphylococcus aureus* in Foods, 3M TECRA STAPH AUREUS Visual Immunoassay.—Study Director DeAnn Benesh. A precollaborative methods comparison study was conducted to compare the TECRA *Staphylococcus aureus* visual immunoassay (VIA), which incorporates enrichment in the proprietary enrichment medium TECRA *Staphylococcus* growth medium (TSGM) and the STAVIA Immunoassay with AOAC reference Method **975.55**. The precollaborative study of this method was approved by the

Methods Committee. A collaborative study of this method was conducted, using a geographically widespread community of collaborators. Unfortunately, organism die-off was seen in the samples sent outside Australia. The Study Director indicates that this method will not be validated through collaborative study; therefore, the General Referee recommends that this topic be discontinued.

(18) **2007.06**, VIDAS® *Staph Enterotoxin II (SET 2) Immunoassay Method for the Detection of Staphylococcal Enterotoxins in Selected Foods*.—Study Directors Robert P. Jechorek, rtech laboratories, PO Box 64101, St. Paul, MN 55164-0101, Tel: 651-481-2236, Fax: 651-486-0837, E-mail: rpjchorek@landolakes.com, and Ronald L. Johnson. This method was approved First Action in 2007 for the detection of *Staphylococcal enterotoxins* in Selected Foods. Continue study.

(19) *OMA-2007-Apr-015, Detection of Enterobacter sakazakii in Powdered Infant Formula by the 3M TECRA HELIX E. sakazakii Method*.—Study Director DeAnn Benesh. 3M participated in the FDA precollaborative study, but has chosen not to validate the method through collaborative study; therefore, the General Referee recommends that this topic be discontinued.

(20) *OMA-2007-Apr-008, Detection of Enterobacter sakazakii in Powdered Infant Formula by the TEMPO Enterobacter sakazakii Method*.—Study Director Ronald L. Johnson. The precollaborative study has been completed and a manuscript is in preparation. Continue study.

(21) *OMA-2007-Apr-009, Detection of Enterobacter sakazakii in Powdered Infant Formula by the MATRIX PSAK50 Method*.—Study Director Adrian Parton, Matrix MicroScience Ltd, Lynx Business Park, Fordham Rd, Newmarket, Cambridgeshire, CB8 7NY, United Kingdom, Tel: 44-0-1638-723110, Fax: 44-0-1638-723111, E-mail: adrian.parton@matrixmsci.com. The precollaborative study has been completed and a manuscript is in preparation. Continue study.

(22) *OMA-2007-Apr-010, Detection of Enterobacter sakazakii in Powdered Infant Formula by the BAX® Assay for the Detection of Enterobacter sakazakii*.—Study Director Morgan Wallace, DuPont Qualicon, ESL-Bldg 400, PO Box 80400, Route 141 and Henry Clay Rd, Wilmington, DE 19880-0400, Tel: 302-695-5473, Fax: 302-695-5277, E-mail: Morgan.Wallace@usa.dupont.com. The precollaborative study has been completed and a manuscript is in preparation. Continue study.

(23) *OMA-2007-Apr-011, Detection of Enterobacter sakazakii in Powdered Infant Formula by the Bioteccon Procedure*.—Study Director Han Joosten, Nestlé Research Center, Quality and Safety Assurance Department, PO Box 44, CH-1000 Lausanne 26, Switzerland, Tel: 41-21-785-8229, Fax: 41-21-785-8553, E-mail: han.joosten@rdls.nestle.com. The precollaborative study has been completed and a manuscript is in preparation. Continue study.

(24) *OMA-2007-Apr-013, Detection of Enterobacter sakazakii in Powdered Infant Formula by the Revised BAM*

*Method.*—Study Director Keith Lampel, U.S. Food and Drug Administration, 5100 Paint Branch Pkwy, HFS-706, College Park, MD 20740, Tel: 301-436-2007, Fax: 301-436-2644, E-mail: Keith.Lampel@fda.hhs.gov. The precollaborative study has been completed and a manuscript is in preparation. Continue study.

(25) *OMA-2007-Apr-007, Detection of Enterobacter sakazakii in Powdered Infant Formula by the Assurance GDS™ for Enterobacter sakazakii.*—Study Director Philip T. Feldsine. The precollaborative study has been completed and a manuscript is in preparation. Continue study.

(26) *2005.03, Detection and Confirmed Quantitation of Coliforms and E. coli in Foods, SimPlate Coliform and E. coli Color Indicator.*—Study Director Philip T. Feldsine. This method was adopted First Action in 2005 and Final Action in 2007. It is recommended that this topic be discontinued.

(27) *OMA-2007-Nov-041, TEMPO® EC for the Enumeration of Escherichia coli in a Variety of Foods and TEMPO® CC for the Enumeration of Total Coliform Counts in a Variety of Foods.*—Study Director Ron Johnson. A collaborative study protocol has been approved by the Methods Committee. Continue study.

(28) *H66, Determination of Escherichia coli in Flesh Foods Using a Visual Immunoassay with a Modified Culture Procedure (3M TECRA E. coli VIA).*—Study Director DeAnn Benesh. Resource allocations, and integration of TECRA International into 3M, have limited the progression of this study through the precollaborative study phase. Continue study.

(29) *OMA-2008-Mar-015, TEMPO® TVC for the Enumeration of Aerobic Mesophilic Flora in Foods.*—Study Director Ron Johnson. A collaborative study manuscript has been submitted to the Methods Committee. Continue study.

(30) *2002.08, Detection of Botulinum Toxins A, B, E, and F from Culture Supernatants, Amplified ELISA Procedure.*—Study Director Susan Maslanka, Centers for Disease Control and Prevention, 1600 Clifton Rd, NCID, Mailstop G-29, Atlanta, GA 30333, Tel: 404-639-0895, Fax: 404-639-4290, E-mail: sht5@cdc.gov. The method was adopted First Action in 2002. The Study Director reports no adverse comments; therefore, the Study Director recommends that this method be adopted Final Action. The General Referee concurs. Continue study.

(31) *OMA-2007-Oct-037, Evaluation of the Botulinum Toxin ELISA for the Detection of Toxins A, B, E, and F in Select Foods.*—Study Director Richard Whiting, U.S. Food and Drug Administration, HFS-300, 5100 Paint Branch Pkwy, College Park, MD 20740, Tel: 301-436-1925, Fax: 301-436-2632, E-mail: Richard.Whiting@fda.hhs.gov. A precollaborative study protocol has been approved by the Methods Committee. Continue study.

### Editorial Additions for the Official Methods of Analysis

**2004.03 Salmonella in Foods, VIDAS Enzyme-Linked Fluorescent Assay (ELFA).**—Add the following to section B.

Reagents: (i) *Rappaport-Vassiliadis R10 Broth, Difco or equivalent (approximate formula per liter).*—4.54 g pancreatic digest of casein, 7.2 g sodium chloride, 1.45 g monopotassium phosphate, 13.4 g magnesium chloride (anhydrous), 36.0 mg malachite green oxalate. *Preparation.*—Suspend 26.6 g commercial Rappaport-Vassiliadis R10 medium in 1 L purified water, mix thoroughly and warm slightly to dissolve. Dispense in 10 mL aliquots and sterilize by autoclaving at 116°C (10 psi pressure) for 15 min. Final pH 5.1 ± 0.2.

### AOAC Research Institute Studies

Since last year's General Referee Report (1), the following studies have been approved by the AOAC Research Institute:

#### Research Institute/Official Methods Program Harmonized Studies

(1) *Assurance GDS™ for Listeria monocytogenes for the Detection of Listeria monocytogenes in Selected Foods and Environmental Surfaces: Harmonized Study.*—The Assurance GDS™ for *Listeria monocytogenes* (BioControl Systems, Inc., 12822 SE 32nd St, Bellevue, WA 98005) is a gene-based assay for the detection of *L. monocytogenes*, from selected foods and environmental surfaces, that incorporates multiple levels of specificity to ensure highly accurate results. The method uses proprietary probes and specific primers directed against a highly conserved DNA sequence of the target organism. GDS also uses a proprietary device and reagents that concentrate populations of target microorganisms and eliminate potential competitive microflora. The method is designed to be highly selective, and does not detect microorganisms that are potential crossreactors in antibody-based assays.

For inclusivity testing, 50 *L. monocytogenes* strains were tested. All strains were positive, giving an inclusivity rate of 100%. For exclusivity testing, 30 non-*Listeria monocytogenes* species (31 isolates) were tested. All strains were negative, giving an exclusivity rate of 100%.

Liquid pasteurized milk, Mexican soft cheese, frankfurter, ready-to-eat turkey meat, raw green beans, raw fish, stainless steel, rubber, concrete, and plastic were selected for the internal methods comparison study. Stainless steel and raw fish were also selected for the independent laboratory methods comparison study. The reference method for liquid pasteurized milk, Mexican soft cheese, raw green beans, and raw fish was the FDA BAM method (2). The reference method for frankfurter, ready-to-eat turkey meat, stainless steel, rubber, concrete, and plastic was the USDA MLG method (3). There was no significant difference between the alternative and reference methods for the recovery of *L. monocytogenes* from liquid pasteurized milk, ready-to-eat turkey meat, raw green beans, raw fish, rubber, and plastic. The alternative method was statistically more productive ( $P < 0.05$ ) than the reference method for Mexican soft cheese, frankfurter, stainless steel, and concrete.

For ruggedness testing, the 3 following parameters were evaluated: (1) incubation temperature (34,  $36 \pm 1$ , and  $38^\circ\text{C}$ ); (2) enrichment time (16,  $21 \pm 3$ , and 26 h); and (3) sample volume (15, 20, and 25  $\mu\text{L}$ ). Variance of these parameters did not significantly affect test kit results.

(2) *Assurance GDS™ for Listeria species for the Detection of Listeria species in Selected Foods and Environmental Surfaces: Harmonized Study*.—The Assurance GDS™ for *Listeria* species (BioControl Systems, Inc.) is a gene-based assay for the detection of *Listeria* species from selected foods and environmental surfaces. Its mode of action is similar to that of the Assurance GDS™ for *Listeria monocytogenes* method for the detection of *L. monocytogenes* from selected foods and environmental surfaces (see 1, above).

For inclusivity testing, 50 *Listeria* spp. strains (including *L. monocytogenes*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi*) were tested. All strains were positive, giving an inclusivity rate of 100%. For exclusivity testing, 30 non-*Listeria* species (31 isolates) were tested. All strains were negative, giving an exclusivity rate of 100%.

Liquid pasteurized milk, Mexican soft cheese, frankfurter, ready-to-eat turkey meat, raw green beans, raw fish, stainless steel, rubber, concrete, and plastic were selected for the internal methods comparison study. Stainless steel and raw fish were also selected for the independent laboratory methods comparison study. The reference method for liquid pasteurized milk, Mexican soft cheese, raw green beans, and raw fish was the FDA BAM method (2). The reference method for frankfurter, ready-to-eat turkey meat, stainless steel, rubber, concrete and plastic was the USDA MLG method (3). There was no significant difference between the alternative and reference methods for the recovery of *L. monocytogenes* from liquid pasteurized milk, Mexican soft cheese, frankfurter, ready-to-eat turkey meat, raw green beans, raw fish, rubber, and plastic. The alternative method was statistically more productive ( $P < 0.05$ ) than the reference method for stainless steel and concrete.

For ruggedness testing, the 3 following parameters were evaluated: (1) incubation temperature (34,  $36 \pm 1$ , and  $38^\circ\text{C}$ ); (2) enrichment time (16,  $21 \pm 3$ , and 26 h); and (3) sample volume (15, 20, and 25  $\mu\text{L}$ ). Variance of these parameters did not significantly affect test kit results.

(3) *IEH E. coli O157 Test System for the Detection of E. coli O157 in Selected Foods: Harmonized Study*.—The IEH Test System detection method (IEH Laboratories & Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155) uses polymerase chain reaction (PCR) technology to amplify unique sequences of DNA present in *E. coli* O157. The specific targets include one gene associated with the O157 antigen, and 3 genes associated with *E. coli* virulence. The amplified products are separated using agarose gel electrophoresis and visualized using a UV transilluminator after staining. Decision tree logic has been defined for the test kit and is used to interpret the pattern of visualized bands to yield presumptive positive or negative results.

For inclusivity testing, 50 *E. coli* O157 strains were tested. All strains were positive, giving an inclusivity rate of 100%. For exclusivity testing, 40 non-*E. coli* O157 species (46 isolates) were tested. All strains were negative, giving an exclusivity rate of 100%.

Raw beef trim, raw ground beef, raw chicken, ready-to-eat chicken, and mixed leafy greens were selected for the internal methods comparison study. Raw beef trim was selected for the independent laboratory methods comparison study. The reference method for raw beef trim, raw ground beef, raw chicken, and ready-to-eat chicken was the USDA MLG method (3). The reference method for mixed leafy greens was the FDA BAM method (2). There was no significant difference between the alternative and reference methods for any of the foods tested.

For ruggedness testing, the 4 following parameters were evaluated: (1) enrichment incubation time (7, 8, and 9 h); (2) enrichment temperature (39, 32, and  $45 \pm 2^\circ\text{C}$ ); (3) sample volume (1.0, 2.0, and 3.0  $\mu\text{L}$ ); and (4) lysate volume (20, 25, and 30  $\mu\text{L}$ ). Variance of these parameters did not significantly affect test kit results.

(4) *TEMPO® CC (Coliform Count) Method for the Enumeration of Coliforms in a Variety of Foods: Harmonized Study*.—The TEMPO CC (bioMérieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042-2320) test consists of a vial of culture medium and a card, which are specific to this test. The culture medium is inoculated with the sample to be tested. The inoculated medium is transferred from the TEMPO Filler into the card containing 48 wells of 3 different volumes. The card contains 3 sets of 16 wells (small, medium, and large) with a 1 log difference in volume for each set of wells.

The card is designed to simulate the most probable number (MPN) method (2). The card is then hermetically sealed in order to avoid any risk of contamination during subsequent handling. The culture medium contains a fluorescent pH indicator which, when its pH is neutral, emits a signal detected by the TEMPO Reader. The coliforms present in the card assimilate the nutrients in the culture medium during incubation, resulting in a decrease in pH and the extinction of the fluorescent signal. After incubation, the cards are transferred to an automated card reader that detects the fluorescent signal. Depending on the number and type of the positive wells in the 3 log dilution range, the TEMPO system calculates the number of coliform bacteria present in the original sample according to a calculation based on the MPN method.

For inclusivity, all 30 coliforms were detected by the TEMPO CC. For exclusivity, none of the 20 noncoliforms were identified as coliforms by the method, giving inclusivity and exclusivity rates of 100%.

Internal testing included method comparison studies for 18 foods, including raw ground pork, fresh ground beef, heat-processed cooked roast beef, fresh ground chicken, frozen cooked chicken, heat-processed grilled chicken, frozen catfish, heat-processed frozen fish, raw cod, bagged salad, frozen green beans, hash brown potatoes, vanilla ice cream, pasteurized milk, milk powder, pasteurized eggs, rice, and dry

pet food. Independent testing included fresh ground beef, frozen cooked chicken, raw cod, and frozen green beans. The TEMPO method was compared to the Standard Methods for the Examination of Dairy Products (4) for dairy products and FDA BAM (2) for all other foods. The TEMPO method was shown to be statistically equivalent (0.05) to the reference method in both the internal and independent studies.

For ruggedness testing, the following 2 parameters were evaluated: (1) temperature of incubation of the test (30–37°C) and (2) time of incubation (21–28 h). Temperature and incubation time had no statistically significant effect ( $P > 0.05$ ) on the TEMPO TVC test kit performance.

(5) *VITEK<sup>®</sup> 2 Gram-Negative (GN) Identification Card Method for the Identification of Selected Gram-Negative Organisms: Harmonized Study*.—The VITEK<sup>®</sup> 2 system (bioMérieux, Inc.) is an automated microbial identification system that uses the VITEK 2 Gram-Negative (GN) Identification card for the identification of most significant fermenting and nonfermenting Gram-negative bacilli, including the select agent organisms, *Brucella melitensis*, *Francisella tularensis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, and *Yersinia pestis*. The VITEK 2 GN card is based on 47 biochemical tests measuring carbon source utilization, inhibition and resistance, and enzymatic activities. Identification results are available in approximately 10 h or less.

Because this is an automated biochemical identification system that is only intended for use with pure cultures, no methods comparison was included in the validation study. A total of 287 Gram-negative organisms were included for inclusivity testing, and 40 organisms were included for exclusivity testing from both the internal and independent laboratory studies. For the internal laboratory study, 257 Gram-negative inclusivity organisms were tested and 30 exclusivity organisms were tested. For the independent laboratory study, 30 Gram-negative inclusivity and 10 exclusivity organisms were tested. A total of 39 select agent strains were tested for identification in a separate study conducted at a Biosafety Level 3 containment facility at the French Agency for Food Safety (AFSSA) in Paris, France. Three strains were tested per organism during internal, independent, and AFSSA testing. All test strains were tested for correct identification from isolates obtained from 3 recommended culture media; Trypticase soy agar (TSA), Columbia agar with 5% sheep blood (CBA), and MAC (MacConkey agar) plates (*Francisella tularensis* strains were cultured on Chocolate Polyvitex agar). Overall correct identification for the select agent strains tested at AFSSA was 100%. Overall correct identifications for all other strains tested in the combined internal and independent studies from TSA, CBA, and MAC cultures were 94.8, 95.1, and 93.5%, respectively.

For ruggedness testing, the following 3 parameters were evaluated: (1) incubation time (17, 20, and 25 h); (2) culture suspension density (0.50, 0.57, and 0.63 McFarland units); and (3) inoculum suspension age (15, 30, and 45 min). Variance of these parameters did not significantly affect test kit results.

(6) *VITEK<sup>®</sup> 2 Gram-Positive (GP) Identification Card Method for the Identification of Selected Gram-Positive Organisms: Harmonized Study*.—The VITEK<sup>®</sup> 2 system (bioMérieux, Inc.) is an automated microbial identification system that uses the VITEK<sup>®</sup> 2 Gram-Positive (GP) Identification card for the identification of select Gram-positive organisms. The VITEK 2 GP card is based on 43 biochemical tests measuring carbon source utilization, enzymatic activities, and resistance. Identification results are available in 8 h or less.

Because this is an automated biochemical identification system that is only intended for use with pure cultures, no methods comparison was included in the validation study. A total of 63 Gram-positive organisms (representing 7 *Listeria* and 4 Staphylococcal species) were included for inclusivity testing, and 40 organisms were included for exclusivity testing from both the internal and independent laboratory studies. For the internal laboratory study, 33 Gram-positive inclusivity organisms were tested and 30 exclusivity organisms were tested. For the independent laboratory study, 30 Gram-positive inclusivity and 10 exclusivity organisms were tested. For both internal and independent inclusivity testing, 3 strains were tested per organism. All strains were grown on the following plating agars incubated for 12 and 48 h: TSA, CBA, and Trypticase soy agar plates with 5% sheep blood (TSAB). The overall identification rates for the combined internal and independent studies from 12 h TSA, CBA, and TSAB cultures were 98, 97, and 98%, respectively. The overall identification rates for the combined internal and independent laboratory comparison studies from 48 h TSA, CBA, and TSAB cultures were 95, 96, and 97%, respectively.

For ruggedness testing, the following 3 parameters were evaluated: (1) incubation time (11, 24, and 50 h); (2) culture suspension density (0.50, 0.57, and 0.63 McFarland units); and (3) inoculum suspension age (15, 30, and 45 min). Variance of culture suspension density and inoculum suspension age did not appear to adversely affect the performance of the method with identification rates of 97 and 93%, respectively. Length of incubation time did appear to have an effect on test results, with 50 h incubation time giving the lowest level of accuracy (89%).

## Research Institute Nonharmonized Studies

(1) *RAPID<sup>®</sup> Salmonella*.—*A Chromogenic Medium for Detection of Salmonella spp. in Selected Foods*.—The RAPID<sup>®</sup> *Salmonella* culture medium (Bio-Rad Laboratories, 1000 Alfred Nobel Dr, Hercules, CA 94547) is a selective and differential medium for both the isolation and the presumptive identification of *Salmonella* species, including lactose-positive *Salmonella*, *S. Typhi*, and *S. Paratyphi* serotypes, from other members of the family *Enterobacteriaceae*. The cultural properties of the medium are a balance of carefully selected growth-promoting nutrients and classical selective ingredients (citrate, surfactants). The presumptive chromogenic identification system relies on a proprietary chromogenic substrate that allows detection of the

*Salmonella* C8-esterase activity. The color of the uninoculated agar is clear to whitish. All the presumptive *Salmonella*-positive colonies are magenta on a clear-white agar background. A second chromogenic substrate, targeting activity of many interfering bacteria, yields blue-colored colonies. Background flora, if not inhibited by the mixture of selective agents, can produce violet to green or colorless colonies. For inclusivity testing, 115 different *Salmonella* serotypes (177 isolates) were tested. Of these, 176 strains produced typical magenta colonies. One *S. Paratyphi* A strain did not produce typical magenta colonies; however, 6 other *S. Paratyphi* A strains all produced typical magenta colonies. The inclusivity rate was 99.4%. For exclusivity, 35 non-*Salmonella* species (66 strains) were tested. Two *Citrobacter koseri* strains produced light magenta colonies. The exclusivity rate was 97%.

Raw chicken breast, eggs, and cantaloupe were selected for internal testing of the RAPID' *Salmonella* agar method. Raw chicken breast was also tested by the independent laboratory. The RAPID' *Salmonella* agar method was compared to the FSIS MLG method (3) for raw chicken breast and to the FDA BAM method (2) for eggs and cantaloupe. In addition, selective enrichments [Rappaport-Vassiliadis Soy (RVS) medium] were incubated for both 6 and 24 h. The RAPID' *Salmonella* agar method did not differ significantly from the reference method for all of the foods tested, except for cantaloupes, where the RAPID' *Salmonella* method detected significantly more ( $P < 0.05$ ) *Salmonella*-positive test portions than did the BAM reference method. This was true for both 6 and 24 h RVS enrichments as well.

For ruggedness testing, the 4 following parameters were evaluated: (1) dehydrated media was used after being aged for varying periods after preparation (0, 1, and 2 days); (2) incubation temperatures (35, 37, and 39°C); (3) incubation time (21, 24, and 27 h); and (4) premade ready-to-use plates were compared to plates made from dehydrated media. Variance of these parameters did not significantly affect test kit results, but there was an arithmetic difference for plates incubated at 39°C. A note has been added to the package insert to ensure proper incubation temperature of the plates is maintained.

(2) *Detection of Salmonella from Selected Foods Using iQ-Check™ Salmonella II Real-Time PCR Test Kit.*—The iQ-Check *Salmonella* II kit (Bio-Rad Laboratories) is a test based on gene amplification and detection by real-time PCR (RTi-PCR). Ready-to-use RTi-PCR reagents contain DNA primers and a DNA probe specific for *Salmonella*, as well as DNA polymerase and nucleotides. In the iQ-Check *Salmonella* II kits, carboxyfluorescein (FAM) is the fluorophore linked to the probe hybridizing to the *Salmonella*-specific DNA sequence. In the absence of target DNA, no fluorescence is detected, and the sample is determined to be negative. As the amount of amplicons increases with each round of amplification, fluorescence intensity also increases. During each PCR cycle, at the annealing step, the RTi-PCR system measures this fluorescence, and the associated software plots the

fluorescence intensity versus number of cycles. This method allows a simple determination of the presence of *Salmonella* in a sample. To monitor for a successful DNA amplification in each reaction tube, a synthetic DNA “internal control” is included in the reaction mix. This control is amplified with a specific probe at the same time as the *Salmonella* target DNA sequence, and is detected by a second fluorophore.

For inclusivity testing, 108 serotypes (147 strains) were tested. All strains were positive, giving an inclusivity rate of 100%. For exclusivity testing, 35 non-*Salmonella* species were tested. All strains were negative, giving an exclusivity rate of 100%.

Raw chicken, raw beef, cantaloupe, and eggs were selected for the internal methods comparison between the iQ-Check *Salmonella* species PCR method and the USDA MLG (3) and FDA BAM (2) reference methods. Raw chicken was also selected for the independent laboratory validation study. There was no significant difference between the alternative and reference methods for any of the 4 foods tested.

For ruggedness testing, the 3 following parameters were evaluated: (1) incubation time (20, 21, and 22 h); (2) cell lysis incubation temperature (95, 100, and 105°C); and (3) lysis time (10, 15, and 20 min). Variance of these parameters did not significantly affect test kit results.

(3) *R.A.P.I.D.® LT Food Security System for Salmonella Detection in Selected Foods.*—The R.A.P.I.D. *Salmonella* LT FSS (Idaho Technology, Inc., 390 Wakara Way, Salt Lake City, Utah 84108) is designed as a qualitative detection method for *Salmonella* in selected foods. It is a PCR method and results can be determined within about 17 h. Results are reported as “positive” or “negative.” The method couples real-time PCR with a short pre-enrichment time. Real-time PCR specifically amplifies target DNA which is detected by fluorescent probes. All required PCR components, including target-specific fluorescent probes, are included in freeze-dried reagent vials for ease of use. The sensitivity of PCR allows for a shorter pre-enrichment than standard cultural methods. Pre-enrichment media for the method are buffered peptone water (BPW) and nonfat dry milk (NFDM). The method has a detection limit of 1 colony-forming unit (CFU) *Salmonella*/25 g food after just a 16 h preenrichment. The R.A.P.I.D. LT instrument has an advantage over traditional PCR instruments because it uses air thermo cycling to heat and cool the sample (within a closed glass reaction vessel) instead of a metal block, allowing for a more rapid heating and cooling. The method involves a series of sequential steps: Sample collection and enrichment, sample processing which includes cell lysis to release DNA, DNA amplification (PCR) in the Idaho Technology R.A.P.I.D. LT instrument, and automatic result interpretation by the R.A.P.I.D. LT FSS software.

For inclusivity, 106 *Salmonella* serotypes (123 strains) were tested. Four different protocols were tested: (1) BPW-PCR (where samples were preenriched in BPW before PCR; 97.6% inclusivity rate); (2) BPW-Pathatrix-PCR (where samples were preenriched in BPW, concentrated with the Pathatrix system, and processed with PCR; 98.4%

inclusivity rate); (3) NFDM-PCR (97.6% inclusivity rate); and (4) NFDM-Pathatrix-PCR (91.9%). Two (*S. Sandiego* and *S. Gallinarum*) of 123 strains could not be detected with any of the 4 protocols. For exclusivity, 30 non-*Salmonella* species (32 isolates) were tested and all were negative (exclusivity rate of 100%).

Raw chicken, cooked-ham, and chocolate were selected for the internal methods comparison study. Cooked ham was selected for the independent laboratory methods comparison. The reference method for cooked ham and raw chicken was the USDA MLG (3). The reference method for chocolate was the FDA BAM method (2). The alternative method was compared to the reference method(s) for both individual and pooled samples. For both individual and pooled samples, the alternative method consisted of pre-enrichment followed by PCR and confirmation. For pooled samples, there was also a second protocol where *Salmonella* was concentrated with the Pathatrix system between the pre-enrichment and PCR analysis steps. There was no significant difference in the detection of *Salmonella* between the alternative and reference methods for any of the 3 foods tested for both pooled and individual samples.

For ruggedness testing, the 4 following parameters were evaluated: (1) storage time of Pathatrix beads before lysis (0, 2, 8, and 16 h); (2) Pathatrix protocol—volume of immunomagnetic beads added to the bead tube (20, 25, and 30  $\mu\text{L}$ ); (3) PCR protocol—volume of incubated pre-enrichment added to the bead tube (5, 10, and 25  $\mu\text{L}$ ); and (4) reagent preparation time (1, 2, and 4 h). Variance of these parameters did not significantly affect test kit results.

(4) *TaqMan*<sup>®</sup> Kit for the Detection of *Salmonella enterica* in Selected Foods and Feed.—The *TaqMan*<sup>®</sup> Pathogen Detection System (Applied Biosystems, 850 Lincoln Centre Dr, Foster City, CA 94404) is based on *TaqMan* Real-Time PCR technology, providing 2 levels of specificity for pathogen detection and combining PCR amplification and signal detection into a single reaction. The first level of specificity is provided by target-specific PCR primers that identify the DNA sequence of the organism in the sample. The identification of the organism is confirmed simultaneously by *TaqMan* probes (second level of specificity). A fluorescent signal is emitted only if the unique genetic signature of the pathogen has been recognized. In addition, each *TaqMan* Pathogen Detection System reaction contains an internal positive control that indicates the presence of inhibitors for reliable negative results. After PCR amplification and detection, reaction tubes remain sealed, thus significantly reducing the potential for contamination (false positives).

For inclusivity, 100 *Salmonella enterica* serotypes were tested and all were positive (inclusivity rate of 100%). For exclusivity, 30 non-*Salmonella enterica* species were tested and all were negative (exclusivity rate of 100%).

Raw ground beef, cheddar cheese, raw chicken wings, and dry dog food were selected for the internal methods comparison study. Raw chicken wings were selected for the independent laboratory methods comparison study. The reference method for the 4 matrixes was ISO

6579:2002(E) (5). There was no significant difference between the alternative and reference methods for any of the 4 tested matrixes.

For ruggedness testing, the 3 following parameters were evaluated: (1) incubation time (21, 24, and 27 h); (2) extraction heating time (7, 10, and 13 min); and (3) sample volume (10, 12, and 14  $\mu\text{L}$ ). Variance of these parameters did not significantly affect test kit results.

(5) *Detection of Listeria monocytogenes from Selected Foods Using iQ-Check*<sup>™</sup> *Listeria monocytogenes* II Real-Time PCR Test Kit.—The *iQ-Check Listeria monocytogenes* II kit (Bio-Rad Laboratories) method is based on gene amplification and detection by RTi-PCR. Its mode of action is similar to that of the *iQ-Check*<sup>™</sup> *Salmonella* II Real-Time PCR Test Kit method for the detection of *Salmonella* from selected foods (see 2, above).

For inclusivity testing, 57 *L. monocytogenes* strains were tested. All strains were positive, giving an inclusivity rate of 100%. For exclusivity testing, 38 non-*L. monocytogenes* species (63 strains), including other species of *Listeria*, were tested. All non-*L. monocytogenes* species were negative, giving an inclusivity rate of 100%.

Smoked salmon, cottage cheese, hot dogs, and deli turkey were selected for the internal methods comparison study. Deli turkey was also used in the independent laboratory validation study. The reference method for smoked salmon was the FDA BAM method (2), for cottage cheese it was AOAC Official Method 993.12 (6), and for hot dogs and deli turkey it was the USDA/FSIS MLG method (3). There was no significant difference between the alternative and reference methods for any of the 4 foods tested.

For ruggedness testing, the 3 following parameters were evaluated: (1) incubation time (24, 25, and 26 h); (2) cell lysis incubation temperature (95, 100, and 105°C); and (3) lysis incubation time (10, 15, and 20 min). Variance of these parameters did not significantly affect test kit results.

(6) *VIDAS*<sup>®</sup> *Listeria DUO (LDUO)* with *Ottaviani Agosti Agar*.—The *VIDAS LDUO* (bioMérieux, Inc.) is a specific enzyme-linked fluorescent immunoassay (ELFA) performed in the automated *VIDAS* instrument for simultaneous detection and differentiation of *Listeria monocytogenes* and *Listeria* species in food. Samples are culturally enriched prior to assay. The assay gives a result for presence and absence of *L. monocytogenes* (DLMO reading), as well as presence or absence of non-*monocytogenes Listeria* species (DLIS). Positive *VIDAS* results are confirmed by cultural identification procedures.

For inclusivity, all 78 *L. monocytogenes* strains and 69 *Listeria* species (non-*L. monocytogenes*) produced positive results with the test kit. For exclusivity, all 34 non-*Listeria* species (54 non-*Listeria* isolates) gave negative results. No cross-reactivity of non-*monocytogenes Listeria* species occurred with the *Listeria monocytogenes* portion of the *LDUO* test.

Camembert cheese, cheddar cheese, yogurt, pasteurized milk, frozen green beans, pasteurized crab-meat, cod fish fillets, shrimp, raw ground beef, meat frankfurters, raw pork,

roast beef, ham, raw ground chicken, and chicken franks were selected for the internal methods comparison study. Vanilla ice cream, cauliflower, fresh cod, and frankfurters were selected for the independent methods comparison study. The reference methods were AOAC Method 993.12 for dairy products (6), FDA BAM method for vegetables and seafood (2), and the USDA MLG method for meat and poultry products (3). Results from the internal method comparison showed close agreement with the reference method, giving a total number of 234 confirmed positive samples with the LDUO method and 220 confirmed positive samples with the reference method. The LDUO method generated significantly better results ( $P < 0.05$ ) than the reference method for cheddar cheese and yogurt. For the independent method comparison, the VIDAS LDUO method and the reference methods detected an equivalent number of positive samples, with 66 confirmed positive results for both methods and only one false-positive result for LDUO.

For ruggedness testing, the following parameters were evaluated: (1) sample volume (450, 500, and 550  $\mu\text{L}$ ); (2) sample boiling time (2, 5, and 10 min); (3) time for reagent temperature equilibration (0, 15, 30, 60, and 120 min); and (4) sample temperature after boiling to target temperatures of 50, 25, and 10°C. Variance of these parameters had no significant effect on test results.

(7) *RAPID' Listeria spp. A Chromogenic Medium for Detection of Listeria spp. from Selected Environmental Surfaces.*—The *RAPID' Listeria* species agar (Bio-Rad Laboratories) is a selective chromogenic plating medium for the isolation and the presumptive identification of all *Listeria* species. The cultural properties of the medium are based on a balance of carefully selected growth-promoting nutrients, enzymatic enhancers, and a mixture of selective agents, including lithium chloride and nalidixic acid. The presumptive chromogenic identification system relies on a chromogenic substrate that allows the detection of the  $\beta$ -*d*-glucosidase activity, an enzyme common to all species of *Listeria*. The hydrolysis of the substrate leads to the formation of a colored precipitate and all presumptive *Listeria* positive colonies are blue. Combined with this principle, *RAPID' Listeria* spp. uses an original selective mixture which enables the inhibition of most background flora. The few non-*Listeria* species that do grow on the agar produce colorless colonies.

For inclusivity testing, 51 food and clinical *Listeria* spp. isolates were tested. One *Listeria grayi* strain produced small, white atypical colonies. After 48 h incubation, the strain produced a typical blue colony. Two additional *L. grayi* strains were tested and produced typical blue colonies after 24 h. The inclusivity rate was 98%. For exclusivity, 52 non-*Listeria* species (83 strains) were tested. None of these isolates produced typical colonies. The exclusivity rate was 100%.

Stainless steel, plastic, ceramic, and sealed concrete were selected for the internal methods comparison between the *RAPID' Listeria* species agar method and the USDA MLG reference method (3). Stainless steel was also selected for the independent laboratory validation study. No significant

differences ( $P < 0.05$ ) were observed between the alternative and reference methods for any of these 4 surfaces.

For ruggedness testing, the 4 following parameters were evaluated: (1) dehydrated media was used after being aged varying periods after preparation (0, 1, and 2 days); (2) incubation temperatures (35, 37, and 39°C); (3) incubation time (21, 24, and 27 h); and (4) premade ready-to-use plates were compared to plates made from dehydrated media. Variance of these parameters did not significantly affect test kit results.

(8) *Detection of Listeria spp. from Selected Environmental Surfaces Using iQ-Check™ Listeria spp. Real-Time PCR Test Kit.*—The *iQ-Check Listeria* spp. kit is a test based on gene amplification and detection by real-time PCR. Its mode of action is similar to that of the *iQ-Check™ Salmonella* II Real-Time PCR Test Kit method for the detection of *Salmonella* from selected foods (see 2, above).

For inclusivity testing, a panel of 94 *Listeria* spp. isolates (food, clinical, and environmental) were tested. All 94 strains gave positive results, giving an inclusivity rate of 100%. For exclusivity testing, 39 non-*Listeria* species (56 isolates) gave negative results. The exclusivity rate was 100%.

Stainless steel, plastic, ceramic, and sealed concrete were selected for the internal methods comparison between the *iQ-Check Listeria* species PCR method and the USDA MLG reference method (3). Stainless steel was also selected for the independent laboratory validation study. For 3 of the surfaces, there was no significant difference between the alternative and reference methods. For plastic, the *iQ-Check Listeria* species PCR method was significantly more productive ( $P < 0.05$ ) than the reference method.

For ruggedness testing, the 3 following parameters were evaluated: (1) incubation time (24, 25, and 26 h); (2) cell lysis incubation temperature (95, 100, and 105°C); and (3) lysis time (10, 15, and 20 min). Variance of these parameters did not significantly affect test kit results.

(9) *DuPont Qualicon BAX® Reverse Transcriptase PCR Assay for the Detection of Listeria species from Stainless Steel Environmental Surfaces.*—The *BAX®* system Reverse Transcriptase PCR assay (Qualicon, Inc., ESL Bldg 400, Rt 141 and Henry Clay, Wilmington, DE 19880) uses the Reverse Transcriptase (RT) and PCR to convert a specific bacterial RNA into DNA, and then amplifies a segment of this fragment to detectable amounts. This fragment is present in large amounts in growing cells and in lesser amounts in sublethally injured cells. The fragment is a genetic sequence that is unique to the genus *Listeria*, thus providing a highly reliable indicator that the organism is present. The *BAX* system simplifies the RT and PCR processes by combining the requisite primers, RT, polymerase and nucleotides into a stable, dry, manufactured tablet prepackaged inside the PCR tubes. Also included in each tablet is an internal positive control (INPC) RNA and primers to serve as a process control which validates the proper functioning of all phases of the assay. After amplification, these tubes remain sealed for the detection phase, thus significantly reducing the potential for contamination with one or more molecules of amplified PCR

product. The automated BAX system uses fluorescent detection to analyze PCR product. Each PCR tablet contains a fluorescent dye, which binds with double-stranded DNA and emits a signal in response to excitation light. During the detection phase, the temperature of the sample is slowly increased to denature the DNA, which in turn, releases the dye and causes a drop in emission signal. The BAX system measures the denaturation temperature and analyzes the magnitude of the fluorescent signal change to determine a positive or negative result.

For inclusivity, 58 *Listeria* species/strains (including *L. monocytogenes*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, *L. innocua*, *L. murrayi*, and *L. grayi*) were tested and all were positive (inclusivity rate of 100%). For exclusivity, 39 non-*Listeria* species (52 isolates) were tested and all were negative (exclusivity rate of 100%).

Three stainless steel surface types (316 2B finish, 316 mirror finish, 304 2B finish) were selected for the internal methods comparison study. One stainless steel surface type (304 2B finish) was used for the independent methods comparison study. The alternative method was significantly ( $P < 0.05$ ) more productive than the USDA MLG reference method (3) for all of the stainless steel surfaces tested, except for stainless steel surface 316 2B finish. The alternative and reference methods did not differ significantly for the detection of *Listeria* spp. on stainless steel surface 316 2B finish.

For ruggedness testing, the 6 following parameters were evaluated: (1) sample volume (980, 1000, and 1020  $\mu\text{L}$ ); (2) incubation temperature (35, 37, and 39°C); (3) incubation time (230, 240, and 260 min); (4) cooling block temperature (1, 4, and 7°C); (5) sample volume to dilution buffer (8, 10, and 12  $\mu\text{L}$ ); and (6) tablet hydration volume (28, 30, and 32  $\mu\text{L}$ ). Variance of these parameters did not significantly affect test kit results.

(10) *Strategic Diagnostics RapidChek Method for the Detection of Listeria species in a Variety of Foods and Selected Environmental Surfaces: Minor Modification*.—The RapidChek method (Strategic Diagnostics Inc., 128 Sandy Dr, Newark, DE 19713) for the enrichment and detection of *Listeria* spp. in a variety of foods and selected environmental surfaces received AOAC certification in 2004 (License No. 02040). The RapidChek *Listeria* method uses a proprietary RapidChek *Listeria* enrichment media for a one-step 40 h enrichment at 30°C and detects *Listeria* on a lateral flow device in 10 min. The lateral flow device filter pad has been modified, but the remaining test strip components have been left unchanged and the instructions for use have been left unchanged.

There was no inclusivity and exclusivity testing, because minor modifications do not require inclusivity and exclusivity testing.

Hot dogs, plastic, and steel were selected for the internal methods comparison study. An independent laboratory methods comparison study was not required. The reference method for the one food and 2 surfaces was the USDA MLG method (3). There was no significant difference between the alternative and reference method for the one food or 2 tested surfaces.

Ruggedness testing was not required.

(11) *BioControl VIP<sup>®</sup> Method for the Detection of Listeria in Selected Foods and on Environmental Surfaces*.—The VIP *Listeria* method (BioControl Systems, Inc.) is a lateral flow test that contains chromogenic particles with bound antibodies that have a high specificity for *Listeria* attached to a solid matrix. Separately, antibodies with similar high specificity are attached to another portion of the same solid matrix. Samples are added to the device, hydrating the chromogenic particles. *Listeria* antigens, if present in the sample, will bind to the particles. These antigen-antibody-chromogen complexes flow across the solid matrix and are captured by the bound antibody, forming a detectable line in the test zone. A line forming in the control zone verifies proper completion of the test. Absence of a line in the control zone invalidates the test. The original VIP *Listeria* method is an OMA method that has gone through a series of AOAC OMA-approved modifications (6). The current modification, reported here, provides an AOAC RI-approved single-step enrichment protocol as an alternative to the 2-step enrichment protocol found in OMA Method 997.03.

For inclusivity, 50 *Listeria* strains were tested and all were positive (inclusivity rate of 100%). For exclusivity, 30 non-*Listeria* species were tested and all were negative (exclusivity rate of 100%).

Liquid milk, Mexican soft cheese, ready-to-eat-turkey meat, raw green beans, raw beef trim, raw fish, stainless steel, rubber, and plastic were selected for the internal methods comparison study. Raw green beans and stainless steel were also selected for the independent laboratory comparison study. The USDA MLG method (3) was the reference method for ready-to-eat turkey meat and beef trim and the environmental surface samples. The FDA BAM method (2) was the reference method for raw fish, raw green beans, liquid milk, and Mexican soft cheese. There was no statistical difference ( $P < 0.05$ ) in the recovery of *Listeria* spp. from any of the foods or surfaces between the alternative and reference methods.

For ruggedness testing, the following 3 parameters were evaluated: (1) incubation temperature (28, 30  $\pm$  1, and 32°C); (2) enrichment time (44, 50  $\pm$  4, and 56 h); and (3) dehydrated media weight (49.5, 55, and 40.5 g/L). Variance of these parameters did not significantly affect test kit results.

(12) *BioControl Assurance<sup>™</sup> Enzyme Immunoassay (EIA) for the Detection of Listeria in Selected Foods and on Environmental Surfaces*.—In the polyclonal EIA *Listeria* method (BioControl Systems, Inc.), proprietary antibodies with a high specificity to *Listeria monocytogenes* and related *Listeria* species are bound to microwell plates. Enriched test suspensions and positive controls are added to the plate. Antigens that are present bind to the antibodies on the microwells, forming antibody-antigen complexes. Nonbinding material is washed away. Specific antibody conjugated to alkaline phosphatase is added and binds to these complexes. After incubation, unbound conjugate is washed away. The substrate, *p*-nitrophenylphosphate, is added, and the absorbance of the resulting colored product is read

spectrophotometrically at 405–410 nm. The original EIA *Listeria* method is an OMA method that has gone through a series of AOAC OMA-approved modifications (6). The current modification, reported here, provides an AOAC RI-approved single-step enrichment protocol as an alternative to the 2-step enrichment protocol found in OMA Method 996.14.

For inclusivity, 50 *Listeria* strains were tested and all were positive (inclusivity rate of 100%). For exclusivity, 30 non-*Listeria* species were tested and all were negative (exclusivity rate of 100%).

Liquid milk, Mexican soft cheese, ready-to-eat-turkey meat, raw green beans, raw beef trim, raw fish, stainless steel, rubber, and plastic were selected for the internal methods comparison study. Raw green beans and stainless steel were also selected for the independent laboratory comparison study. The USDA MLG method (3) was the reference method for ready-to-eat turkey meat and beef trim and the environmental surface samples. The FDA BAM method (2) was the reference method for raw fish, raw green beans, liquid milk, and Mexican soft cheese. There was no statistical difference ( $P < 0.05$ ) in the recovery of *Listeria* spp. from any of the foods or surfaces between the alternative and reference methods.

For ruggedness testing, the following 3 parameters were evaluated: (1) incubation temperature (28,  $30 \pm 1$ , and  $32^\circ\text{C}$ ); (2) enrichment time (44,  $50 \pm 4$ , and 56 h); and (3) dehydrated media weight (49.5, 55, and 40.5 g/L). Variance of these parameters did not significantly affect test kit results.

(13) *RAPID' E. coli O157:H7: A Chromogenic Medium for Detection of E. coli O157:H7 in Raw Ground Beef and Fresh Spinach.*—The *RAPID' E. coli O157:H7* (Bio-Rad Laboratories) is a selective chromogenic plating medium for the rapid isolation and presumptive identification of *E. coli O157:H7*. Characteristic *E. coli O157:H7* colonies appear as dark blue with a black precipitate around the edge after 24 h incubation at  $37^\circ\text{C}$ . This chromogenic medium differentiates *E. coli O157:H7*, including atypical  $\beta$ -glucuronidase-positive or sorbitol-positive *E. coli O157:H7* isolates, from other *E. coli* strains. The cultural properties of the medium are based on a balance of carefully selected growth-promoting nutrients and classical selective ingredients. Potassium tellurite and novobiocin are used in addition to enhance the selectivity of the medium by inhibiting interfering flora. The presumptive identification system relies on differential characteristics driven by proprietary chromogenic substrates coupled with both carbohydrate fermentation and pH modification. Background microflora, when not inhibited, give rise to red, green, or colorless colonies on this agar.

For inclusivity, 50 *E. coli O157:H7* strains were tested on *RAPID' E. coli O157:H7* agar. Every strain tested produced typical blue-black colonies. The inclusivity rate was 100%. For exclusivity, 54 non-*E. coli O157:H7* organisms (23 non-*E. coli* species and 26 non-*O157:H7 E. coli* strains) were selected from the culture collection of Adria Development Laboratory. *E. coli O55:H6* and *E. coli*

*O92:H33* produced false-positive results (blue-black colonies). The exclusivity rate was 96%.

Raw ground beef and fresh spinach were selected for internal testing of the *RAPID' E. coli O157:H7* agar method. Raw ground beef was also used in the independent laboratory validation. The reference methods for raw ground beef and spinach were the USDA MLG method (3) and FDA BAM method (2). No significant differences ( $P < 0.05$ ) were observed between the alternative and reference methods for either of these 2 foods.

For ruggedness testing, the 3 following parameters were tested: (1) dehydrated media was used after being aged varying periods after preparation (0, 1, and 2 days); (2) incubation temperatures (35, 37, and  $39^\circ\text{C}$ ); (3) incubation time (21, 24, and 27 h). Variance of these parameters did not significantly affect test kit results.

(14) *Detection of E. coli O157:H7 from Selected Foods Using iQ-Check™ E. coli O157:H7 Real-Time PCR Test Kit.*—The *iQ-Check E. coli O157:H7* (Bio-Rad Laboratories) is a real-time PCR kit for detection of *E. coli O157:H7* from selected foods. Its mode of action is similar to that of the *iQ-Check™ Salmonella II Real-Time PCR Test Kit* method for the detection of *Salmonella* from selected foods (see 2, above).

For inclusivity testing, 60 *E. coli O157:H7* strains were tested. All strains were positive, giving an inclusivity rate of 100%. For exclusivity testing, 36 species were tested (48 non-*E. coli O157:H7* strains). All non-*E. coli O157:H7* species were negative, giving an inclusivity rate of 100%.

Ground beef, apple cider, and fresh spinach were selected for the internal methods comparison study. Ground beef was also used in the independent laboratory comparison study. The reference method for ground beef was the USDA MLG method (3) and the reference method for apple cider and fresh spinach was the FDA BAM method (2). Three *iQ-Check E. coli O157:H7* protocols were tested in this study. Eight and 24 h primary enrichments in BPW were validated. The kit was also validated by testing with the MLG and BAM reference method enrichment broths as well. For the 8 h BPW enrichment, there were no significant differences between the alternative and reference methods for ground beef and apple cider. For spinach, the alternative method was significantly more productive than the reference method ( $P < 0.05$ ). The 24 h BPW enrichment results were the same as the 8 h BPW results. There were no significant differences between the alternative and reference methods for all of the foods tested with the reference method enrichments, though the alternative method detected more *E. coli O157:H7*-positive samples than did the reference method (40 positive samples for the alternative method versus 29 for the reference method).

For ruggedness testing, the 4 following parameters were evaluated: (1) sample volume (90, 100, and 110  $\mu\text{L}$ ); (2) cell lysis incubation temperature (95, 100, and  $105^\circ\text{C}$ ); (3) lysis incubation time (5, 10, and 15 min); and (4) with and without a grinding step. Variance of these parameters did not significantly affect test kit results.

(15) *DuPont Qualicon BAX<sup>®</sup> System Q7 Real-Time PCR Assay for the Detection of Staphylococcus aureus in Ground Beef and Milk and Soy-Based Powdered Infant Formula.*—The BAX system (Qualicon, Inc.) uses PCR to amplify specific DNA fragments, which are stable and unaffected by growth conditions. The BAX system simplifies the PCR process by combining the requisite PCR reagents into a stable, dry, manufactured tablet prepackaged inside the PCR tubes. After these tablets are hydrated with prepared samples, the tubes remain sealed to reduce the potential for contamination. The BAX system PCR tablets used in real-time assays, including this test kit, contain dye-linked probes. Intact probes are short oligonucleotides with quencher dye at one end that absorbs the signal from fluorescent reporter dye at the opposite end. During PCR cooling cycles, probes bind to a specific area within the targeted fragment. During extension, DNA polymerase encounters the probe in its path and breaks the probe apart. This releases the reporter dye, resulting in increased fluorescent signal. The BAX system Q7 instrument uses multiple filters to measure signal at the end of each cycle and report results for each target in less than 90 min.

For inclusivity, 53 *S. aureus* strains were tested and all were positive (inclusivity rate of 100%). For exclusivity, 33 non-*S. aureus* species (44 isolates) were tested and all were negative (exclusivity rate of 100%).

Ground beef, milk-based powdered infant formula (PIF), soy-based PIF, and soy protein isolate (ingredient in soy-based PIF) were selected for the internal methods comparison study. Milk-based PIF was also used for the independent laboratory methods comparison study. The reference method for ground beef was ISO 6888-1:2003(E) (7); for soy- and milk-based PIF it was ISO 6888-3:2003(E) (8); and for soy protein isolate it was the FDA BAM method (2). There was no significant difference between the alternative and reference methods for the detection of *S. aureus* in any of the 3 matrixes when diluted (1:10) sample matrixes were used. For PIF, an additional comparison was conducted with nondiluted sample material added directly to enrichment media. For the reference method, samples are diluted and the diluent is enriched. In this case, undiluted samples were analyzed with the alternative method, and diluted samples were analyzed with the reference method. Under these conditions, during the internal study, the alternative method was significantly ( $P < 0.05$ ) more effective than the reference method for the 2 infant formula matrixes. For the independent laboratory testing, there was no significant difference between the alternative and reference methods for these 2 foods. The probable explanation for this was that the inoculation levels were too high: 17 of 20 diluted reference method test portions were positive, whereas 20 of 20 alternative method test portions were positive. It is not possible to statistically differentiate methods when the level of positive test portions is so high.

For ruggedness testing, the 8 following parameters were evaluated: (1) sample volume (3, 5, and 7  $\mu\text{L}$ ); (2) lysis buffer volume (180, 200, and 220  $\mu\text{L}$ ); (3) lysate incubation

temperature (53, 55, and 58°C); (4) lysate incubation time (56, 60, and 66 min); (5) lysate inactivation temperature (91, 95, and 99°C); (6) lysate inactivation time (8, 10, and 12 min); (7) total hydration volume (28, 30, and 32  $\mu\text{L}$ ); and (8) enrichment temperature (35, 37, and 39°C). Variance of these parameters did not significantly affect test kit results.

(16) *Pathogen Detection Systems Automated Method for the Detection of Escherichia coli and Coliforms in Water.*—The Pathogen Detection Systems' (PDS) *E. coli* and coliform kit (Pathogen Detection Systems, Inc., Suite 1619, Biosciences Complex, 116 Barrie St, Kingston, ON, Canada K7L 3N6) is designed for the simultaneous detection of *E. coli* and coliforms in water and other selected matrixes. The PDS test uses standard solution culture broth medium (LB Lennox) composed of ingredients selected from methods already in use in laboratories accredited for testing drinking water. Therefore, the PDS test is an adaptation of current tests, and performs similarly to standard methods in terms of detecting particular organisms, excluding "nontarget" organisms, and recovering stressed organisms.

The most significant difference between the PDS medium and the conventional tests is the specific substrates used as *E. coli* (EC) and total coliform (TC) indicators. Current tests use a variety of molecules as specific substrates. The common feature for all EC indicator substrates is a glucuronic acid molecule which is conjugated onto an aromatic molecule. The product of the enzyme reaction is a free aromatic molecule with color or fluorescence properties which must be different from the substrate in order for the product to be visually differentiated. The EC indicator substrate in the PDS test is also a glucuronic acid molecule conjugated onto an aromatic molecule; however, both the product and substrate are fluorescent. The difference is that the free aromatic molecule from the PDS substrate is extracted into a small, localized, clear polymer element within the test cartridge while the substrate is not. Similarly, current TC tests and the PDS TC test use aromatic molecules conjugated onto a galactose molecule. Both indicators are fluorescent; however, the EC and TC indicators emit 2 different wavelengths. Using a single excitation light source for both the EC and TC products, a CCD spectrometer is used to simultaneously resolve the fluorescence of both products within the polymer. Isolating optical detection to the polymer makes the test resistant to optical interference from the sample matrix.

For inclusivity, 25 *E. coli* strains and 25 non-*E. coli* coliforms were tested. All 25 *E. coli* strains were identified as *E. coli* and all 25 non-*E. coli* coliforms were identified as coliforms, but not as *E. coli*. The inclusivity rate was 100%. For exclusivity, 30 non-*E. coli*/coliform species were tested. None were misidentified as *E. coli*, for an exclusivity rate of 100% for *E. coli*. Two were misidentified as coliforms, giving an exclusivity rate of 93.3% for coliforms.

Tap water, well water, distilled water, lake water, vegetable wash water, bottled water, remineralized bottled water, and iced tea were selected for the internal methods comparison study. Bottled water was selected for the independent methods comparison study. The reference method for tap water, well

water, surface water, and vegetable wash water was EPA method 1604 (membrane filter with MI medium; 9). The reference method for bottled water and iced tea was the FDA BAM method (2). There was no significant difference between the alternative and reference methods for any of the 8 tested matrixes.

For ruggedness testing, the 2 following parameters were evaluated: (1) sample volume (80, 100, and 120 mL); and (2) incubation temperature (33, 35, and 37°C). Variance of these parameters did not significantly affect the performance of this kit, but the 33°C test times were about 1 h longer and the 37°C test times were about 1 h shorter than the 35°C test times. The longest detection times were almost 3 h below the recommended 18 h test time necessary to declare a sample as “negative.”

(17) *Bio-Theta DOX-60F/30F Total Aerobic Viable Count Test for Selected Foods*.—The Bio-Theta DOX instrument (Daikin Industries, Ltd, 1000-2, Ohtani, Okamoto-cho, Kusatsu, Shiga 525-8526, Japan) provides a quantitative estimate of bacterial counts based on oxygen depletion by respiration. An oxygen electrode measures the level of dissolved oxygen in a sample diluted with media. As the concentration of bacteria in the test medium increases with time, electrode current decreases with decreasing dissolved oxygen. A high bacterial load sample will cause the current value to decrease to a predetermined threshold value faster than a low bacterial load sample. The time required to reach the threshold level correlates to the level of bacteria in the sample. By creating a standard calibration curve for each food matrix, the level of bacterial contamination can be estimated for unknown samples.

Inclusivity and exclusivity testing were not required for this method, because it is a viable count method.

Raw beef, raw ground beef, and raw chicken carcass rinse were selected for the internal methods comparison study. Raw ground beef was selected for the independent laboratory methods comparison study. The reference method for the validation study was AOAC OMA Method 966.23 (6). The alternative method did not differ significantly from the reference method for any of the 3 foods tested.

For ruggedness testing, the following 4 parameters were evaluated: (1) sample volume (0.9, 1.0, and 1.1 mL); (2) media volume (0.9, 1.0, and 1.1 mL); (3) incubation temperature (33, 35, and 37°C); and (4) ambient temperature (15, 20, 25, and 30°C). Variance of these 4 parameters did not significantly affect test kit results, except for incubation temperature, where results for 33 and 37°C were significantly different ( $P < 0.05$ ) from those at 35°C. Because incubation temperature is not controlled by the user, this is not a significant issue with the method.

## References

- (1) Hammack, T.S. (2008) *J. AOAC Int.* **91**, 23B–31B
- (2) U.S. Food and Drug Administration (2001) *Bacteriological Analytical Manual Online*, <http://www.cfsan.fda.gov/~ebam/bam-toc.html>
- (3) U.S. Department of Agriculture, Food Safety and Inspection Service (2002) *Microbiology Laboratory Guidebook Online*, <http://www.fsis.usda.gov/OPHS/microlab/mlgbook.html>
- (4) *Standard Methods for the Examination of Dairy Products* (2004) 17th Ed., American Public Health Association, Washington, DC
- (5) International Organization for Standardization (2002) *Horizontal Method for the Detection of Salmonella spp.*, ISO 6579:2002E
- (6) *Official Methods of Analysis* (2008) 18th Ed. and Suppl., AOAC INTERNATIONAL, Gaithersburg, MD
- (7) International Organization for Standardization (2003) *Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (Staphylococcus aureus and Other Species)—Part 1: Technique Using Baird-Parker Agar Medium*, ISO 6888-1:2003E
- (8) International Organization for Standardization (2003) *Horizontal Method for the Enumeration of Coagulase-Positive Staphylococci (Staphylococcus aureus and Other Species)—Part 3: Detection and MPN Technique for Low Numbers*, ISO 6888-3:2003E
- (9) U.S. Environmental Protection Agency (2002) *Test Method 1604: Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)*, EPA 821-R-02-024, Washington, DC