

GENERAL REFEREE REPORTS

Committee on Microbiology and Extraneous Materials

Food Microbiology, Nondairy

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Collaborative Studies

BioControl Assurance GDS™ Method for the Detection of E. coli O157:H7 in Selected Foods.—Study Director, Philip T. Feldsine (see *Recommendations*, 19). This system (BioControl Systems, Inc., Bellevue, WA) is an automated gene-based assay that uses proprietary probes and specific primers directed against a highly conserved DNA sequence of the target organism. This assay detects strains of *Escherichia coli* O157:H7 and toxigenic *E. coli* O157:NM (nonmotile). The assay is designed to be highly selective and does not detect microorganisms that are potential cross-reactors in antibody-based assays, including *E. coli* O157 that are neither H7 nor NM, and other microorganisms that express O157 antigen but are not *E. coli* O157:H7. The Assurance GDS system also uses a proprietary device and reagents that concentrate populations of target microorganisms, thereby providing increased sensitivity and elimination of potential competitive microflora.

This system has been subjected to 2 separate phases of testing. The first phase was an inhouse methods comparison study in which 6 food types (raw ground beef, beef trim, orange juice, apple juice, lettuce, and sprout process water) were analyzed. The alternative method provided results equivalent to those of the reference culture method for the 6 food types. For inclusivity testing, the alternative method correctly identified 50 of 50 *E. coli* O157:H7 strains for a sensitivity rate of 100%. For exclusivity testing, the alternative method gave negative results for 175 of 175 non-*E. coli* O157:H7 organisms for a specificity rate of 100%.

A collaborative study was conducted to compare the alternative method and the reference culture method for the detection of *E. coli* O157:H7 in orange juice, raw ground beef, and fresh lettuce. Fifteen laboratories participated in the study. Overall, there were valid data from the analysis of 642 test portions for the alternative method. The results generated in this study demonstrated a high level of correlation between the alternative method and the confirmed results. For the alternative method, agreement between the alternative method and confirmed results was 97%. The method was highly sensitive. The alternative method was significantly more effective ($P < 0.05$) than the reference method for the

detection of *E. coli* O157:H7 in orange juice and ground beef (both high and low levels) based on the Chi square results. The alternative method was significantly more effective ($P < 0.05$) than the reference method for the detection of *E. coli* O157:H7 in lettuce (high level) and equivalent to reference method in lettuce (low level). This method was adopted First Action.

Biocontrol Assurance GDS™ Method for the Detection of Shigatoxin Genes from Escherichia coli O157:H7 in Selected Foods.—Study Director Philip T. Feldsine (see *Recommendations*, 19). The Assurance GDS™ system (BioControl Systems, Inc., Bellevue, WA) is an automated gene-based assay that uses proprietary probes and specific primers directed against a highly conserved DNA sequence of the target organism. This assay specifically measures the presence of shigatoxin genes from *E. coli* O157:H7. It has been reported that 99.6% of *E. coli* O157:H7 isolates contained at least one of the two shigatoxin genes, *stx*₁ and *stx*₂. This assay may be useful when information regarding the presence of Shiga-like toxin genes in *E. coli* O157:H7 is desired. Both precollaborative and collaborative studies were conducted. In the precollaborative study, the alternative method was equivalent to the U.S. Department of Agriculture (USDA) reference culture method (1) for the detection of *E. coli* O157:H7 (containing shigatoxin genes) in ground beef and beef trim. To determine inclusivity for *E. coli* O157:H7 that contain shigatoxin genes, the alternative method was compared to the BAM shigatoxin reference method (2). Fifty-three motile and nonmotile *E. coli* O157:H7 strains were tested with both the alternative method and with the reference shigatoxin method. All 53 strains were positive with the alternative method for one, or both, of the shigatoxin genes for a specificity rate of 100%. The alternative and reference methods were in 100% agreement as to the specific shigatoxin genes present in each of the *E. coli* O157:H7 isolates. For exclusivity testing, none of 51 non-*E. coli* O157:H7 isolates was identified as *E. coli* O157:H7 (containing shigatoxin genes) for a specificity rate of 100%.

Fifteen laboratories participated in the collaborative study, which analyzed raw ground beef, orange juice, and lettuce. The alternative method was significantly more productive ($P < 0.05$) than the reference method for the detection of *E. coli* O157:H7 (containing shigatoxin genes) in orange juice and ground beef (both high and low levels) based on the Chi square results. The alternative method was significantly more productive ($P < 0.05$) than the reference method for the detection of *E. coli* O157:H7 (containing shigatoxin genes) in lettuce (high level) and equivalent to reference method in lettuce (low level) based on the Chi square results. This method was adopted First Action.

BioControl SimPlate CEC Quantitative Method for Total Coliforms and E. coli in Foods.—Study Director Philip T.

Feldsine (see *Recommendations*, 19). SimPlate (BioControl Systems, Inc., Bellevue, WA) is a unique delivery system for quantification of microorganisms. This assay is specifically formulated for the quantification of total coliform bacteria and *E. coli*. A 1 mL of diluted test portion is added to the test kit device with culture media. The results are read at 24–28 h. The alternative method has a much wider counting range than other plating methodologies, thereby generally requiring fewer serial dilutions. Also, the data are recorded by reading a binary reaction in each of the device test wells. The effectiveness of this method was examined in a precollaborative study with 21 foods. The relative effectiveness of the alternative method was compared to the AOAC 3-tube most probable number (MPN) methods for enumerating and confirming of coliforms and *E. coli* in foods (Official Methods **966.23** and **966.24**; 3). Precollaborative study results showed a high correlation between this assay and the AOAC reference methods. The assay produced equivalent or higher mean log counts for the majority of food types analyzed, and the repeatability standard deviation values of this assay were generally lower than those of the reference method.

In the collaborative study, 6 food types were artificially contaminated with coliform bacteria and *E. coli*: frozen burritos, frozen broccoli, fluid pasteurized milk, whole almond nutmeats, cheese, and powdered cake mix. Fifteen laboratories participated in the study. Overall, the alternative method demonstrated <0.3 log difference for total coliform and *E. coli* counts compared to the AOAC reference methods for the majority of food types and levels analyzed. In all cases, the repeatability and reproducibility of the alternative method were not different from those of the reference method and, in certain cases, were statistically better than those for the AOAC reference method. These results indicate that the alternative method and the reference culture methods are comparable for enumeration of both total coliforms and *E. coli* in foods. This method was adopted First Action.

Recommendations

(1) **2000.07** Modified 5/9/01, *Salmonella* in Foods, Rapid Colorimetric TECRA Unique Test.—Study Directors Matthew P. Turner, TECRA International Pty Ltd, 13 Rodborough Rd, Frenchs Forest, NSW 2100, Australia, Tel: 61-2-8977-3052, Fax: 61-2-9453-3522, E-mail: matthew.turner@tecra.net and Ian Garthwaite, TECRA International Pty Ltd, Tel: 61-2-8977-3032, Fax: 61-2-997-27869, E-mail: ian.garthwaite@tecra.net. Continue study.

(2) *H66 Determination of Escherichia coli in Flesh Foods Using a Visual Immunoassay with a Modified Culture Procedure*.—Study Directors Matthew P. Turner and Ian Garthwaite. Continue study.

(3) *H71 Staphylococcus aureus in Foods, TECRA STAPH AUREUS Visual Immunoassay*.—Study Directors Matthew P. Turner and Ian Garthwaite. Continue study.

(4) **995.22** Modified 2/6/01, *Listeria spp., TECRA Visual Immunoassay for Environmental Surfaces*.—Study Directors Matthew P. Turner and Ian Garthwaite. Continue study.

(5) *H17 Listeria in Selected Foods by TECRA Unique 2000 Listeria Method*.—Study Directors Matthew P. Turner and Ian Garthwaite. Continue study.

(6) **955.22** Modified 5/23/01, *TECRA Enrichment for Listeria in Foods*.—Study Directors Matthew P. Turner and Ian Garthwaite. This method is now in Official Final Action status. It is recommended that this topic be discontinued.

(7) **998.06** Modified 3/13/03 *Validation Study to Demonstrate Equivalence of a Minor Modification to 998.09 with the Reference Culture Method*.—Study Directors Matthew P. Turner and Ian Garthwaite. Continue study.

(8) **2000.07** Modified 2/15/01 *TECRA Unique Salmonella Test*.—Study Directors Matthew P. Turner and Ian Garthwaite. Continue study.

(9) Modified **999.06** *Evaluation of VIDAS Listeria (LIS) immunoassay*.—Study Director Karen M. Silbernagel, Land O'Lakes, MS 0075, PO Box 64101, St. Paul, MN 55164-0101, Tel: 651-766-1303, Fax: 651-486-0837, E-mail: kmsilbernagel@landolakes.com. It is recommended that this topic be deleted because the Study Director has resigned and it is a duplicate of topic number 35, which is to be continued.

(10) *H16 Improved Analysis of Food Samples for Total Escherichia coli Populations to Determine Whether 10⁴ CFU/g Action Levels Have Been Exceeded*.—Study Director Michael A. Grant, U.S. Food and Drug Administration, 22201 23rd Dr, SE, Bothell, WA 98021-4421, Tel: 425-402-3179, Fax: 425-483-4996, E-mail: mgrant@ora.fda.gov. Continue study.

(11) **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. Continue study.

(12) **996.08** *VIDAS SLM Method for Detection of Salmonella in Foods*.—Study Directors Wendy A. McMahon and Ronald L. Johnson, bioMerieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042-2320, Tel: 314-506-8182, Fax: 314-506-8182, E-mail: Ron.johnson@na.biomerieux.com. This method has been adopted Final Action. It is recommended that this topic be discontinued.

(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 foods in general in 2004. Thus, it is recommended that the title of the topic be changed to “*Salmonella* in Foods by Immunoconcentration *Salmonella* (ICS) and Enzyme-Linked Immunofluorescent Assay (ELFA).” Continue study.

(14) *H7 Clostridium botulinum Toxins A, Proteolytic B, and E, ELCA Enzyme Immunoassay*.—Study Director Wendy A. McMahon. A precollaborative study report has been approved by the Committee, and a collaborative study is planned once the Committee approves the protocol. Continue study.

(15) **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 “foods” in general in 2004. Thus, it is recommended that the title of this topic be changed to “*Salmonella* in Foods by Immuno-Concentration (ICS) and Selective Plate (BS, HE, SMID) Procedure.” Continue study.

(16) **2001.08** *Salmonella in Selected Foods by Immunocentration (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 foods in general in 2004. Thus, it is recommended that the title of this topic be changed to “*Salmonella* in Foods by Immunocentration (ICS) and Selective Plate (BS, HE, XLD) Procedure.” Continue study.

(17) **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. Continue study.

(18) **2002.08** *Detection of Botulinum 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.

(19) **2002.10** *ISO vs AOAC Reference Culture Methods for the Detection of Motile and Nonmotile Salmonella in Selected 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. A collaborative study report, consisting of inclusivity data and a collaborative study involving fresh cheese, dried egg powder, and raw poultry meat was approved. This effort was the result of a cooperative effort between AOAC and the organizers of the Central European Commission project to develop performance data on ISO 6579 (4). The ISO 6579 method was adopted First Action in 2002. This method is widely used, and the Study Director recommends that this method be adopted Final Action. The co-General Referees concur.

(20) **2002.07** *SimPlate Total Plate Count Color Indicator (TPC-CI) for the Enumeration of Total Aerobic Microorganisms in Foods*.—Study Director Philip T. Feldsine. This method was adopted Final Action in 2004. It is recommended that this topic be discontinued.

(21) **2002.11** *SimPlate Yeast and Mold Color Indicator (Y&M-CI) Method for the Enumeration of Yeasts and Molds in Foods*.—Study Director Philip T. Feldsine. This method

was adopted Final Action in 2004. It is recommended that this topic be discontinued.

(22) **996.10** *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 2004. Thus, it is recommended that this topic be deleted.

(23) **2005.06** *SimPlate CEC Quantitative Method for Total Coliforms and Escherichia coli in Foods*.—Study Director Philip T. Feldsine. A collaborative study has been performed, and the method has been adopted First Action (*see Collaborative Studies*).

(24) **996.14** *Assurance Polyclonal Enzyme Immunoassay for the Detection of Listeria monocytogenes and Related Listeria Species in Selected Foods*.—Study Director Philip T. Feldsine. This method for selected foods was adopted First Action in 1996 and Final Action in 1998. Subsequently, the applicability of the method was expanded to include the monitoring of environmental surfaces. This method for environmental surfaces was adopted First Action in 2001 and Final Action in 2003. Thus, it is recommended that this topic be discontinued.

(25) **996.09** *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 2004. Thus, it is recommended that this topic be deleted.

(26) **997.03** *Visual Immunoprecipitate Assay for the Detection of Listeria monocytogenes and Related Listeria Species in Selected Foods*.—Study Director Philip T. Feldsine. This method was adopted First Action in 1997 and Final Action in 1999. Subsequently, the applicability of the method was expanded to include the monitoring of environmental surfaces. This method for environmental surfaces was adopted First Action in 2001 and Final Action in 2003. Thus, it is recommended that this topic be discontinued.

(27) **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. Continue study.

(28) **2000.13** *Eight-Hour REVEAL Screening Test for the Detection of Escherichia coli O157:H7 in Selected Foods*.—Study Director Mark Mozola. Continue study.

(29) **2003.01** *Enterobacteriaceae in Foods, Dry Rehydratable Film Method*.—Study Director Deborah A. McIntyre, rtech Laboratories, MS 0075, PO Box 64101, St. Paul, MN 55164-0101, Tel: 651-481-2636, Fax: 651-486-0837. There have been no adverse reports

concerning this First Action method. The Study Director recommends that this method be adopted Final Action. The co-General Referees concur.

(30) **2004.02 VIDAS *Listeria monocytogenes* II (LMO2) Immunoassay for the Detection of *Listeria monocytogenes* in Foods.**—Study Directors Deborah A. McIntyre and Ronald L. Johnson. Continue study.

(31) **2003.09 BAX System with Automated Detection of *Salmonella* in Foods.**—Study Director Deborah A. McIntyre. There have been no adverse reports concerning this method. The Study Director recommends that this method be adopted Final Action. The co-General Referees concur.

(32) **2003.07 3M Petrifilm Staph Express Count Plate Method for the Enumeration of *Staphylococcus aureus* in Selected Processed and Prepared Foods.**—Study Director Deborah A. McIntyre. There have been no adverse reports concerning this method. The Study Director recommends that this method be adopted Final Action. The co-General Referees concur.

(33) **2003.12 BAX System for Detection of *Listeria monocytogenes* in Foods.**—Study Director Deborah A. McIntyre. There have been no adverse reports concerning this method, the Study Director recommends that this method be adopted Final Action. The co-General Referees concur.

(34) **2000.15 Coliform Counts in Foods, Dry Rehydratable Film Method.**—This method is in Final Action status. It is recommended that this topic be discontinued.

(35) **2004.06 (formerly 999.06) VIDAS *Listeria* (LIS) Immunoassay for the Detection of *Listeria* Species in Foods Using Demi-Fraser and Fraser Enrichment Broths.**—Study Directors Deborah A. McIntyre and Ronald L. Johnson. Continue study.

Editorial Additions for Official Methods of Analysis

(1) **2003.09 *Salmonella* in Selected Foods, BAX Automated System.**—Add the following section:

H. Confirmation.

Presumptive positive test portions must be confirmed culturally as described in **967.26** (see 17.9.02), **967.27** (see 17.9.03), and **967.28** (see 17.9.07).

(2) **2003.12 *Listeria monocytogenes* in Foods, BAX Automated System.**—Add the following section:

H. Confirmation

Presumptive positive tests must be confirmed using culture methods as described in the current edition of the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (<http://www.cfsan.fda.gov/~ebam/bam-10.html>) or the USDA, Food Safety Inspection Service's *Microbiology Laboratory Guidebook* (http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp). Isolate from previously enriched MOPS-BLEB tubes.

AOAC Research Institute Studies

The following studies have been approved by the AOAC Research Institute:

(1) **BBL CHROMagar *Salmonella* Prepared Plated and Difco CHROMagar *Salmonella* Dehydrated Culture Media:**

Comparison with USDA and BAM Reference Culture Methods.—These 2 agars (BD Diagnostic Systems, Hunt Valley, MD) permit the detection and presumptive identification of *Salmonella* species through the incorporation of specific chromogenic substrates and inhibitory agents, including amphotericin B, cefsulodin, and novobiocin. As a result of the hydrolysis of chromogenic substrates, most *Salmonella* species produce a mauve (moderate rose to moderate purple) colony. Organisms, other than *Salmonella*, that are not suppressed appear as blue-green, tan, white, or colorless colonies.

For the inclusivity study, a total of 118 cultures was used. Seven of these cultures produced a false-negative reaction for a sensitivity rate of 94%.

For the exclusivity study, 65 cultures were used. Four of these cultures produced a false-positive reaction for a specificity rate of 94%.

CHROMagar *Salmonella* commercially prepared plates (CS PPM) and CHROMagar *Salmonella* laboratory-prepared plates from dehydrated media (CS DCM) were compared with the plating media recommended in the USDA (1) or BAM (2) reference culture methods, depending on the food type examined. The raw chicken and raw ground beef were analyzed according to the USDA reference culture method, with brilliant green sulfa agar and double modified lysine iron agar used as the selective plating media. The raw fish, lettuce, and shell eggs were analyzed according to the BAM reference culture method, with bismuth sulfite (BS), Hektoen enteric (HE), and xylose lysine desoxycholate (XLD) agars used as the selective plating media. CS PPM and CS DCM plates were compared to the reference culture method plates for the isolation of *Salmonella*. In the internal study, both the CS PPM and the CS DCM plates produced results comparable to those of the reference method for the analysis of raw chicken, resulting in a method agreement of 100% based on Chi square results. In the independent study, both the CS PPM and CS DCM plates produced comparable results for the analysis of raw ground beef, raw fish, lettuce, shell eggs, and raw chicken, resulting in a method agreement of 100% based on Chi square results.

With respect to ruggedness testing, 2 *Salmonella* strains and one *Escherichia coli* strain were used to evaluate 3 incubation temperatures (32, 35, and 38°C) and 4 incubation periods (14, 24, 48, and 60 h). At 14 h incubation, minimal growth was observed with both *Salmonella* strains at 32°C. Colonies that did grow were not mauve. When incubated at 35 and 38°C, plates inoculated with *Salmonella* had light mauve or mauve colonies. The *E. coli* strain was not recoverable at any of the incubation temperatures.

At 24 h incubation, both *Salmonella* strains incubated at 35 and 38°C produced light mauve or mauve colonies. When incubated at 32°C, 10 of 30 plates failed to produce colonies. The *E. coli* strain was recovered and produced blue-green colonies at all temperatures.

At 48 and 60 h incubation, both *Salmonella* strains produced mauve colonies at each incubation temperature. The

E. coli strain produced blue to blue-green colonies at all temperatures.

(2) *BBL CHROMagar Salmonella Prepared Plated Culture Medium*.—Comparison with ISO Reference Culture Method.—This validation study was similar to the previous validation study (see 1), except that only the CS PPM medium (BD Diagnostic Systems) was validated, and the alternative method medium was compared to the ISO reference culture method (4) rather than to the USDA and BAM reference culture methods.

For the inclusivity study, 127 cultures were used. Four of these cultures produced a false-negative reaction for a sensitivity rate of 97%.

For the exclusivity study, 65 cultures were used. Four of these cultures produced a false-positive reaction for a specificity rate of 94%.

In the independent validation study, the performance of CS PPM agar was compared to that of the ISO-recommended agars (HE and XLD) for the analysis of raw ground beef, raw fish, lettuce, shell eggs, and raw chicken. There was a method agreement of 100% based on Chi square results.

Ruggedness testing was performed as described previously (see 1) with identical results obtained in the 2 studies.

(3) *Singlepath Salmonella Assay for the Detection of Salmonella Species in Selected Foods*.—This assay (Merck KGaA, Darmstadt, Germany) is an immunochromatographic test which uses gold-labeled antibodies. The test device has a circular sample port and an oval-shaped test (T) and control (C) window. After pre-enrichment in buffered peptone water (BPW) and selective enrichment in Rappaport-Vassiliadis Soya (RVS) medium, the selective enrichment is boiled, cooled to room temperature, and applied to the nitrocellulose membrane pad via the circular sample port. The test portion is absorbed through the pad to the reaction zone containing colloidal, gold-labeled antibodies specific to *Salmonella* species. Any *Salmonella* antigen present complexes with the gold-labeled antibody and migrates along the membrane until it encounters the binding zone in the test (T) area. The binding zone (T) contains another anti-*Salmonella* antibody, which immobilizes any *Salmonella* antigen-antibody complex present. Due to the gold labeling, a distinct red line is then formed. The remainder of the test portion continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). At the control zone, an antibody directed against the gold-labeled antibody, is immobilized onto the membrane. Regardless of whether any *Salmonella* antigen is present, this distinct red line is always formed in the control (C) zone within 20 min, ensuring that the test is working properly. When *Salmonella* organisms are present in the test portion, 2 distinct red lines are formed. Only one red line (control) is observed when no *Salmonella* organisms are present or when *Salmonella* organisms are present below detectable levels.

For inclusivity testing, all of 105 *Salmonella* strains were reactive with the test kit device. With exclusivity testing, 58 cultures not belonging to the genus *Salmonella* were used. When these cultures were grown in a nonselective medium

(buffered peptone water), 4 cultures cross-reacted in the test kit device. However, when these cultures were grown in the selective RVS broth, there were no cross-reactions.

In the internal study, the performance of the alternative method was compared to that of the ISO reference culture method (4) for the analysis of nonfat dried milk, ground black pepper, desiccated coconut, cooked prawns, and dried dog food. Recovery rates of *Salmonella* were identical with those of the alternative and reference methods for each of the food types examined.

In the independent study, there was no significant difference in the recovery of *Salmonella* from raw ground beef and raw ground turkey by the alternative and reference culture methods.

For the results of the internal and independent studies combined, the alternative method had a false-negative rate of 2.5% and a false-positive rate of 7.3%.

With respect to ruggedness testing, the effects of variation of 3 parameters on method performance were investigated: (1) test portion time in boiling water bath (0, 15, and 25 min); (2) time for observation of test portion result (20 ± 5 min); and (3) volume of test portion for assay (160 ± 10 μ L). For day 1, 2 positive strains, *S. Enteritidis* and *S. Typhimurium*, were cultured from cryobeads to BPW, enriched at 37°C for 20 h, inoculated into RVS broth, and enriched at 41.5°C for 24 h. The positive strains were diluted to 10^5 CFU/mL in RVS broth, which was used to study all 3 method parameters. For day 1, one negative strain, *E. coli*, was cultured from cryobeads to BPW and enriched at 37°C for 20 h. The negative strain was used undiluted.

For day 2, the 2 positive strains were prepared by inoculating fresh RVS broth with 1% of the primary BPW culture used to prepare day 1 cultures and incubating overnight at 41.5°C. The negative strain, *E. coli*, was prepared by inoculating fresh BPW with the day 1 *E. coli* culture and incubating overnight at 37°C.

For the evaluation of variation in test portion time in boiling water bath, on day 1, when tests were read at 20 min, the test kit device detected both positive strains at all 3 boiling times. On day 2, *S. Typhimurium* was not detected by the test kit device when unboiled (0 min boiling time). On day 2, *S. Enteritidis* was detected at all 3 boiling times and the negative strain was negative at all 3 boiling times.

For the evaluation of variation of time for observation of test portion result, on day 1 and day 2, the test kit device detected both positive strains when read at all 3 time intervals. The negative strain was negative at all 3 time intervals.

For the evaluation of variation of volume of test portion for assay, on days 1 and 2, the test kit device detected both positive strains when tested with all 3 test portion volumes. The negative strain was negative with all 3 test portion volumes.

(4) *Genevision™ Salmonella Assay for the Detection of Salmonella Species in a Variety of Foods*.—The Genevision™ Pathogen detection kit (Warnex Diagnostics, Inc., Laval, Quebec, Canada) provides fast, accurate, and highly specific results using customized microplates and real-time polymerase chain reaction (PCR) technology. Each

microplate contains all the reagents required for the detection of pathogens. An assessment of pathogenic contamination is performed by specifically identifying the DNA signature of a pathogen in a series of sequential steps that include sample collection, enrichment, DNA extraction and DNA amplification by real-time PCR.

The fluorescence of the molecular beacons occurs once they recognize the targeted bacterial DNA sequence. The amount of fluorescence produced is proportional to the amount of DNA amplified and recognized by the beacon. In the absence of the target bacteria in food samples, no fluorescence signal is detected.

For the inclusivity study, 166 isolates were tested (105 *Salmonella* serovars). All 166 *Salmonella* isolates yielded positive PCR results for a sensitivity rate of 100%.

For the exclusivity study, 112 non-*Salmonella* spp. were tested. None of the non-*Salmonella* spp. yielded a positive PCR result for a specificity rate of 100%.

For the internal validation study, the selected foods were clam chowder, cottage cheese, tortellini, pork cubes, ground beef, and frozen chicken breast. The alternative method was compared to the appropriate reference method: the BAM method (2) for the analysis of clam chowder, cottage cheese, and tortellini; the USDA method (1) for the analysis of pork cubes, ground beef, and chicken breast. The alternative method was equivalent to the reference method for all of the foods tested based on Chi square results. For the external validation study, the foods were cooked ham, apple juice, chicken wings, and cauliflower. The reference method for the analysis of apple juice and cauliflower was the BAM method (2). The reference method for the analysis of cooked ham and chicken wings was USDA method (1). The alternative method was equivalent to the reference method for all foods, except for apple juice where the alternative method was significantly less effective than the reference method ($P < 0.05$). Consequently, the alternative method was modified by changing the pre-enrichment broth for apple juice from nutrient broth to Universal Pre-enrichment broth. After this modification, the alternative method was equivalent to the reference method for the analysis of apple juice.

Ruggedness was tested by (1) rehydrating the Extraction-2 (EX-2) reagent with half the required amount of Extraction-1 (EX-1) buffer; (2) transferring in the PCR plate 20% of the extracted DNA; and (3) inverting the volumes of detection (DT) solution and DNA to reconstitute the PCR reaction (10 μ L of DT solution and 15 μ L of test portion instead of 15 μ L DT solution and 10 μ L test portion). All deviations were tested in pentuplicates for each test portion. The ruggedness experiments were repeated once. None of the 3 deviations had a significant effect on the performance of the method.

(5) *Pathatrix Salmonella Species Pooling Test Method for Selected Foods*.—The Pathatrix *Salmonella* Test System (Matrix MicroScience Ltd, Newmarket, Cambridgeshire, UK) is comprised of a pre-programmed workstation and a consumable pack and uses magnetic beads coated with antibodies specific to the target organism. The food test portion is stomached in BPW, and the stomacher bag is then

incubated overnight at 37°C. After incubation, the bag is placed on the Pathatrix instrument in a thermally controlled pot at 37°C, and magnetic beads, coated with antibodies to *Salmonella*, are added to the test portion homogenate. The consumable pack is then loaded into the instrument, and the pre-programmed run is started. The liquid test portion is then continuously re-circulated over the phase from the stomacher bag by a peristaltic pump via tubing. Within this closed loop system is a plastic phase that incorporates a sloped face, which becomes magnetized and captures the beads on the face's surface as they pass.

After continuously circulating the test portion through the system for 30 min, the target organisms are bound to the magnetic beads on the phase. Any residue and food debris are removed from the phase by a subsequent wash step. The beads from the capture phase are then eluted into a wash vessel and concentrated using a magnetic rack. After completion of the capture step, the test portion is then directly plated onto selective agar plates for isolation of colonies.

A modification of the above method, involving pooling of test portion pre-enrichments, was validated. Pooling involves taking 50 mL volumes from 5 individual test portions and pooling them to create a 250 mL composite which can be analyzed by a single Pathatrix run. If the composite is positive, then the individual test portions can be re-tested separately by the Pathatrix system to determine which test portions are positive. If the wet composite is negative, then all 5 test portions can be discarded. This system is unique in that it is the only microbial detection system that can analyze the entire 225 mL + 25 g test portion simultaneously by re-circulating the test portion through the "capture phase" where the antibody-coated magnetic beads are immobilized.

An in-house study was conducted in which the Pathatrix system and the BAM method were compared for the analysis of individual and composited test portions of 5 food types. For each food type, 10 sets of test portions were analyzed. Each set consisted of one positive test portion and 4 negative test portions. The data showed that any individual or pooled positive test portion was always positive by both the Pathatrix and BAM methods, i.e., there was 100% correlation between methods.

(6) *Pathatrix Listeria Species Pooling Test Method for Selected Foods*.—This method (Matrix MicroScience Ltd, Newmarket, Cambridgeshire, UK) operates on the same principle as the Pathatrix *Salmonella* Species Pooling Method for selected foods (see 5). The food test portions are stomached in half-Fraser broth, and the stomacher bags are incubated at 30°C for 24 h. After incubation, the bag is placed on the Pathatrix instrument in a thermally controlled pot at 30°C, and magnetic beads, coated with antibodies to *Listeria*, are added to the test portion homogenate. The subsequent operation of the instrument and the validation protocol for the pooling of test portion homogenates are the same as described for the Pathatrix *Salmonella* Species Pooling Method for selected foods (see 5).

An in-house study was conducted in which the Pathatrix system and the BAM method were compared for the analysis of individual and composited test portions of 5 food types. For each

food type, 10 sets of test portions were analyzed. Each set consisted of one positive test portion and 4 negative test portions. The data showed that any individual or pooled positive test portion was always positive by both the Pathatrix and BAM methods, i.e., there was 100% correlation between methods.

(7) *Genevision™ Listeria Assay for the Detection of Listeria monocytogenes in a Variety of Foods*.—The mode of action for the Genevision system (Warnex Diagnostics, Inc., Laval, Quebec, Canada) for the detection of *L. monocytogenes* in a variety of foods is the same as the Genevision *Salmonella* assay for the detection of *Salmonella* spp. in a variety of foods (see 4).

For the inclusivity study, 51 *L. monocytogenes* strains were tested. All 51 *L. monocytogenes* strains yielded a positive PCR signal for a sensitivity rate of 100%.

For the exclusivity study, 129 nontarget bacterial strains were tested. Of the 129 nontarget bacterial strains tested, none gave a positive PCR result for a specificity rate of 100%.

For the internal validation study, the effectiveness of the method for pork, sausages, beef cubes, green beans, and yogurt was examined. The alternative method was compared to the USDA reference method (1) for ground pork, sausages, and beef cubes. The alternative method was compared to the BAM method (2) for green beans and to the AOAC Official Method 993.12 (3) for yogurt. Food lots were artificially inoculated with pure *L. monocytogenes* cultures and held at 4°C for 2 to 3 days. Following storage, the food was divided into 25 g portions and tested by the alternative method and by the corresponding reference culture method. The alternative method was equivalent to the reference method for foods tested by the internal laboratory, except for green beans. For green beans, at the high level of inoculation, the alternative method was significantly more productive ($P < 0.05$) than the reference method based on Chi square results. For the external validation study, the effectiveness of the alternative method for raw boneless pork chops, mayonnaise, raw cod fillet, fruit salad, and Brie was examined. The alternative method was compared to the USDA reference method for pork chops. It was compared to the BAM method for mayonnaise, raw cod fillet, and fruit salad. It was compared to Official Method 993.12 for Brie. The method was equivalent to the reference method for the foods tested by the external laboratory based on Chi square results.

Ruggedness testing was previously described (see 4). None of the 3 deviations had a significant effect on the performance of the method.

(8) *BBL CHROMagar Listeria Medium: Comparison with USDA, BAM, AOAC, and ISO Reference Culture Methods*.—This agar (BD Diagnostic Systems, Hunt Valley, MD) permits the detection and presumptive identification of *L. monocytogenes* and *L. ivanovii* from food through the use of specific chromogenic substrates and inhibitory agents for Gram-negative bacteria, yeasts, and fungi. The chromogenic substrate produces a blue-green colored compound when hydrolyzed by an enzyme specific to *Listeria* species. A specific enzyme found in *L. monocytogenes* and *L. ivanovii* acts upon the phospholipid substrate in BBL CHROMagar *Listeria*

(CL agar) producing an opaque, white halo around the blue-green colonies. The growth of a blue-green colony with well-defined edges surrounded by an opaque, white halo is presumptive for *L. monocytogenes* or *L. ivanovii* on CL agar.

For the inclusivity study, 57 cultures was used. None of these cultures produced a false-negative reaction for a sensitivity rate of 100%.

For the exclusivity study, of 56 cultures was used. None of these cultures produced a false-positive reaction for a specificity rate of 100%.

The internal portion of the method comparison used test portions of raw ground beef and smoked salmon. The ground beef was processed according to the USDA (1) and ISO (5) reference methods. The smoked salmon was processed according to the BAM (2) and ISO (5) reference methods. Of 115 test portions examined, 59 test portions were positive by the alternative method and 57 were positive by the reference culture methods. A Chi square analysis demonstrated no significant difference between the 2 methods for each of the 2 matrixes examined. The sensitivity and specificity of the alternative method were 98.3 and 100%, respectively.

In the independent validation study, test portions of lettuce, smoked salmon, and Brie were examined. The lettuce was processed according to the BAM and ISO reference methods; the salmon by the BAM and ISO reference methods; and the Brie by the AOAC and ISO methods. Of 75 test portions examined, 45 test portions were positive by both the alternative and reference methods. A Chi square analysis demonstrated no significant difference between the alternative and appropriate reference culture methods for each of the 3 matrixes examined. Both the sensitivity and specificity of the alternative method were 100%.

For ruggedness testing, 2 strains of *L. monocytogenes* and one strain of *L. innocua* were used to evaluate 3 incubation temperatures (32, 35, and 38°C) and 4 incubation periods (22, 24, 48, and 50 h). At 22 h of incubation, the *L. monocytogenes* and *L. innocua* strains produced blue-green colonies without halos at 32°C. At 35 and 38°C, the 2 *L. monocytogenes* produced blue-green colonies with halos, while the *L. innocua* strain produced blue-green colonies without halos.

At 24 h of incubation, one *L. monocytogenes* strain produced blue-green colonies without halos, whereas the second *L. monocytogenes* strain produced blue-green colonies with halos at 32°C. The *L. innocua* strain produced blue-green colonies without halos. At 35 and 38°C, the *L. monocytogenes* strains produced blue-green colonies with halos, and the *L. innocua* strain produced blue-green colonies without halos.

At 48 and 50 h of incubation, both *L. monocytogenes* strains produced blue-green colonies with halos, while the *L. innocua* strain produced blue-green colonies without halos at 32, 35, and 38°C.

(9) *Paradigm Diagnostics Listeria Indicator Broth (PDX-LIB) for Detection of Listeria monocytogenes, L. innocua, L. ivanovii, and L. welshimeri on Selected Environmental Surfaces*.—This system (Paradigm Diagnostics, Inc., St. Paul, MN) contains a patented formula of antibiotics, growth enhancers, and color changing

compounds. The antibiotics function synergistically to inhibit most non-*Listeria* microorganisms. Indicator compounds will turn the broth from yellow to black by utilizing the beta-glucosidase enzyme produced by *Listeria* species. A brown-to-black color that appears after incubation at 37°C for 30–48 h indicates a presumptively positive test. Applicability of the method is limited to selected *Listeria* species (*L. monocytogenes*, *L. innocua*, *L. ivanovii*, and *L. welshimeri*) on selected environmental surfaces (ceramic tile, stainless steel, plastic, and sealed concrete).

A total of 55 *Listeria* spp. cultures was used for inclusivity testing. One culture (*L. murrayi grayi*) gave a false-negative result.

For exclusivity testing, 46 cultures belonging to genera other than *Listeria* were used. Six cultures gave false-positive reactions: *Enterococcus hire* (4 cultures), *E. avium* (1 culture), and *E. casseliflavus* (1 culture).

Internal testing involved the use of *L. innocua* on ceramic tile, *L. welshimeri* (plus *Staphylococcus aureus* at a 10-fold higher concentration) on plastic, and *L. ivanovii* on stainless steel. On the basis of a Chi square analysis, there were no significant differences in the alternative method and the USDA reference culture method for each of the environmental surfaces.

Independent laboratory validation involved the use of *L. monocytogenes* on sealed concrete and *L. innocua* on ceramic tile. There was no significant difference in the performance of the 2 methods on the basis of a Chi square analysis.

For ruggedness testing, 2 positive controls (*L. monocytogenes* and *L. innocua*) and one negative control (*S. aureus*) were used to study the effect of variations in incubation time (22, 26, and 32 h) and in incubation temperature (36 and 38°C). None of these variations affected the performance of this medium.

(10) *DuPont Qualicon BAX Assay for the Detection of Listeria Species from Environmental Surfaces.*—This assay (DuPont Qualicon, Wilmington, DE) 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 *Listeria*, thus providing a highly reliable indicator that the organism is present. This assay simplifies the PCR process by combining the requisite primers, polymerase, and nucleotides into a stable, dry tablet already packaged inside the PCR tubes. After amplification, these tubes remain sealed for the detection phase, thus reducing the potential for contamination with one or more molecules of the amplified PCR product. This system uses fluorescent detection to analyze the 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 test portion is slowly increased to denature the DNA which, in turn, releases the dye and causes a drop in the emission signal. The denaturation temperature is measured and the magnitude of the fluorescent signal change is analyzed to determine a positive or negative result.

For inclusivity testing, this system was 97.8% accurate in detecting 174 of 178 *Listeria* species cultures. All 4 cultures

that were not detected were *L. grayi*. Exclusivity testing was 100% accurate in giving negative results for all 50 cultures not belonging to the genus *Listeria*.

In the internal study, the alternative and USDA reference culture methods were compared for detecting *Listeria* species on 7 surfaces: ceramic, cast iron, plastic, concrete, rubber, air filter, and painted wood. A Chi square analysis demonstrated no significant difference in the performance of the 2 methods.

In the independent study, these 2 methods were compared for the detection of *Listeria* species on unpainted wood and stainless steel. The 2 methods did not differ significantly for the analysis of the unpainted wood surface, but, on the basis of a Chi square analysis, the USDA method was significantly better than the alternative method for the analysis of stainless steel surfaces. Because of this finding, stainless steel has been removed from the product claim.

Because the amplification and detection phases are fully automated, 8 variables for ruggedness testing were selected from the enrichment and test portion preparation phases: (1) test portion volume (4, 5, and 6 μ L); (2) incubation temperature (52, 55, and 58°C); (3) incubation time (50, 60, and 70 min); (4) inactivation temperature (92, 95, and 98°C); (5) inactivation time (8, 10, and 12 min); (6) total hydration volume (45, 50, and 55 μ L); (7) enrichment time (24, 26–30, and 32 h); and (8) enrichment temperature (33, 36, and 39°C). For inoculated test portions, strains of *L. monocytogenes* and *L. innocua* were used. Non-*Listeria* strains that were used included *Staphylococcus wamari* and *Enterococcus faecalis*. All of the *Listeria*-inoculated test portions were positive and all of the non-*Listeria*-inoculated test portions were negative, indicating that none of the variables affected the performance of the alternative method within the ranges tested.

(11) *Microwell DNA Probe Assay for Detection of Listeria in Selected Foods and Selected Environmental Surfaces.*—This assay (Neogen Corp., Lansing, MI) is a DNA probe-based diagnostic in test kit format, which permits rapid detection of *Listeria* species in selected foods and selected environmental surfaces. Following test portion pre-enrichment and selective enrichment, target bacteria are lysed enzymatically at 37°C, and *Listeria*-specific oligonucleotide probes are added for a 60 min hybridization incubation at 45°C. If *Listeria* ribosomal RNA (rRNA) is present in the test portion, the detector probe, labeled with a horseradish peroxidase (HRP), and a polydeoxyadenylic acid (poly dA)-tailed capture probe will hybridize to the target organism rRNA sequences. Concurrently, base pairing between the poly dA-tailed capture probe and the polydeoxythymidylic (poly dT) coated polystyrene microwells facilitates solid phase capture of probe-target molecules. Unbound probe is removed by washing, and then substrate chromogen is added to react with HRP to yield a blue color. The reaction is stopped by the addition of sulfuric acid, which changes the color of the substrate from blue to yellow. A microwell plate or microwell strip reader (A₄₅₀) measures absorbance. An absorbance in excess of the threshold value indicates the presence of *Listeria* in the test portion. Positive assay results must be confirmed by standard culture methods.

Inclusivity testing was conducted with 52 strains representing all of the *Listeria* species, and all of the strains were reactive with the assay. Exclusivity testing was conducted with 33 strains other than *Listeria* species, and all of these strains were nonreactive.

An internal comparative study was conducted comprising 15 food types (representing meats, seafood, dairy products, and fruits and vegetables food categories). Performance of the alternative method was compared to that of the USDA reference method (1) for the analysis of raw and cooked meat and poultry products, and to the BAM method (2) for the analysis of dairy products, seafood, and vegetables.

Statistical analysis of the results indicated that the alternative method was equivalent to the reference culture methods. On the basis of the internal validation study, the sensitivity and specificity of the alternative method was at least 97 and 98%, respectively. An independent study was performed using 2 food types (cottage cheese and mayonnaise) which provided additional data supporting product claims.

In addition to the 2 food types mentioned previously, 6 environmental surface types (stainless steel, ceramic, cast iron, plastic, concrete, and painted wood) were used in the independent study. In this study the performance of the alternative method was compared to that of the USDA reference culture method (1). There were 2 false-negative results (both on plastic) with the alternative method. Thus, sensitivity rates were 97.7% for the alternative method and 100% for the reference method. There were 5 false-negative results (all on ceramic) with the alternative method for a specificity of 83.3%. Overall agreement between the 2 methods was 98.7%.

For ruggedness testing, the effects of variations on 5 parameters were investigated: (1) number of mixing steps of the lysed test portion and the probe/hybridization solution (0, 5, and 10 times); (2) volume of the pre-mixed probe/hybridization solution (0.1, 0.125, and 0.15 mL); (3) number of wash steps (4, 5, and 6 times); (4) hybridization incubation temperature (43, 45, and 47°C); and (5) hybridization incubation time (45, 60, and 75 min). Even though mixing did not seem to be an important contributor to the appearance of background, the manufacturer recommends mixing the lysed test portions with the probe/hybridization 5 times to ensure a complete distribution of the formamide in the solution. Ruggedness testing of a variation in the volume of the premixed probe/hybridization solution demonstrated that a volume of 0.125 mL of probe/hybridization solution is needed. Without the proper concentration of formamide (hybridization solution) in the hybridization reaction, the assay conditions do not have the proper stringency, and false-positive reactions may occur due to mismatching of the probe sequences with nontarget rRNA sequences. With respect to variation in the number of wash steps, the manufacturer recommends that the wells be washed 5 times before the addition of the substrate chromogen solution. With respect to ruggedness testing of a variation in hybridization incubation temperature, the manufacturer recommends a

temperature of 45°C. Ruggedness testing of a variation in hybridization incubation times indicated that a variation of up to 15 min above or below the recommended time of 60 min does not affect performance of the test.

(12) *Microgen Listeria ID (MID-67) Assay for the Identification of Listeria Species from Foods*.—The Microgen Listeria ID (MID-67) assay (Microgen Bioproducts Ltd, Camberley, Surrey, UK) is a miniaturized biochemical identification system designed to identify individual *Listeria* species using single colonies on selective agar plates. This method uses classical dehydrated substrates and an in-well hemolysis reaction to produce 12 individual results, which are then used to produce a 4-digit code that is processed by a dedicated software program which calculates the percentage possibility that the test isolate is a particular *Listeria* species.

Inclusivity testing was conducted with 91 strains representing all of the *Listeria* species, and all of the strains were reactive with the assay. Exclusivity testing was conducted with 32 strains other than *Listeria* spp., and all of these strains were negative with the assay.

An in-house comparative study was conducted comparing this assay with the BAM method for the identification of a panel of cultures consisting of *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. ivanovii*, and *L. grayi*. The 2 methods were in 100% agreement as to the identity of the isolates.

An independent laboratory study of this method was conducted in which the alternative method and the BAM method were compared for the identification of a panel of *Listeria* species cultures. Both methods correctly identified all the *Listeria* cultures. However, one strain of *L. welshimeri* was positive for xylose fermentation by the BAM method but negative by the alternative method. This negative reaction may have been due to the fact that xylose fermentation by this particular strain of *L. welshimeri* took longer than 24 h. Thus, the alternative method would not have detected this fermentation as the microwell strip was read after 18–24 h incubation, whereas xylose fermentation by the BAM method was read after 7 days incubation. Nonetheless, the alternative method correctly identified this strain as *L. welshimeri*.

With respect to ruggedness testing, the variation in the following parameters were investigated: (1) type of selective agar (Oxford, modified Oxford, PALCAM, and ALOA) used to produce colonies for identification; (2) inoculum density (the optimal number of cells, as well as one-fifth and 5 times the optimal number of cells); (3) test portion volume (50, 100, and 150 µL); (4) substrate volume (volume of individual substrates dispensed into the multi-well strips prior to drying in the vacuum ovens; 10, 25, and 50 µL); (5) incubation temperature (33 and 37°C); (6) open pouch stability (determination if the test strips could be used up to 14 days after opening the desiccant pouch and just closing the pouch with tape); (7) temperature encountered during shipping (–20, 4, 25, and 37°C). Results of the ruggedness testing showed that the performance of the method was not affected by the described variations in any of these parameters.

(13) *Genevision™ Listeria Assay for the Detection of Listeria Species in Selected Foods*.—The mode of action for

the Genevision system (Warnex Diagnostics, Inc., Laval, Quebec, Canada) for the detection of *Listeria* spp. in different food matrixes is the same as the Genevision *Salmonella* assay for the detection of *Salmonella* spp. in a variety of foods (see 4).

For the inclusivity study, 79 *Listeria* spp. were tested. All of the *Listeria* spp. generated positive results for a sensitivity rate of 100%.

For the exclusivity study, 288 non-*Listeria* spp. were tested. All of the non-*Listeria* spp. generated negative results for a specificity rate of 100%.

For the internal validation study, cooked sliced ham, ground pork, sausages, pre-cut lettuce and yogurt were tested. The alternative method was compared to the USDA reference method (1) for sliced ham, ground pork, and sausages. It was compared to the BAM method (2) for pre-cut lettuce and yogurt. The foods were artificially inoculated with *Listeria* spp. as previously described (see 7). The alternative method was equivalent to the appropriate reference method for cooked sliced ham, ground pork, sausages, and yogurt based on Chi square results. The alternative method was significantly more productive than the reference method for pre-cut lettuce ($P < 0.05$). For the external validation study, the selected foods were raw beef, mayonnaise, green beans, and raw cod fillet. The method was compared to the USDA reference method for raw beef. The method was compared to the BAM method for mayonnaise, green beans, and raw cod fillet. The alternative method was equivalent to the reference method for raw beef, mayonnaise, green beans, and raw cod fillet.

Ruggedness testing was previously described (see 4). None of the 3 deviations had a significant effect on the performance of the method.

(14) *Genevision™ Method Extension from "Selected Foods" to "Variety of Foods" for the Genevision™ Listeria Assay for the Detection of Listeria Species in Selected Foods.*—The mode of action for the Genevision system (Warnex Diagnostics, Inc.) for the detection of *Listeria* spp. in raw shrimp and imitation crabmeat is the same as the Genevision for the detection of *L. monocytogenes* in a variety of foods (see 7).

Inclusivity and exclusivity data were as previously described (see 13).

For the internal validation study, the selected foods were raw shrimp and imitation crab meat. Foods were inoculated and handled as previously described (see 7). The alternative method was compared to the BAM method for these 2 foods. The alternative method was significantly more sensitive than the reference method ($P < 0.05$) by Chi square analysis for both the shrimp and imitation crab meat at the low levels of inoculation.

Ruggedness testing was as previously described (4). None of the 3 deviations had a significant effect on the performance of the method.

(15) *Genevision™ Minor Method Modification from the Microplate Format to PCR Strip Tubes Format for the Following Assays: Escherichia coli O157 in Ground Beef, Escherichia coli O157:H7 in Ground Beef, Listeria monocytogenes in a Variety of Foods, Listeria species in a Variety of Foods, and Salmonella spp. in a Variety of Foods.*—The mode of action for the Genevision system (Warnex Diagnostics, Inc., Laval, Quebec, Canada) for the 5 pathogen detection systems is the same as the Genevision *Salmonella* assay for the detection of *Salmonella* spp. in a variety of foods (see 4). In this study, the system was modified by going from a microplate system to an 8-well strip tube system. All of the other parameters of the system remained the same.

Inclusivity and exclusivity results were reported previously for *E. coli* O157 and *E. coli* O157:H7 (6). Inclusivity and exclusivity data were previously described for *Listeria monocytogenes* (see 7), *Listeria* spp. (see 13), and *Salmonella* spp. (see 4).

For *E. coli* O157 and *E. coli* O157:H7, the alternative method was compared to the Canadian reference method (7) for the detection of these pathogens in raw ground beef. The alternative methods were comparable to the reference method for *E. coli* O157 and *E. coli* O157:H7 in raw ground beef on the basis of Chi square results. For *L. monocytogenes*, the alternative method was compared to the USDA reference method (1) for the detection of *L. monocytogenes* in ground pork. The alternative method was comparable to the reference method for *L. monocytogenes* in raw ground pork on the basis of Chi square results. For *Listeria* spp., the alternative method was compared to the USDA reference method for the detection of *Listeria* spp. in sausages. The alternative method was comparable to the reference method for the detection of *Listeria* spp. in sausages on the basis of Chi square results. For *Salmonella* spp., the alternative method was compared to the USDA reference method for the detection of *Salmonella* in pork cubes. The alternative method was comparable to the reference method for the detection of *Salmonella* in pork cubes based on Chi square results.

Ruggedness testing was previously described (see 4). None of the 3 deviations had a significant effect on the performance of the method.

(16) *BAX System Multiplex Assay for the Detection of Escherichia coli O157:H7 in Raw Ground Beef and Raw Beef Trim.*—This assay (DuPont Qualicon) operates on the same principle as that of the DuPont Qualicon BAX assay for the detection of *Listeria* species from environmental surfaces (see 10).

Inclusivity results were 100% positive for 96 *E. coli* O157:H7 and 5 *E. coli* HNM (non-motile) strains. Exclusivity results demonstrated 99.2% accuracy when tested against a panel of 98 *E. coli* isolates other than O157:H7 and O157:HNM and 28 other diverse bacterial isolates. The only

cross-reactivity observed was with an enteropathogenic *E. coli* O55:H7 isolate.

Because the amplification and detection phases are fully automated and not subject to user modification, 8 variables for ruggedness testing were chosen from the enrichment and test portion preparation phases: (1) test portion volume (15–25 μ L); (2) incubation temperature (34–40°C); (3) incubation time (17–23 min); (4) inactivation temperature (92–98°C); (5) inactivation time (8–12 min); (6) total hydration volume (45–55 μ L); (7) enrichment time (7–9 h); and (8) enrichment temperature (39–45°C). For inoculated test portions, 2 *E. coli* O157:H7 strains and 2 non-*E. coli* O157:H7 strains (*Lactobacillus curvatus* and *Pseudomonas aeruginosa*) were used. Except for the high enrichment temperature, all *E. coli* O157:H7-inoculated test portions were positive, and all non-*E. coli*-inoculated test portions were negative, indicating that the variability in these factors did not affect the performance of the assay. The only factor that affected results was elevated enrichment temperature (45°C), which led to 1 false-negative result at 7 h.

(17) *bioMérieux VIDAS Assay for the Detection of Escherichia coli O157 in Ground Beef*. The VIDAS *E. coli* O157 assay (bioMérieux, Inc., Hazelwood, MO) is an ELFA test designed for use with the automated VIDAS or mini-VIDAS instruments for the specific detection of *E. coli* O157. 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-*E. coli* O157 antibodies. Reagents for the assay are ready-to-use and predisposed in the sealed reagent strips.

The instrument performs all of the assay steps automatically. The user places the test portion into the reagent strip. Then the test portion is cycled in and out of the SPR for a specific length of time. *E. coli* O157 antigens present in the sample will bind to the anti-O157 monoclonal antibodies, which are coated on the interior of the SPR. Unbound test portion components are washed away. Alkaline phosphatase-labeled antibodies are cycled in and out of the SPR and will bind to any O157 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.

There are 2 alternative methods available to the user: an 8 h enrichment method and a 24 h enrichment method. The 8 h enrichment uses pre-warmed (41 \pm 1°C) mTSB without novobiocin, and homogenized samples are incubated for 8 h at 42 \pm 1°C. The 24 h enrichment uses pre-warmed mTSB (41 \pm 1°C) containing novobiocin, and homogenized samples are incubated 6–8 h at 42 \pm 1°C. A 1.0 mL aliquot of the mTSB + novobiocin broth is then transferred into 9.0 mL CT-MAC broth, and incubation is continued for 18–24 h at 35 \pm 1°C. After either the 8 h or 24 h enrichment procedure, an aliquot of sample is boiled, cooled to room temperature, and then 500 μ L is added to the reagent strip-sample well. Standards and

controls are analyzed in the same run. The strips and SPRs are inserted into the instrument, and the assay is initiated according to the *Operator's Manual*. Once initiated, the instrument will complete the assay in about 45 min.

When the assay is completed, the instrument analyzes the results automatically and a test value is generated. This value is compared to a threshold and each result is interpreted as positive or negative, and a printed report is generated.

For inclusivity testing, 43 *E. coli* O157:H7 strains and 10 *E. coli* O157:non-H7 strains were tested with both the 8 h and 24 h methods. All *E. coli* O157 strains provided positive results with both the 8 h and 24 h methods.

For exclusivity testing, 46 non-*E. coli* O157 isolates were tested with both the 8 h and 24 h methods. The assay provided negative results for all of the exclusivity organisms except 2 Group N *Salmonella* isolates. This result was probably due to the fact that Group N *Salmonella* serovars have the same lipopolysaccharide (LPS) antigen as *E. coli* O157.

In the independent study, the 8 h and 24 h methods were compared to the USDA method (1) for ground beef. In addition, samples analyzed by the alternative methods were confirmed by streaking to CT-O157:H7 ID agar or CT-SMAC agar followed by biochemical and serological testing recommended by the USDA reference method. Both the 8 h and 24 h alternative methods were statistically equivalent ($P < 0.05$) to the reference method based on Chi square results.

The following parameters were tested for ruggedness testing: (1) test portions were tested after boiling for 10, 15, or 20 min to determine the effect of boiling time on the assay; (2) differing sample volumes (300, 440, 450, 500, 550, and 600 μ L) were tested in the strips to test the effect of differing sample volume on the assay; (3) boiled test portions, cooled to 10, 25, or 45°C, were added to the test strips to test the effect of test portion temperature on the assay; and (4) test portions were tested with test strips/reagents that had been stored at 2–8°C and equilibrated at room temperature for 0, 30, or 240 min to determine if variation in this parameter would affect the performance of the test kit. None of these deviations significantly affected the performance of the kit.

(18) *Sanita-kun Coliforms Test*.—This system (Chisso Corp., Tokyo, Japan) is used for the enumeration of coliforms in foods. The device consists of a transparent cover film, an adhesive sheet, a layer of nonwoven fabric, and a water-soluble film containing nutrients for coliform bacteria and 5-bromo-4-chloro-3-indoyl-beta-D-galactopyranoside (X-gal). Coliforms contain beta-galactosidase, an enzyme that will hydrolyze X-gal to produce a visible blue dye.

Test portion dilutions containing bacteria are deposited on the fabric portion of the device. These dilutions diffuse throughout the entire pad to dissolve and release the nutrients. Bacteria migrate to the surface of the fabric where coliforms metabolize the nutrients and X-gal to produce a visible dye during incubation. Growth of non-coliforms is inhibited by selective agents.

For inclusivity testing, all of 34 coliform strains were positive with the alternative method, indicating a sensitivity rate of 100%. With respect to exclusivity testing, a total of

42 strains of noncoliform bacteria were used. Two strains of *Serratia marcescens* and one strain of *Serratia rubidaea* were positive with the alternative method, indicating a specificity rate of 92.9%.

In the internal study, the alternative method was compared to the violet red bile (VRB) agar method of the BAM (2) for the enumeration of coliforms in test portions of 26 food types belonging to 9 food categories (meat, poultry, fish and seafood, fruits and vegetables, dairy, bakery products, animal feeds, pasta, and miscellaneous). There was no significant difference in coliform counts given by the 2 methods for 84.6% of the test portions examined. The linear correlation coefficient for the 2 methods was 0.94.

In the independent study, the 2 methods were compared for the enumeration of 3 levels of coliforms in raw ground beef and raw milk. With ground beef, the counts given by the 2 methods were not significantly different at the low and high levels of inoculation. At the mid level of inoculation, the alternative method gave a significantly higher count of coliforms than did the reference method. With raw milk, significantly higher counts were obtained with the reference method at the low level of inoculation and with the alternative method at the mid and high levels of inoculation.

With respect to ruggedness testing, variations in incubation times (20, 24, and 28 h) and temperatures (30, 33, 35, and 37°C) indicated no significant effect on method performance.

(19) *Compact Dry CF Method for Enumeration of Total Coliforms in Raw Meats*.—This system (Nissui Pharmaceutical Co., Tokyo, Japan) consists of a plastic device containing a sheet of dehydrated nonselective medium containing nutrients (yeast extract and peptone), selective agents, phosphate buffer, a chromogenic enzyme substrate (5-bromo-4-chloro-3-indoyl-beta-D-galactoside), a cold water-soluble gelling agent (algae polysaccharides), and an indicator dye. A buffering solution is added to the test portion and homogenized. Serial 10-fold dilutions are made, and 1 mL inoculum is pipeted into the middle of the dry sheet. The inoculum diffuses evenly throughout the sheet, transforming it to a gel within seconds. During incubation viable coliform organisms react with the indicator dye to produce blue-green colonies. These colonies are counted as in the conventional pour plating procedure in Official Method 966.23 (3).

For inclusivity testing, 50 of 51 (98%) target strains produced a positive result with the test kit. For exclusivity testing, 49 of 52 (94.2%) nontarget stains produced a negative result with the test kit.

This method was validated in the internal laboratory with 4 different types of raw meat (raw ground pork; raw pork; raw lamb, and raw veal) and in the independent laboratory with raw ground beef. A correlation coefficient of 0.91 was observed between the alternative method and Official Method 966.24 (3).

Ruggedness testing involved the study of the effect of variations in 3 parameters on the performance of the method: (1) test portion volume (0.9, 1.0, and 1.1 mL); (2) incubation temperature (33, 35, and 37°C); and (3) colony count procedure [whole plate (1 × 1 cm count × 20) or grid count (0.5 × 0.5 cm count × 80)]. None of these variations had any significant effect on the final colony counts.

(20) *Compact Dry EC Method for Enumeration of Escherichia coli and Other Coliforms in Raw Meats*.—The principle of this method (Nissui Pharmaceutical Co.) is the same as that described for the Compact Dry CF method (see 19), except that the chromogenic enzyme substrates are 5-bromo-6-chloro-3-indoxyl-beta-D-galactopyranoside (turns red/pink for coliforms) and 5-bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid (turns blue/blue purple for *E. coli*).

For inclusivity testing, 50 of 53 (94.3%) target strains produced a positive result with the test kit. For exclusivity testing, 48 of 51 (94.1%) nontarget strains produced a negative result with the test kit.

This method was validated in the internal laboratory with 4 different types of raw meat (raw ground pork, raw pork, raw lamb, and raw veal), and in the independent laboratory with raw ground beef. Correlation coefficients of 0.93 (*E. coli*) and 0.93 (*coliforms*) were observed between the alternative method and Official Method 966.24.

Ruggedness testing involved the study of the effect of variations in 3 parameters on the performance of the method: (1) test portion volume (0.9, 1.0, and 1.1 mL); (2) incubation temperature (33, 35, and 37°C); and (3) colony count procedure [whole plate (1 × 1 cm count × 20) or grid count (0.5 × 0.5 cm count × 80)]. None of these variations had any significant effect on the final colony counts.

(21) *SinglePath Campylobacter Assay for the Detection of Campylobacter in Raw Ground Chicken, Raw Ground Turkey, and Pasteurized Whole Milk*.—The principle for this system (Merck KgaA) is the same as that described previously (see 3).

For inclusivity testing, all of 50 strains of *Campylobacter* provided a positive signal at the test and control zones of the test kit. For exclusivity testing, positive signals were obtained in the control zone for all cultures. One strain of *S. aureus*, however, cross-reacted when grown in a nonselective medium. When grown in selective Bolton broth, this strain did not produce a cross-reaction.

In the internal study, the performance of the alternative method was compared with that of the ISO reference culture method (8) for the detection of *C. jejuni* in chicken and *C. coli* in milk. On the basis of a Chi square analysis, there was no significant difference in the recovery of *Campylobacter* species by the 2 methods. Similarly, in the independent study, there was no significant difference in the 2 methods in their ability to detect *C. jejuni* in turkey.

For ruggedness testing, the effects of variations in 3 method parameters were investigated on 2 different days:

(1) test portion time in boiling water bath (0, 15, and 25 min); (2) device temperature (10, 20, and 30°C); and (3) time for observation of test portion result (20 ± 5 min). For day 1, 2 positive cultures (*C. jejuni* and *C. coli*) were cultured from cryobeads to Bolton broth, enriched microaerobically at 37°C for 4 h, and then at 41.5°C for 44 h. At 48 h, the 2 strains were subcultured to Bolton broth, and enriched as described previously. On day 1, the positive strains were diluted to 10⁶ CFU/mL in Bolton broth. This dilution was used to analyze all 3 method parameters. Remaining undiluted stock was stored at 4°C for use on day 2.

For day 1, the negative culture (*E. coli*) was cultured from cryobeads to Bolton broth and enriched at 37°C for 24 h. The negative strain was used for analysis undiluted in Bolton broth. Remaining undiluted stock was stored at 4°C for use on day 2.

For day 2, the stored 4°C stock of the 2 positive strains was diluted to 10⁶ CFU/mL in Bolton broth for analysis. The stored 4°C of the negative strain was used undiluted for analysis.

With respect to variation in test portion time in boiling bath, the test kit device detected both positive strains at all 3 boiling times on days 1 and 2. On day 1, at 20 min, the unboiled negative *E. coli* strain gave a ± result for 5 replicates with the test kit device. The *E. coli* strain was negative after boiling for 15 min and 25 min. On day 2, the negative *E. coli* strain was negative with the test kit device at all 3 boiling times.

With respect to variation in device temperature, on days 1 and 2, at 20 min, the test kit device detected both positive strains at all 3 device temperatures. The negative strain was negative at all 3 device temperatures.

With respect to variation in time for observation of test kit result, on days 1 and 2, the test kit device detected both positive strains when read at all 3 times. The negative strain was negative with the test kit device when read at all 3 times.

(22) *Compact Dry YM Method for the Enumeration of Yeasts and Molds in Fruit and Fruit Products*.—The principle of this method (Nissui Pharmaceutical Co.) is the same as described previously (see 19), except that the chromogenic substrate is 5-bromo-4-chloro-3-indoxyl phosphate, *p*-toluidine salt, which is turned blue by yeast and mold colonies.

For inclusivity testing, 61 of 61 (100%) of target strains produced a positive result, and for exclusivity testing, 65 of 69 (95.6%) nontarget strains produced a negative result.

This method was validated in the internal laboratory with 4 matrixes (fresh apples, frozen blueberries, dried banana chips, and orange juice) and in the independent laboratory with fresh grapefruit. A correlation coefficient of 0.98 was observed between the alternative method and the BAM reference method (2).

Ruggedness testing involved an investigation of the effect of variation of 3 factors on the performance of the method: (1) test portion volume (0.9, 1.0, and 1.1 mL); (2) incubation temperature (20, 22, and 25°C); and (3) colony count procedure [whole plate (1 × 1 cm × 20) and grid count (0.5 ×

0.5 cm × 80)]. The performance of the method was not significantly affected by a variation in any of these factors.

(23) *bioMérieux VIDAS Assay for the Detection of Staphylococcal Enterotoxin II (SET2) in a Variety of Foods*.—The bioMérieux VIDAS Staphylococcal Enterotoxin II (SET2) assay (bioMérieux, Inc.) is an ELFA test used with the automated VIDAS or mini-VIDAS instruments, which have the same mode of action, and uses the bioMérieux VIDAS Assay for the detection of *E. coli* O157 in ground beef (see 17). This EFLA assay is reactive to staphylococcal enterotoxins (SETs) A, B, C (C1, C2, C3), D, and E in the extracts of a variety of foods.

No inclusivity or exclusivity testing was performed with this assay because the target analyte is a toxin rather than a microorganism.

For the internal validation study, 15 foods were tested with the method (frozen lasagna, chocolate éclair, canned mushrooms [post-retort spiking], powdered eggs, roast beef, cooked chicken, ham, unpasteurized milk, cheddar cheese, yogurt, Italian salami, smoked salmon, potato salad, ice cream, and non-instant nonfat dry milk). Foods were spiked with individual SETs at the 2.00, 1.00, and 0.25 ng/g levels. Each toxin serotype was tested in 3 of the 15 foods, so that each of the serotypes would be equally represented in the study; C1, C2, and C3 SETs were counted as a single serotype, because they are all equally reactive with serogroup C antisera. Portions (25 g each) were individually spiked with the appropriate amount of diluted toxin, and the test portions were extracted after spiking. Five replicate test portions, at each level, were examined with the alternative method. There were 5 unspiked controls per food. All 5 replicate test portions gave positive results with the assay for each of the foods, except ice cream without trichloroacetic acid (TCA), at the 0.25 ng level. When TCA was added to the ice cream extract, all 5 test portions were positive at the 0.25 ng level. It was recommended that TCA be added to the ice cream extracts. The independent laboratory tested smoked salmon spiked with SET B at 2.00, 1.00, and 0.25 ng/g. The assay detected SET B in all 5 of the replicate test portions at the lowest level of detection (0.25 ng/g level). The percentage of spiked test portions detected by both the internal and independent laboratories was 100%. None of the unspiked test portions gave a positive result with the assay for a specificity rate of 100%.

For ruggedness testing, the following 3 parameters were examined: (1) test portion volumes (300, 400, 450, 500, 550, and 600 µL) of SET A (0.2 ng/mL); SET B (0.075 ng/mL); SET C (0.2 ng/mL); SET D (0.25 ng/mL); and SET E (0.25 ng/mL) were added to the test strips and the assay was initiated; (2) test kits/reagents were allowed to equilibrate for 0, 10, 20, 30, 120, and 240 min before initiation of analysis; and (3) the final pH of 3 different food extracts (cocoa, canned mushrooms, and Brie), with SET levels as low as 0.5 ng/g, were adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5 before being tested with the assay. Deviations in sample volume and test kit

equilibration time did not significantly affect the performance of the method. When the pH was varied between 6.0 and 8.5 for each of the 3 sample types, the test values showed a plateau in the range of pH 7.0 to 8.5. If the pH is <7.0, then false-negative results may occur. Consequently, it was recommended that the final pH of the extract fall between 7.5 and 8.0.

(24) *Duopath Verotoxin Assay for the Detection of Verotoxins 1 and 2 from Verotoxigenic Escherichia coli Isolated from Food Enrichments*.—This assay (Merck KgaA) is an immunochromatographic rapid test based on gold-labeled antibodies. The test device has a circular sample port and an oval-shaped test (VT1 and VT2) and control (C) window. After an appropriate enrichment, the test portion broth is applied to the nitrocellulose membrane via the circular sample port. The test portion is absorbed through the pad to the reaction zone containing colloidal gold-labeled antibodies specific to Verotoxins 1 and 2. Any verotoxin antigen present complexes with the gold-labeled antibody and migrates over the membrane until it encounters the binding zones in the test (VT1 and VT2) area. The binding zones (VT1 and VT2) contain another anti-VT1 or VT2 antibody, which immobilizes any verotoxin-antibody complex present. At the control zone, an antibody directed against the gold-labeled antibody, is immobilized onto the membrane. Regardless of whether any verotoxin is present, this distinct red line is always formed in the control zone, thus ensuring that the test is working properly. When VT1 and/or VT2 is/are present in the test portion, the formation of one or 2 distinct red lines is observed in the test zone. Only one red line (control zone) is observed when no verotoxin is present or the verotoxin level is below the detectable level.

For inclusivity testing in the internal laboratory, all 55 verotoxin-producing *E. coli* O157 and non-O157 isolates gave a positive signal in the test and control zones of the test kit. For exclusivity testing in the internal laboratory, 30 bacterial species belonging to other members of the *Enterobacteriaceae*, as well as other Gram-negative and Gram-positive bacteria, were analyzed for cross-reactivity with the alternative method. All strains provided a signal in the control zone but no signal in the test zone.

For the independent laboratory validation, inclusivity testing included 10 verotoxin-producing *E. coli* O157 and non-O157 isolates. Exclusivity testing included 5 species belonging to other members of the *Enterobacteriaceae*, as well as other Gram-negative and Gram-positive bacteria. All target analytes were reactive with the test kit, whereas nontarget analytes were nonreactive.

With respect to ruggedness testing, the effect of 3 variations on the performance of the test kit was studied: (1) lapse of time of beginning the test after opening the pouch (2 and 2.5 h); (2) temperature of conducting test (18, 22, and 25°C); and (3) lapse of time for reading results (15, 20, and

25 min). None of these variations had any effect on the performance of the test kit.

(25) *Roche/BIOTECON Diagnostics LightCycler-Listeria monocytogenes Detection Kit for Listeria monocytogenes in Combination with the ShortPrep Foodproof II Kit for the Detection of L. monocytogenes in a Variety of Foods*.—This method is based on a real-time PCR. The method has been designed to reduce the time necessary to achieve results from PCR reactions and to enable the user to monitor the amplification of the PCR product simultaneously in real time. After DNA isolation, using the Roche/BIOTECON ShortPrep Foodproof II Kit (BIOTECON, Potsdam, Germany) which is designed for the rapid preparation of *L. monocytogenes* DNA for direct use in PCR, the real-time detection of *L. monocytogenes* DNA is performed with the Roche/BIOTECON LightCycler-*L. monocytogenes* detection kit.

For inclusivity testing, 50 *L. monocytogenes* isolates were tested with the alternative method. All 50 isolates gave positive results with the alternative method for a sensitivity rate of 100%. For exclusivity testing, 30 non-*L. monocytogenes* organisms were tested with the alternative method. All 30 isolates gave negative results with the alternative method for a specificity rate of 100%.

For the in-house validation study, 20 different foods were tested with the alternative method and with either the USDA (1) or the BAM (2) reference method. The foods that were tested in this study were peanut butter, dried whole eggs, dry whole milk, dry pet food, milk chocolate, melon cubes, white cabbage, pizza, vanilla ice cream, paprika emulsion dye, spaghetti, sausage, gravlax, raw ground chicken, raw ground pork, bean sprouts, parsley flakes, ham, and “Harzer” cheese. The alternate method was equivalent to the reference method on the basis of Chi square results. For the independent laboratory validation, frankfurters were tested with the alternative method and with the BAM reference method. The 2 methods did not differ significantly based on Chi square results.

The ruggedness of the ShortPrep foodproof II kit was measured by varying the concentration of the lysis buffer for the 2 lysis components and by varying the mechanical cell disruption devices (Disruptor Genie vs Mixer Mill). Neither of these deviations significantly impaired the performance of the kit. The ruggedness of the Lightcycler *L. monocytogenes* detection kit was measured by: (1) using 2 different testers with the same lightcycler apparatus; (2) testing the kit with 2 slightly different lightcycler apparatuses; and (3) varying the test portion volumes of 5 different food matrixes. Deviations of these 3 parameters did not impair the performance of the kit.

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