

GENERAL REFEREE REPORTS

Committee on Microbiology and Extraneous Methods

Food Microbiology, Nondairy

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Recommendations

(1) **2000.07**, *Modified 5-9-01, Salmonella in Foods, Rapid Colorimetric TECRA UNIQUE Test (3M TECRA Salmonella IC)*.—Study Director Ian Garthwaite, 3M Microbiology, 13 Rodborough Rd, Frenchs Forest, NSW 2086, Australia, Tel: 61(0)-2-8977-3032, Fax: 61(0)-2-9453-3422, E-mail: ian.garthwaite@mmm.com. The TECRA UNIQUE *Salmonella* test was approved First Action Method **2000.07**. This study goal was to validate a format change of the UNIQUE product. The original 3 food study was broadened after a full claims review when TECRA was acquired by 3M. These studies showed the method to be not as consistently productive as the BAM method for a number of foods. Therefore, 3M has chosen to withdraw AOAC OMA approval for this method. Delete method.

(2) *H66, Determination of Escherichia coli in Flesh Foods Using a Visual Immunoassay with a Modified Culture Procedure (3M TECRA E. coli VIA)*.—Study Director Ian Garthwaite. Resource allocations and integration of TECRA International into 3M have limited the progression of this study through the precollaborative study phase. Plans are to expand this study to incorporate immunocapture of the target organism. Continue study.

(3) *H71, Staphylococcus aureus in Foods, 3M TECRA STAPH AUREUS Visual Immunoassay*.—Study Director Ian Garthwaite. 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 on this method was conducted, using a geographically widespread community of collaborators. Unfortunately, organism die-off was seen in the samples sent outside Australia. Continue study.

(4) **995.22**, *Modified 2/6/01, Listeria spp., 3M TECRA Visual Immunoassay for Environmental Surfaces*.—Study Director Ian Garthwaite. 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

plans 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. There has been difficulty in initiating the collaborative study for environmental samples. This will be addressed. Continue study.

(5) *H17, Listeria in Selected Foods by TECRA Unique Listeria Method (3M TECRA Listeria IC)*.—Study Director Ian Garthwaite. The precollaborative and collaborative study protocols have received approval from the Methods Committee and are in progress. Continue study.

(6) **998.09**, *Modified 3/13/03, Validation Study to Demonstrate Equivalence of a Minor Modification to 998.09 with the Reference Culture Method*.—Study Director Ian Garthwaite. This assay, using Rappaport-Vassiliadis (RV) [R10] medium as a single selective enrichment broth, has Final Action approval (Official Method **998.09**). TECRA developed a protocol (3M TECRA ULTIMA) that includes a test portion additive that allows the direct analysis of RV [R10] medium in the VIA without subsequent post-enrichment in M broth. The TECRA ULTIMA protocol, as an additional option within the method, was granted modified First Action status in 2004. Continue study.

(7) **2000.07**, *Modified 2/15/00, TECRA Unique Salmonella Test (3M TECRA Salmonella IC)*.—Study Director Ian Garthwaite. This assay was approved First Action for all foods, except raw flesh foods. 3M Microbiology plan to extend the applicability of the method to all foods using a single module incubation temperature. The protocols for the precollaborative and collaborative studies have received approval from the Methods Committee. Discontinue study.

(8) *OMA-2007-Apr-015, Detection of Enterobacter sakazakii in Powdered Infant Formula by the 3M TECRA HELIXE. sakazakii Method*.—Study Director Ian Garthwaite. A precollaborative study protocol has been approved by the Committee. Continue study.

(9) *H-16, Improved Analysis of Food Samples for Total Escherichia coli Populations to Determine Whether 10⁴CFU/g Action Levels Have Been Exceeded*.—Study Director Michael A. Grant, U.S. Food and Drug Administration (FDA), 22201 23rd Dr, SE, Bothell, WA 98021-4421, Tel: 425-402-3179, Fax: 425-483-4966, E-mail: mgrant@ora.fda.gov. The Study Director indicates that this method will not be validated through collaborative study, so the General Referee recommends that this topic be discontinued.

(10) **2003.11**, *3M Petrifilm Staph Express for Staphylococcus aureus in Meat, Seafood, and Poultry*.—Study Director Wendy A. McMahon, Silliker, Inc., Research Center, 160 Armory Dr, South Holland, IL 60473, Tel: 708-756-3210, Fax: 708-756-0049, E-mail: wendy.mcmahon@silliker.com. Official Method **2003.11** was adopted First Action for the specific and exclusive analysis of cooked diced chicken, cured ham, smoked salmon, and pepperoni. This method was granted Final Action status in 2006; therefore, the General Referee recommends that this topic be discontinued.

(11) *OMA-2007-Apr-008, Detection of Enterobacter sakazakii in Powdered Infant Formula by the TEMPO Enterobacter sakazakii Method*.—Study Director Ronald L. Johnson, bioMérieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042-2320, Tel: 314-506-8182, Fax: 314-731-8276, E-mail: ron.johnson@na.biomerieux.com. A precollaborative study protocol has been approved by the Committee. Continue study.

(12) *OMA-2007-Jan-004.R1 Evaluation of the VIDAS Staph Enterotoxin II (SET 2) Immunoassay Method for the Detection of Staphylococcal Enterotoxins in Selected Foods: Collaborative Study*.—Study Directors Ronald L. Johnson and 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. The SET 2 collaborative study was performed in 2006 and collaborative study manuscript has been submitted to the Methods Committee for review. Continue study.

(13) **2001.09**, *Salmonella in Selected Foods by Immuno-Concentration Salmonella (ICS) and Enzyme-Linked Immunofluorescent Assay (ELFA)*.—Study Directors Wendy A. McMahon and Ronald L. Johnson. This method was approved First Action for selected foods in 2001 and subsequently approved First Action for all foods in 2004. This method was granted Final Action status in 2006; therefore, the General Referee recommends that this topic be discontinued.

(14) **2001.07**, *Salmonella in Selected Foods by Immuno-Concentration (ICS) and Selective Plate (BS, HE, SMID) Procedure*.—Study Directors Wendy A. McMahon and Ronald L. Johnson. This method was approved First Action for selected foods in 2001 and subsequently approved First Action for all foods in 2004. This method was granted Final Action status in 2006; therefore, the General Referee recommends that this topic be discontinued.

(15) **2001.08**, *Salmonella in Selected Foods by Immuno-Concentration (ICS) and Selective Plate (BS, HE, XLD) Procedure*.—Study Directors Wendy A. McMahon and Ronald L. Johnson. This method was approved First Action for selected foods in 2001 and subsequently approved First Action for all foods in 2004. This method was granted Final Action status in 2006; therefore, the General Referee recommends that this topic be discontinued.

(16) **2004.03**, *Evaluation of VIDAS Salmonella (SLM) Immunoassay Method with Rappaport-Vassiliadis (RV) Medium for the Detection of Salmonella in Foods*.—Study Directors Wendy A. McMahon and Ronald L. Johnson. This

method was granted Final Action status in 2006; therefore, the General Referee recommends that this topic be discontinued.

(17) **2002.08**, *Detection of Botulinal Toxins A, B, E, and F from Culture Supernatants, Amplified ELISA Procedure*.—Study Director Joseph L. Ferreira, Centers for Disease Control and Prevention, 1600 Clifton Rd, NCID, Mailstop G-29, Atlanta, GA 30333, Tel: 404-639-0896, Fax: 404-639-4290, E-mail: jferreira@cdc.gov. Continue study.

(18) **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, 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 2005. 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.

(19) **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. 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.

(20) **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. 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.

(21) **1996.10**, *Modified 9/21/00, Assurance Enzyme Immunoassay for the Detection of Escherichia coli O157:H7 in Ground 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.

(22) **1996.09**, *Visual Immunoprecipitate Assay for the Analysis of Ground 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. 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.

(23) OMA-2007-Apr-007, *Detection of Enterobacter sakazakii in Powdered Infant Formula by the Assurance GDS™ for Enterobacter sakazakii*.—Study Director Philip T. Feldsine. A precollaborative study protocol has been approved by the Committee. Continue study.

(24) 2000.14, *Twenty-Hour REVEAL Screening Test for Detection of Escherichia coli O157:H7 in Selected Foods and Environmental Surfaces*.—Study Director Mark Mozola, Neogen Corp., 620 Leshar Pl, Lansing, MI 48912, Tel: 517-372-9200, Fax: 517-372-0108, E-mail: mmozola@neogen.com. This method was adopted First Action in 2000 and Final Action in 2005; therefore, the General Referee recommends that this topic be discontinued.

(25) 2007.02, *Salmonella spp. in Select Foods, GeneQuence® Salmonella DNA Hybridization with 24 H Enrichment*.—Study Director Mark Mozola. This method was adopted First Action in 2007. Continue study.

(26) 2004.02, *VIDAS Listeria monocytogenes II (LMO2) Immunoassay for the Detection of Listeria monocytogenes in Foods*.—Study Director Ronald L. Johnson. This method was adopted First Action in 2004. 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.

(27) 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 First Action in 2004. 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.

(28) 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, UK, Tel: 44 (0) 1638 723110, Fax: 44 (0) 1638 723111, E-mail: adrian.parton@matrixmsci.com. A precollaborative study protocol has been approved by the Committee. Continue study.

(29) 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. A precollaborative study protocol has been approved by the Committee. Continue study.

(30) OMA-2007-Apr-011, *Detection of Enterobacter sakazakii in Powdered Infant Formula by the Zurich Procedure*.—Study Director Han Joosten, Quality and Safety

Assurance Department, Nestlé Research Center, 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. A precollaborative study protocol has been approved by the Committee. Continue study.

(31) 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-710, College Park, MD 20740, Tel: 301-436-2007, Fax: 301-436-2644, E-mail: Keith.Lampel@fda.hhs.gov. A precollaborative study protocol has been approved by the Committee. Continue study.

AOAC Research Institute Studies

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

(1) Roche/BIOTECON *Diagnosics LightCycler foodproof Salmonella Detection Kit for Salmonella spp. in combination with ShortPrep foodproof I Kit: Matrix Extension*.—The ShortPrep foodproof I Kit (BIOTECON Diagnostics GmbH, Hermannswerder Haas 17, 14473 Potsdam, Germany) was extended to include the detection of *Salmonella* spp. on environmental surfaces. The detection method is based on real-time polymerase chain reaction (PCR). In <20 min the kit generates PCR templates from 50 µL of bacterial enrichment culture, using a prefilled special lysis reagent that eliminates the need for hazardous organic extractions or chaotropic agents. The DNA can then be used directly in PCR using the LightCycler System. The LightCycler foodproof *Salmonella* Detection Kit detects *Salmonella* specific DNA by means of real-time PCR using the LightCycler Instrument. Using the kit's supplied sequence-specific primers, the LightCycler Instrument and its associated reagents amplify and simultaneously detect fragments of a *Salmonella*-specific gene. The LightCycler Instrument detects these amplified fragments in real time through fluorescence generated by their corresponding pair of sequence-specific HybProbe probes. For each amplicon, one HybProbe Probe is labeled at the 5'-end with a LightCycler Red fluorophore (LightCycler Red 640 or LightCycler Red 705 for the detection of the IC); it is also 3'-phosphorylated, so it cannot be extended. The other oligonucleotide probe is labeled at the 3'-end with fluorescein. When hybridized to the template DNA, the 2 probes are close enough to allow fluorescence resonance energy transfer (FRET) between the 2 fluorophores. During FRET, fluorescein (the donor fluorophore) is excited by the light source of the LightCycler Instrument. Fluorescein transfers part of this excitation energy to the LightCycler Red dye (the acceptor fluorophore). The LightCycler Instrument measures the emitted fluorescence of the LightCycler Red fluorophores.

For the inclusivity study, all 125 *Salmonella* serotypes (707 isolates) generated positive results with the test kit. For the exclusivity study, all 51 of the non-*Salmonella* species gave negative results.

Internal and independent laboratory comparison studies showed that the ShortPrep foodproof I Kit was equivalent to the ISO 6579:2002 (2) for the detection of *Salmonella* on stainless steel, plastic (polypropylene), glass, painted surfaces, wood, sealed concrete, cast iron, and air filter material.

Ruggedness testing was not required for this matrix extension.

(2) *RapidChek SELECT Salmonella for the Detection of Salmonella spp. in Raw Ground Beef, Raw Ground Chicken, Chicken Carcass Rinsates, Liquid Eggs, and Sliced Cooked Turkey.*—The RapidChek SELECT *Salmonella* Test Kit method (Strategic Diagnostics Inc., 128 Sandy Dr, Newark, DE 19713) is an immunoassay-based test that uses anti-*Salmonella* spp. antibodies and colloidal gold-antibody conjugates incorporated into a lateral flow test strip. The method utilizes 2 proprietary enrichment broths (primary and secondary). Following the primary enrichment of the food sample, an aliquot is transferred to a tube containing 1 mL of secondary enrichment broth. The incubation time is dependent upon the matrix. The test strip is placed into the tube containing the secondary enrichment broth. The liquid sample flows through the test strip where it rehydrates antibody-coated colloidal gold reagents specific to *Salmonella* spp. impregnated in the strip. If antigens are present in the sample, they will bind to the antibody-gold conjugate to form an antigen/antibody complex. As this complex migrates through the nitrocellulose matrix, it passes a zone of anti-*Salmonella* antibody immobilized on the nitrocellulose membrane. If antigen is present, the complex is captured in this zone and is visualized by the formation of a red line. A second zone on the membrane is designed to capture any antibody-gold complex not bound in the first zone. As a result, when *Salmonella* antigen is present, the formation of 2 red lines is observed, whereas when *Salmonella* is not present, only 1 line forms.

One hundred and thirteen *Salmonella* isolates representing 18 somatic antigen serogroups were tested for inclusivity. The test method gave positive results for all but 2 isolates, *S. Boecker* and *S. Urbana*. For exclusivity, 50 non-*Salmonella* bacterial isolates representing 11 genera commonly found in food were grown for 24 h in nonselective media. All 50 isolates gave negative results with the test method.

In internal and independent laboratory studies, 185 samples were tested by both the test method and the U.S. Department of Agriculture (USDA) reference method (3) using both naturally contaminated (ground chicken) and artificially contaminated food matrixes. Several major *Salmonella* serogroups found in food (B, C₃, D₁, and E₁) were used for inoculation. Overall, 90 samples were found to be positive by the RapidChek SELECT *Salmonella* method and 74 were found to be positive by the reference method. There were no false positives or false negatives found with the test method in the method comparison studies.

For ruggedness testing, the following method parameters were investigated: (1) primary enrichment temperature (40, 42, and 44°C); (2) the use of autoclaved primary enrichment

media base and boiled secondary media; (3) sample volume (0.5, 1.0, 1.1, and 1.25 mL); (4) operating temperature of the test strip assay (4, 15, room temperature, and 37°C); and, (5) variations in time of reading the test strip (immediately, 5, 10, and 20 min). Results demonstrated that method performance was not affected by variation in the parameters with the exception of the operating temperature of the test strip. Test strip results were equivalent at temperatures from 15–37°C. For test strips that were run at 4°C, the liquid sample moved more slowly through the test strip matrix resulting in the development of test signals that were lighter in intensity than test strips that were run at temperatures ≥15°C. However, at 4°C, the ultimate test result was not affected.

(3) *VIDAS[®] Listeria species Xpress (LSX) with Ottaviani Agosti Agar: Matrix Extension.*—The VIDAS *Listeria* species Xpress (LSX; bioMérieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042-2320) test is an enzyme-linked fluorescent immunoassay (ELFA) designed for use with the automated VIDAS[®] or mini-VIDAS[®] instruments for the specific detection of *Listeria* species. Meat and dairy products require 2-step 30 h enrichments in proprietary LX broth. Environmental swabs and sponges require 1-step 24 h enrichments in LX broth. The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. The SPR is coated with anti-*Listeria* antibodies. Reagents for the assay are ready-to-use and predispensed in the sealed reagent strips. The instrument performs all of the assay steps automatically. The user places the sample into the reagent strip. Then the sample is cycled in and out of the SPR for a specific length of time. *Listeria* antigens present in the sample will bind to the anti-*Listeria* monoclonal antibodies, which are coated on the interior of the SPR. Unbound sample components are washed away. Alkaline phosphatase-labeled antibodies are cycled in and out of the SPR and will bind to any *Listeria* antigens captured on the SPR wall. Further wash steps remove unbound conjugate. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The bound enzyme conjugate catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. When the assay is completed, the results are analyzed automatically by the instrument and a test value is generated for each sample. This value is then compared to an internal reference (threshold) and each result is interpreted (positive, negative).

For the inclusivity study, all 50 *Listeria* strains gave positive results. For the exclusivity study, all 70 non-*Listeria* species gave negative results.

A matrix extension study was conducted to validate poultry, vegetable, and seafood products for a modification to the previously AOAC RI validated VIDAS *Listeria* species Xpress (LSX) method (1). The LSX method was previously validated with Ottaviani Agosti Agar as an AOAC Performance-Tested Method for use with meat products and dairy products and 8 environmental surfaces. In this study, results from the internal and independent laboratories showed that the VIDAS LSX was significantly ($P < 0.05$) more

sensitive than the USDA *Microbiology Laboratory Guidebook* method (3) for raw pork, plastic, rubber, ceramic, and cast iron, and was significantly more sensitive than AOAC Official Method 993.12 (4) for Camembert cheese. Confirmation of presumptive results was compared using Ottaviani Agosti Agar (OAA) versus the reference method agar [modified Oxford agar (MOX) for USDA method and Oxford Agar (OXA) for AOAC 993.12 (4)] was shown to be equivalent. In the matrix extension study, the VIDAS LSX was significantly more sensitive than BAM method (5) for the detection of *L. grayi* in pasteurized crabmeat and OAA was significantly more sensitive than OXA in confirming *L. grayi* from pasteurized crabmeat. For all other foods including seafood, poultry, and vegetable products tested in the method extension study, the VIDAS LSX with Ottaviani Agosti chromogenic agar was equivalent to the reference methods.

For ruggedness testing, the following 4 parameters were varied as follows: (1) sample volume (450, 500, 550 µL); (2) sample boiling time (10, 15, 20 min); (3) sample temperature (10, 25, 50°C); and (4) instrument variability (5 different VIDAS instruments). Deviations of these 4 parameters did not impair the performance of the test kit.

(4) *Reveal Listeria Test for Detection of Listeria spp. in Foods and Selected Environmental Surfaces: Method modification.*—The Reveal *Listeria* Test (Neogen Corp., 620 Leshar Pl, Lansing, MI 48912) is a lateral-flow format, immuno-diagnostic test that permits rapid and accurate detection of *Listeria* spp. in foods and environmental samples. A preheated portion (135 µL) of the final enrichment culture is placed into the sample port of the Reveal test device. This sample is wicked through a reagent zone, which contains specific antibodies (anti-*Listeria*) conjugated to blue latex particles. If antigens are present in the sample, they will bind to the blue latex conjugated antibodies. This antigen-antibody complex then leaves the reagent zone and travels through the nitrocellulose membrane, which contains a zone of anti-*Listeria* antibodies. The immune complex with blue latex conjugate is captured and aggregates in this zone, thus displaying a visible line. The remainder of the sample continues to migrate to the end of the membrane where it will eventually be deposited into a waste reservoir.

The reagent zone also contains blue latex conjugate of a proprietary antigen (color indicator), which is eluted by the sample solution regardless of the presence of *Listeria* antigen. The blue latex-conjugated control indicator migrates through the membrane to the positive control capture zone (antibody to the proprietary antigen), where it is captured and aggregated to form a visible line. Regardless of the presence or absence of the *Listeria* antigen in the sample, the control line will form in the control zone, ensuring the test is working properly. Positive assay results must be confirmed by standard culture methods.

The method was modified by changing the enrichment for environmental surfaces from a 2-step to a single-step procedure. The current version specifies a 2-stage enrichment procedure, first in half-Fraser broth and then in buffered *Listeria* enrichment broth, with a total incubation time of 42 to

48 h. The new single-step enrichment procedure, for environmental samples, utilizes *Listeria* enrichment single step (LESS) broth and has an enrichment period of as little as 25 h.

For the inclusivity study, all 52 *Listeria* strains gave positive results. Exclusivity testing was performed in the original PTM study (960701).

Results from the internal laboratory methods comparison study showed that the Reveal method was more productive ($p < 0.05$) than the reference USDA culture procedure (3) for 2 surfaces (stainless steel and plastic), and statistically comparable to the reference method for the other 3 surfaces (cast iron, ceramic tile, and sealed concrete). Overall, sensitivity of the Reveal method at 24 h compared to plating from LESS broth to Oxford agar was 87%, while compared to the USDA culture procedure the Reveal method detected 74% more positive samples. An independent laboratory trial with ceramic tile inoculated with *L. monocytogenes* confirmed the effectiveness of the Reveal method at the 24 h time point. Of a total of 40 inoculated surface samples at 2 levels, the Reveal method produced 34 positives (versus 38 by plating from LESS broth to Oxford agar for a sensitivity of 89%). This compared favorably to the 28 positives detected by the USDA reference culture procedure. There were no false-positive results with the Reveal test in either the internal or independent laboratory trials, therefore the test specificity was 100%. The new, abbreviated enrichment procedure for environmental samples allows results to be obtained in as little as 25 h from sample collection. The enrichment procedure for food samples remains as previously described.

Ruggedness testing was not performed for this study.

(5) *DuPont Qualicon BAX[®] System PCR Assay for Screening Listeria genus: Q7 BAX[®] Instrument Modification.*—The BAX[®] System (Qualicon, Inc., ESL Bldg 400, Rt 141 and Henry Clay, Wilmington, DE 19880) uses PCR to amplify a specific fragment of bacterial DNA, which is stable and unaffected by growth environment. 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 measures the denaturation temperature and analyzes the magnitude of the fluorescent signal change to determine a positive or negative result. The system combines primers, polymerase, and nucleotides needed for PCR into a single tablet. The specificity of a PCR assay is determined by the DNA sequences of the primers used.

Most chemical and operational parameters of the new instrument/assay kit combination are the same with the new instrument as with the current instrument. All currently available assays will be compatible with both instruments. All kit reagents are identical to those used in the kits previously AOAC validated. All components of the BAX tablets including PCR primers, Taq, nucleotides and bulking agents are the same. Kits are thus fully compatible using both the current BAX and Q7 instruments. The only operational difference between the 2 instruments is in the way that the signal is recorded. Emission of bound SYBR Green is

detected using a photomultiplier tube in the current BAX platform. In the Q7 instrument, a charged coupled device camera is used to detect the emitted light.

In the inclusivity study, all 30 *Listeria* spp. strains tested were positive with the assay. *L. grayi* was not included and it is stipulated based on the previous study that some *L. grayi* strains are not detected by BAX assay. In the exclusivity study, all 21 non-*Listeria*, grown in brain heart infusion (BHI) broth, were negative with the assay.

An interlaboratory method comparison study was done with samples from 3 environmental surfaces: ceramic tile, plastic (polypropylene), and unpainted wood. Each of these surfaces was inoculated with a different *Listeria* strain. The alternative method was compared to the USDA reference culture method (3). There were no significant differences between the alternative and reference methods for the recovery of *Listeria* spp. from these 3 surfaces.

No ruggedness data were required, because this was a minor modification.

(6) *DuPont Qualicon BAX[®] system L. monocytogenes: Q7 BAX[®] Instrument Modification.*—The mode of action of the BAX[®] System (Qualicon, Inc.) for the detection of *L. monocytogenes* in foods is the same as that of the BAX[®] System for the detection of *Listeria* spp. on environmental surfaces (see 5).

In the inclusivity study, all 30 *L. monocytogenes* strains tested were positive with the assay. In the exclusivity study, all 21 non-*L. monocytogenes* strains, grown in BHI broth, were negative with the assay.

An interlaboratory method comparison study was done with frankfurters, frozen peas, and soft cheese. The alternative method was compared to the USDA reference culture method (3) for frankfurters and to the BAM reference method (5) for frozen peas and soft cheese. There were no significant differences between the alternative and reference methods for the recovery of *L. monocytogenes* from these 3 foods.

No ruggedness data were required because this was a minor modification.

(7) *Profos AG Profos Listeria Capture Kit.*—The Profos *Listeria* Capture Kit (Profos AG, Josef-Engert-Strasse 11, D-93053 Regensburg, Germany) is based on bacteriophage technology which applies magnetic beads and *Listeria* binding protein for the selective capture of *Listeria* spp. The main components of the kit are biotinylated binder molecules derived from bacteriophages and streptavidin-coated magnetic beads. The *Listeria* binding proteins in free solution attach to the *Listeria* organisms which mediates the attachment of *Listeria* to streptavidin-coated magnetic beads. The bead-protein-bacteria complexes are then separated from the sample with an applied magnetic field. The complexes are washed in order to remove possible food residues and can be directly plated on a *Listeria* selective agar (as was validated), or alternatively detected with PCR or enzyme-linked immunosorbent assay.

In the inclusivity study, all 60 *Listeria* spp. strains tested were positive with the assay. In the exclusivity study, all

42 non-*Listeria* strains grown in BHI broth were negative with the assay.

In internal and independent laboratory studies, the alternative method compared favorably to the ISO 11290-1:1996 method (6) for the detection and enumeration of *L. monocytogenes*. Salami, smoked salmon, and Camembert were naturally and artificially contaminated with different *Listeria* species. No significant difference was detected between the methods. The overall sensitivity rate was 97% and the specificity was 100%.

For ruggedness testing, the following parameters were examined: (1) sample pre-enrichment time (14, 18, and 26 h); (2) volume of *Listeria* Binding Protein (8, 10, and 12 μ L); (3) volume of Magnetic Beads (8, 10, and 12 μ L); and, (4) time the samples are mixed on the circular laboratory rotator (15, 20, and 30 min). A decrease in the amounts of *Listeria* Binding Protein and Magnetic Beads as well as of incubation time in the laboratory rotator slightly lowered the capture efficiency of *Listeria* Capture Kit in *Listeria* test samples but did not affect test results.

(8) *Roche Diagnostics/BIOTECON Diagnostics LightCycler foodproof E. coli O157 Detection Kit in combination with ShortPrep foodproof II Kit.*—The ShortPrep foodproof II Kit (BIOTECON Diagnostics GmbH) is used for the isolation of *E. coli* O157 DNA from the enriched food sample. Cells are lysed and their DNA is extracted in the ShortPrep foodproof II Kit Resuspension buffer. The LightCycler Instrument, with the LightCycler foodproof *E. coli* O157 Detection Kit, uses sequence-specific primers in a PCR to simultaneously amplify and detect fragments of a gene located in the O antigen gene cluster of *E. coli* O157. The co-amplification of an internal control prevents misinterpretation of false-negative results due to inhibition of the amplification. The LightCycler Instrument detects both amplification products in real time through fluorescence generated by sequence-specific pairs of hybridization probes. For each amplicon, one hybridization probe is labeled at the 5'-end with a LightCycler Red fluorophore (LightCycler Red 640 for the detection of the *E. coli* O157 amplicon, LightCycler Red 705 for the detection of the internal control). The 3'-end is modified by phosphorylation to avoid extension. The other hybridization probe is labeled at the 3'-end with fluorescein. During the annealing phase of each PCR cycle, these probes hybridize to an internal sequence of the amplicon. Only while hybridized in close proximity to each other, fluorescein, the donor fluorophore, which is excited by the light source of the LightCycler Instrument, transfers part of the excitation energy to the LightCycler Red, the acceptor fluorophore (fluorescence resonance energy transfer). The LightCycler Instrument measures the emitted fluorescence of the LightCycler Red fluorophores.

For the inclusivity study, extracts from all 60 *E. coli* O157 isolates gave positive results. For the exclusivity study, extracts from all 120 non-*E. coli* O157 strains and species gave negative results.

Internal and independent laboratory comparison studies showed that the ShortPrep **foodproof** II Kit was equivalent to the FDA BAM method (5) or to ISO 16654:2001 (7) for the detection of *E. coli* O157 in mayonnaise, Camembert, coalfish, egg salad, raw vegetable salad, frozen salmon, apple juice, raw milk, minced meat, and large frankfurters. Both methods produced identical results in 7 foodstuffs (70 %): mayonnaise, egg salad, frozen salmon, apple juice, minced meat, coalfish, and large frankfurters. In 4 foodstuffs the alternative PCR method generated more positive results than the reference method in both inoculation levels. These foodstuffs were Camembert, coalfish, raw vegetables salad, and raw milk. Altogether, it was observed that the bacterial background had a bigger effect on the cultural reference methods than on the PCR method. Regarding the PCR, only the crossing points (cp-values) were influenced, whereas a distinctive competitive flora could influence the results of analysis of the reference methods.

For ruggedness testing, variations of the following parameters were examined: (1) variation of sample volumes (2, 5, and 7 μ L); (2) variation of analysts (30 replicates were tested by 3 different users with 1 instrument) and, (3) variation of instruments (4 different LightCycler instruments were tested by 1 analyst). Deviations of these 3 parameters did not impair the performance of the kit.

(9) *DuPont Qualicon BAX[®] System E. coli O157:H7 PCR Assay: Addition of Immunomagnetic Capture Step Modification.*—The BAX[®] System (Qualicon, Inc.) uses the PCR to amplify a specific fragment of bacterial DNA, which is stable and unaffected by growth environment. The fragment is a genetic sequence that is unique to the genus *E. coli* O157:H7 serotype, thus providing a highly reliable indicator that the organism is present. The BAX system simplifies the PCR process by combining the requisite primers, polymerase and nucleotides into a stable, dry, manufactured tablet already packaged inside the PCR tubes. The system uses fluorescent detection to analyze PCR product.

The BAX System *E. coli* O157:H7 PCR Assay was paired with an immunomagnetic separation (IMS) step and was evaluated for the detection of *E. coli* O157:H7 in 25 g ground beef samples. Paramagnetic beads are coated with antibody specific for *E. coli* expressing the O157 cell surface determinate. These beads can be used to separate small amounts of the analyte from a large amount of other extraneous material. The addition of the IMS step saves approximately 1½ h with respect to time to result when compared with the original validated protocol.

For inclusivity testing, 100 of 101 *E. coli* O157:H7 strains and 3 of 3 *E. coli* O157:HNM strains gave positive results. For exclusivity testing, all 30 non-O157 strains gave negative results.

Results from the internal study indicated no significant difference between the USDA culture based reference method (3) and the BAX system method using a 6 h enrichment and IMS prior to assay for 25 g ground beef samples. In the independent study, the BAX assay with a 6 h

enrichment and IMS prior to testing performed significantly better than the USDA method, producing a χ^2 value of 10.

With respect to ruggedness testing, the following parameters were studied: (1) temperature of enrichment at time sample begins IMS (38, 42, and 46°C); (2) sample volume (0.4, 0.5, and 0.6 mL); (3) Dynal[®] bead volume (16, 20, and 24 μ L); (4) mixing time (12, 15, and 18 min); and (5) lysis time (8, 10, and 12 min). Variation of these parameters did not impair method performance.

(10) *TEMPO[®] EC (E. coli) Method for the Enumeration of Escherichia coli in a Variety of Foods.*—The TEMPO EC test (bioMérieux, Inc.) 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 wells) with a 1 log difference in volume for each set of wells. The card is designed to simulate the Most Probable Number (MPN) method (8, 9). The card is then hermetically sealed in order to avoid any risk of contamination during subsequent handling. Based on β -glucuronidase activity, *Escherichia coli* present in the card reduce the substrate in the culture medium during incubation and cause a fluorescent signal to appear, which is detected by the TEMPO Reader. Depending on the number and type of the positive wells in the 3 log dilution range, the TEMPO system calculates the number of *Escherichia coli* present in the original sample according to a calculation based on the MPN method.

In the inclusivity study, all 30 *E. coli* strains tested were detected with TEMPO EC. For exclusivity, none of the 20 non-*E. coli* strains, belonging to 19 different species, were detected with the TEMPO EC system.

Internal and independent laboratory method comparison studies showed that the TEMPO method was statistically equivalent ($p < 0.05$) to AOAC OMA methods **966.23** and **966.24** (4) for the enumeration of *E. coli* in raw fresh ground pork, raw fresh ground veal, fresh ground beef, frozen ground beef, frozen chicken nuggets, raw fresh ground turkey, frozen turkey breast, raw milk, vanilla ice cream, mozzarella cheese, yoghurt, raw white fish fillet, raw salmon steak, frozen cooked white fish, iceberg lettuce, fresh strawberries, dry pet food, and pasteurized eggs.

For ruggedness testing, the following parameters were evaluated: (1) sample incubation temperature (34, 35, 36, 37, and 38°C); (2) sample incubation time (21, 23, 24, 25, 26, and 27 h); and (3) waiting time before incubation of the test (0, 30, 60, 90, and 120 min). Temperature, incubation time, and delay in the tested interval had no statistically significant effect ($p < 0.05$) on the TEMPO EC test kit performance.

(11) *RAPID[®] Staph[™]: A Medium for Differentiation and Enumeration of Coagulase-Positive Staphylococcus aureus in Selected Foods.*—The principle of RAPID[®] Staph medium (Bio-Rad Laboratories, 2000 Alfred Nobel Dr, Hercules, CA 94547) relies on the capacity of *S. aureus* to reduce tellurite (production of black colonies) and to provoke proteolysis of egg yolk (production of clear halo around the colony). The

proprietary peptone mixture, in addition to the meat and yeast extract, provides nutrients to the bacteria, allowing for growth in 24 h. Glycine and lithium chloride inhibit the growth of competitors adding to the selectivity of the medium while sodium pyruvate stimulates the growth of *S. aureus*, increasing sensitivity.

For inclusivity, 30 *S. aureus* strains were tested on RAPID[®]Staph agar. All 30 strains produced typical black colonies with clear halo. For exclusivity, 40 non-*S. aureus* strains were tested on RAPID[®]Staph agar. Thirty-seven strains were completely inhibited. *Staphylococcus hyicus*, *Listeria ivanovii*, and *Listeria monocytogenes* were the only organisms that grew on RAPID[®]Staph. The colonies were pinpoint and gray to black with no halo. This morphology was not typical of *S. aureus*, therefore these colonies would not have been mistaken for the target organism.

Internal and independent laboratory method comparison studies showed that the RAPID[®]Staph agar method was equivalent to AOAC Method 975.55 (4) for the identification and enumeration of coagulase-positive *S. aureus* in pasteurized whole milk, custard pie, processed ham, and smoked salmon.

For ruggedness testing, variations in the following parameters were examined: (1) incubation time (21, 24, and 27 h); (2) incubation temperature (35, 37, and 39°C); and (3) a comparison of dehydrated media and ready-to-use plates. Deviations from the method did not impair the performance of the kit. There was no statistical difference ($p > 0.05$) between the dehydrated and the ready-to-use formulations of the media.

(12) *DuPont Qualicon BAX[®] System Real-Time PCR Assay for Campylobacter jejuni/coli/lari*.—The BAX[®] System (Qualicon, Inc.) uses the PCR to amplify a specific fragment of bacterial DNA, which is stable and unaffected by growth environment. The fragment is a genetic sequence that is unique to *Campylobacter jejuni*, *C. coli*, and *C. lari*, thus providing a highly reliable indicator that one or more of these organisms are present. The BAX system simplifies the PCR process by combining the requisite PCR reagents into a stable, dry, manufactured tablet already packaged inside the PCR tubes. The tablets used in the BAX system real-time assays also contain multi-dye 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.

BAX system inclusivity results were 100% accurate for 52 *Campylobacter* strains of the target species (18 *C. jejuni*, 15 *C. coli*, and 19 *C. lari*). Exclusivity results were 100% accurate for 35 nontarget strains.

Results from the internal and independent method comparison studies demonstrate that the BAX system performance is not statistically different from ISO 10272-1:2006 (10) for the detection of *Campylobacter* in artificially contaminated, sliced, vacuum-packaged turkey and naturally contaminated chicken carcass rinses.

Eight variables were evaluated for ruggedness: (1) sample volume (3, 5, and 7 μ L); (2) lysis buffer volume (180, 200, and 220 μ L); (3) incubation temperature of lysate (35, 37, and 39°C); (4) incubation time of lysate (16, 20, and 24 min); (5) inactivation temperature of lysate (91, 95, and 99°C); (6) inactivation time of lysate (8, 10, and 12 min); (7) total hydration volume (28, 30, and 32 μ L); and (8) enrichment temperature (40, 42, and 44°C). Initial ruggedness testing revealed that incubation only slightly above the originally suggested incubation temperature of $42 \pm 2^\circ\text{C}$ gave inconsistent results at the high incubation temperature abuse condition of 45°C . In order to reduce the risk of similar issues as users run the assay, and to highlight the sensitivity of this portion of the assay, the suggested incubation range was tightened to $42 \pm 1^\circ\text{C}$. Incubations inoculated with low levels of *Campylobacter* and maintained at 44°C gave consistently positive results by the test kit. The BAX[®] System User Guide was edited to reflect this change in temperature tolerance. Other variations in assay conditions tested did not have an effect on the assay results.

(13) *TEMPO[®] TVC (Total Viable Count) Method for the Enumeration of Aerobic Mesophilic Flora in a Variety of Foods*.—The TEMPO TVC test (bioMérieux, Inc.) 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 wells) with a 1 log difference in volume for each set of wells. The card is designed to simulate the MPN method (8, 9). The card is then hermetically sealed in order to avoid any risk of contamination during subsequent handling. During incubation, the microorganisms present in the card reduce the substrate in the culture medium and cause a fluorescent signal to appear, which is detected by the TEMPO Reader. Depending on the number and type of the positive wells in the 3 log dilution range, the TEMPO system calculates the number of aerobic bacteria present in the original sample according to a calculation based on the MPN method.

Internal and independent laboratory method comparison studies showed that the TEMPO TVC method was statistically equivalent ($p < 0.05$) to the Standard Methods for the Examination of Dairy Products (11) for dairy products and to AOAC Method 966.23 (4) for the other naturally contaminated foods that were tested (raw ground pork, frozen ground beef, heat-processed cooked roast beef, smoked turkey, fresh ground chicken, frozen cooked chicken nuggets, heat-processed grilled chicken, frozen catfish, heat-processed frozen fish, heat-processed crab cakes, bagged salad, frozen green beans, vanilla ice cream, pasteurized milk, hash brown

potatoes, processed frozen egg omelet, raw cod, and raw bean sprouts).

For ruggedness testing, variations in the following parameters were examined: (1) incubation temperature of the test (33, 34, 35, 36, and 37°C); and (2) incubation time of the test (40, 42, 44, 46, and 48 h). Temperature and incubation time had no significant effect ($p < 0.05$) on the TEMPO TVC test kit performance.

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