

METHODS COMMITTEE REPORTS

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Food Microbiology—Non-Dairy, Wallace H. Andrews

(1) **998.08 Enumeration of *Escherichia coli* in Poultry, Meat, and Seafood Products, Dry Rehydratable Film Method (PetriFilm *E. coli*/Coliform Count Plate Method):** Study Directors Sonya A. Gambrel-Lenarz, 3M Microbiology Products, 3M Center, Bldg 260-6B-01, St. Paul, MN 55144-1000, Tel: +1-651-733-0913, Fax: +1-651-733-1804, E-mail: SAGambrel-Lenarz1@mmm.com and Michael S. Curiale, Silliker Laboratories Group, Inc., 160 W. Armory Dr, South Holland, IL 60473, Tel: +1-708-225-1435, Fax:

+1-708-225-1536, E-mail: michael.curiale@silliker.com. Continue study.

(2) **996.08 *Salmonella* in Foods, VIDAS SLM Method:** Study Directors Wendy A. Lepper, Silliker Laboratories Group, 160 Armory Dr, South Holland, IL 60473, Tel: +1-708-225-1435, Fax: +1-708-225-1536, E-mail: wendy.lepper@silliker.com and Ronald L. Johnson, bioMerieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042-2320, Tel: +1-314-506-8182, Fax: +1-314-506-8182, E-mail: ron_johnson@na.biomerieux.com. A precollaborative study is being conducted to obtain approval of the VIDAS SLM method utilizing the selective enrichment tetrathionate broth and Rappaport-Vassiliadis medium (in place of the currently recommended selenite cystine broth). A collaborative study protocol has been submitted for review. Continue study.

(3) **997.16 LOCATE-ELISA Immunoassay for Identification of *Salmonella* in Foods:** Study Director Michael S. Curiale (see 1). Method is recommended for Final Action. Continue study.

(4) **Detection of Botulinum Toxins A, B, E, and F from Culture Supernatants, Amplified ELISA Procedure:** Study Directors Joseph L. Ferreira, U.S. Food and Drug Administration, 60 8th St, Atlanta, GA 30309, Tel: +1-404-253-2216, Fax: +1-404-253-1210, E-mail: jferreir@ora.fda.gov; Susan Maslanka, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA, Tel: +1-404-639-0895, Fax: +1-404-639-3333, E-mail: sht5@cdc.gov; Eric Johnson, University of Wisconsin, 1925 Willow Dr, Madison, WI 53706, Tel: +1-608-263-6949, Fax: +1-608-263-1114, E-mail: eajohnso@facstaff.wisc.edu. Associate Referee Michael Goodnough, University of Wisconsin, 1925 Willow Dr, Madison, WI 53706, Tel: +1-608-263-6949, Fax: +1-608-263-1114, E-mail: mgoodnou@facstaff.wisc.edu. On the basis of a completed collaborative study, the Study Directors have recommended the adoption of this assay as a First Action method, and the General Referee concurs. Continue study.

(5) **996.09 *Escherichia coli* O157:H7, Visual Immuno-precipitate Assay:** Study Director Philip T. Feldsine, Biocontrol Systems, Inc., 12822 SE 32nd St, Bellevue, WA 98005, Tel: +1-425-603-1123, Fax: +1-425-603-0070, E-mail: ptf@biocontrolsys.com. This assay was adopted First Action in 1996 and Final Action in 1998. A method applicability statement modification was submitted to revise the enrichment protocol for raw and cooked beef products only so as to allow for an 8 h enrichment. This modification was approved following the completion of a collaborative study, and the method was adopted Revised First Action in 2002. Continue study.

(6) **999.09 VIP for the Detection of Motile and Non-Motile *Salmonella* in Foods:** Study Director Philip T. Feldsine (see 5). This assay was adopted Final Action in 2001. Continue study.

(7) *ISO Versus AOAC Reference Culture Methods for the Detection of Motile and Non-Motile Salmonella in Selected Foods*: Study Director Philip T. Feldsine (see 5). The Study Director has been requested to repeat a segment of the study, and the results are pending. Continue study.

(8) **996.14** *Listeria monocytogenes and Related Listeria Species Detection in Selected Foods, Assurance Polyclonal Enzyme Immunoassay Method*: Study Director Philip T. Feldsine (see 5). This method was adopted Final Action in 1998. A method applicability modification to include the monitoring of environmental surfaces was validated and approved in 2001. Continue study.

(9) *Probelia PCR Method for Salmonella*: Study Director Philip T. Feldsine (see 5). A precollaborative study manuscript has been prepared by the Study Director and submitted for review. The Study Director has decided to place this study on inactive status. However, the topic itself should be continued.

(10) **999.08** *Assurance Gold Salmonella EIA for the Visual or Instrumental Detection of Motile and Non-Motile Salmonella in Foods*: Study Director Philip T. Feldsine (see 5). The method was adopted Final Action in 2001. Continue study.

(11) **996.10** *MOD 9/21/00-AEI (sic) for the Analysis of Ground Beef for Escherichia coli O157:H7*: Study Director Philip T. Feldsine (see 5). **996.10** was originally adopted as a First Action method for the analysis of selected foods in 1996 and Final Action in 1998. A method applicability modification was submitted to revise the enrichment protocol for raw and cooked beef products only to allow for an 8 h enrichment. This modification was approved following a collaborative study, and the method was adopted Revised First Action in 2002. The General Referee recommends that the title for this topic be changed to "*Escherichia coli* O157:H7, Assurance Polyclonal Enzyme Immunoassay." Continue study.

(12) **992.11** *MOD 12/00 Salmonella in Foods, Assurance Enzyme Immunoassay*: Study Director Philip T. Feldsine (see 5). **992.11** was originally adopted First Action in 1992 and Final Action in 1996. This method was adopted Revised First Action in 1999 following a change in reagent format. The Revised method has been widely used since that time with favorable results and it is now recommended for Final Action. A proposed method modification protocol to examine alternative enrichments is awaiting Committee input regarding the level of validation required to support an enrichment protocol modification. Continue study.

(13) *IDEXX SimPlate for Coliforms and Escherichia coli*: Study Director Philip T. Feldsine (see 5). An inclusivity study has been completed and a methods comparison study is in progress. The General Referee recommends that the title of this topic be changed to "SimPlate Cec Quantitative Method for Total Coliforms and *Escherichia coli* in Foods." Continue study.

(14) **997.03** *Listeria monocytogenes and Related Listeria species in Selected Foods, Visual Immunoprecipitate Assay*: Study Director Philip Feldsine (see 5). This method was adopted Final Action in 1999. A method applicability modification to include the monitoring of environmental surfaces was validated and approved in 2001. Continue study.

(15) **991.38** *Salmonella in Foods, Automated Conductance Method*: Study Director Donald M. Gibson, BIODON International, 43 Brighton Pl, Aberdeen AB10-6RT, United Kingdom, Tel: +44-1224-322777, Fax: +44-1224-322777, E-mail: dm Gibson@sol.co.uk. This method was adopted Final Action in 1996. Discontinue topic.

(16) **997.11** *Escherichia coli O157:H7 Counts in Foods, Hydrophobic Grid Membrane Filter (ISO-GRID) Method Using SD-39 Agar and Serological Confirmation*: Topic is vacant, and it is recommended that it be discontinued.

(17) **2000.06** *Detection of Salmonella in Foods with a Low Microbial Load, Rappaport-Vassiliadis Medium Method*: Study Director Thomas S. Hammack, U.S. Food and Drug Administration, 5100 Paint Branch Pkwy, College Park, MD 20740-3835, Tel: +1-301-436-2010, Fax +1-301-436-2644, E-mail: thomas.hammack@cfsan.fda.gov. Continue study.

(18) *Salmonella in Foods, Reveal for Salmonella Test System*: Study Director Mark Mozola, Neogen Corp., 620 Leshar Pl, Lansing, MI 48912, Tel: +1-517-372-9200, Fax: +1-517-372-0108, E-mail: mmozola@neogen.com. A precollaborative study has been completed and is being reviewed by the General Referee. Continue study.

(19) **2000.14** *Escherichia coli O157:H7 in Foods, 20-Hour REVEAL Screening Test*: Study Director Mark Mozola (see 18). Continue study.

(20) **2000.13** *Escherichia coli O157:H7 in Foods, 8-Hour REVEAL Screening Test*: Study Director Mark Mozola (see 18). Continue study.

(21) *Salmonella in Foods, Alert for Salmonella Test System*: Study Director Mark Mozola (see 18). Continue study.

(22) *Rapid Presence/Absence Screen for Listeria monocytogenes in Foods Using HGMF with LM-137 Agar*: Topic is vacant, and it is recommended that it be discontinued.

(23) *Twenty-Four Hour Presumptive Enumeration of Listeria monocytogenes Using HGMF Procedure with LM-137 Agar*: Topic is vacant, and it is recommended that it be discontinued.

(24) *Twenty-Four Hour Rapid Presence/Absence Screen for Salmonella in Foods Using HGMF*: Topic is vacant, and it is recommended that it be discontinued.

(25) **997.02** *Yeast and Mold Counts in Foods, Dry Rehydratable Film Method*: Study Director Sonya A. Gambrel-Lenarz (see 1). This method was adopted Final Action in 2000. Discontinue topic.

(26) *Clostridium botulinum Toxins A, Proteolytic A, B, and E, ELCA Enzyme Immunoassay*: Study Director Wendy Lepper (see 2). A precollaborative study report has been approved by the Methods Committee, and a collaborative study protocol is under review by the Committee. Continue study.

(27) **2001.07** *Salmonella in Selected Foods by Immuno-Concentration Salmonella (ICS) and Selective Plate (HE, BS, and SMID) Procedure*: Study Directors Wendy Lepper and Ronald Johnson (see 2). Precollaborative and collaborative studies have been completed. This method has been adopted First Action for selected foods. Studies are underway to extend the method applicability to all food matrixes. Continue study.

(28) **2001.08** *Salmonella in Selected Foods by Immuno-Concentration Salmonella (ICS) and Selective Plate*

(HE, XLD, BS) Procedure: Study Directors Wendy Lepper and Ronald Johnson (*see* 2). Precollaborative and collaborative studies have been completed. This method has been adopted First Action for selected foods. Studies are underway to extend the method applicability to all food matrixes. Continue study.

(29) **2001.09** *Salmonella in Selected Foods by Immuno-Concentration Salmonella (ICS) and Enzyme-Linked Immunofluorescent Assay (ELFA)*: Study Directors Wendy Lepper and Ronald Johnson (*see* 2). This method has been adopted First Action for selected foods. Studies are underway to extend the method applicability to all food matrixes. Continue study.

(30) **990.13** *Salmonella in Foods, Colorimetric Deoxyribonucleic Acid Hybridization Method (GENE-TRAK)*: Study Director Mark Mozola (*see* 18). A precollaborative study was conducted to validate alternative enrichment protocols for use with Final Action Method **990.13**, and the GENE-TRAK Salmonella DLP assay (AOAC Research Institute Performance Tested Method No. 961101), both dipstick-format DNA hybridization assays. The alternative enrichment protocols utilize the combination of tetrathionate broth and Rappaport-Vassiliadis medium for selective enrichment rather than the combination of tetrathionate broth and selenite cystine broth. Although results were favorable, study on this topic is being discontinued in favor of further development and validation of a new microwell-format assay, which already employs the combination of tetrathionate broth and Rappaport-Vassiliadis medium for selective enrichment. This method was adopted Final Action in 1996. Discontinue topic.

(31) *Detection of Salmonella, GENE-TRAK Systems*: Study Director Mark Mozola (*see* 18). This topic is too broad and, as such, should be discontinued. When new validation studies are initiated, more specific topic titles can be applied.

(32) *Determination of Escherichia coli in Flesh Foods Using Visual Immunoassay with a Modified Culture Procedure*: Study Director Denise Hughes, TECRA International Pty Ltd, 13 Rodborough Rd, Frenchs Forest, NSW, 2086, Australia, Tel: +61-2-8977-3000, Fax: +61-2-9453-3422, E-mail: denise.hughes@tecra.net. A collaborative study protocol has been approved by the Methods Committee. Continue study.

(33) **995.22** *Listeria in Foods, Colorimetric Polyclonal Enzyme Immunoassay Screening Method (TECRA Listeria Visual Immunoassay [TLVIA])*: Study Director Denise Hughes (*see* 32). This method was adopted First Action in 1995 and Final Action in 1998. A new enrichment procedure, not containing the highly toxic antifungal agent, cycloheximide, was subsequently validated for use with this method. Details of these precollaborative and collaborative validation studies were included in last year's General Referee Report (8). The modified enrichment methods are recommended for First Action approval. It is intended that the original Final Action method **995.22** be retained and that the new enrichment methods be designated by a separate method number, with applicability for raw meats, fresh produce/vegetables, processed meats, seafoods, dairy cultured/non-cultured products, and fruit/fruit juices. Continue study.

(34) **995.22 MOD 2/6/01** *Listeria in Foods, Colorimetric Polyclonal Enzyme Immunoassay Screening Method (TECRA Listeria Visual Immunoassay [TLVIA]) for Environmental Surfaces*: Study Director Denise Hughes (*see* 32). Precollaborative and collaborative studies are planned to extend the applicability of **995.22** to the analysis of environmental surfaces. The precollaborative study manuscript has been submitted for approval, and a collaborative study protocol is in review. Continue study.

(35) **2000.07** *Salmonella in Foods, Rapid Colorimetric TECRA Unique Salmonella Test*: Study Director Denise Hughes (*see* 32). The Study Director reports that this method has gained wide acceptance in the food industry. This method was independently validated by Campden and Chorleywood Food Research Association, United Kingdom, and recently received approval according to the European Microbiological Assessment Scheme. This First Action method is now recommended for Final Action status. Continue study.

(36) **2000.07 MOD (2-15-01)** *TECRA Unique Salmonella Test*: Study Director Denise Hughes (*see* 32). TECRA is planning to modify the enrichment methods and the module format. The Committee has approved a precollaborative study protocol to validate changes in the enrichment protocols, as well as to allow manual and automated reading of results. Continue study.

(37) **2000.07 MOD (1/24/01)** *Salmonella in Foods (Juice) by TECRA Unique Salmonella Test*: Study Director Denise Hughes (*see* 32). An in-house validation study to validate a minor modification of **2000.07** has been approved by the Methods Committee. This modification involves incubation of the Unique test module at 42°C instead of the current module incubation at 37°C. The study showed complete agreement between the modified method and Method **2000.07**. This minor modification is recommended for First Action approval as an alternative method to the current Method **2000.07** for juice. Continue study.

(38) *Listeria in Selected Foods by TECRA Unique 2000 Listeria Method*: Study Director Denise Hughes (*see* 32). Precollaborative and collaborative study protocols have been approved by the Methods Committee. Continue study.

(39) *Staphylococcus aureus in Foods, TECRA S. aureus Visual Immunoassay*: Study Director Denise Hughes (*see* 32). The Study Director has requested: (a) the use of a 3 g sample with the TECRA method and (b) the use of surface plating Method **975.55** rather than **987.09** as the reference culture method. A collaborative study protocol has been approved by the Methods Committee, and the precollaborative study protocol is awaiting approval by the Methods Committee. Continue study.

(40) **993.10** *Clostridium perfringens, Iron Milk Test for Recovery from the Marine Environment*: Study Director Carlos Abeyta, Jr, U.S. Food and Drug Administration, 22201 23rd Dr, SE, Bothell, WA 98021-4421, Tel: +1-425-483-4890, Fax: +1-425-483-4996, E-mail: cabeyta@ora.fda.gov. This method was adopted Final Action in 1999. Discontinue topic.

(41) **2000.15** *Coliform Counts in Foods, Dry Rehydratable Film Method*: Study Director Karen Silbernagel, tech Labora-

ories, MS 0075, PO Box 64101, St. Paul, MN 55164-0101, Tel: +1-651-766-1303, Fax: +1-651-486-0837, E-mail: ksib@landolakes.com. Continue study.

(42) **2001.05** *Rapid Enumeration of Staphylococcus aureus in Selected Foods, Dry Rehydratable Film Method*: Study Director Karen Silbernagel (see 41). Continue study.

(43) *Enumeration of Enterobacteriaceae in Foods, Dry Rehydratable Film Method*: Study Director Karen Silbernagel (see 41). A collaborative study has been conducted, and the Study Director recommends that the method be adopted First Action. The General Referee concurs. Continue study.

(44) *Evaluation of BAX for the Detection of Listeria monocytogenes in Foods*: Study Director Karen Silbernagel (see 41). A precollaborative study manuscript has been prepared and is being revised. The collaborative study protocol is being reviewed by the Methods Committee. Continue study.

(45) *Detection of Listeria in Foods Using ALOA Medium*: Study Director Sebastien Manuel, AES-Chemunex, Inc., 301 N. Harrison St, Suite 109, Princeton, NJ 08540, Tel: +1-450-689-8754, Fax: +1-450-689-8754, E-mail: s.manuel@aeschemunex.com. Continue study.

(46) *Determination of Microbial Load on Stainless Steel Surfaces Using Hygicult TPC Dipslide*: Study Director Gun L. Wirtanen, VTT Biotechnology and Food Research, PO Box 1500 (Tietotie 2), Espoo, FIN-02044, Finland, Tel: +358-9-456-5222, Fax: +358-9-455-2103, E-mail: gun.wirtanen@vtt.fi. A collaborative study has been conducted, and the Study Director recommends that the method be adopted First Action. The General Referee has recommended revisions in the manuscript. Continue study.

The following topics contain methods that are currently in the validation process and need to be added to the official roster of Study Director topics:

(47) *Detection of Listeria monocytogenes in Foods, VIDAS Listeria monocytogenes II (LMO2) Immunoassay Method*: This topic represents a renaming of the current topic, *VIDAS Listeria monocytogenes (LMO) Immunoassay Method for Detection of Listeria monocytogenes in Foods*. Study Director Karen Silbernagel (see 41). Continue study.

(48) *Enumeration of Total Aerobic Microorganisms in Foods, SimPlate Total Plate Count Color Indicator Method*:

Study Director Philip T. Feldsine (see 5). A precollaborative study has been approved by the Methods Committee. Moreover, a collaborative study has been approved by the Methods Committee and is awaiting review by the Official Methods Board. It is recommended that the SimPlate 35 method be adopted First Action. Continue study.

(49) *Enumeration of Yeasts and Molds in Foods, SimPlate Yeast and Mold Color Indicator (Y & M-CI) Method*: Study Director Philip T. Feldsine (see 5). A precollaborative study manuscript has been submitted for review. Continue study.

(50) *Determination of Actionable Levels ($>10^4$ organisms/g) of Escherichia coli with Two Membrane Filtration Methods* (this topic is now referred to as *Improved Analysis of Food Samples for Total Escherichia coli Populations to Determine Whether 10^4 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: +1-425-402-4421, E-mail: mgrant@ora.fda.gov. A protocol for a precollaborative study has been approved by the Methods Committee. Continue study.

References

- (1) *Compendium of Methods for the Microbiological Examination of Foods* (2001) 4th Ed., F.P. Downes and K. Ito (Eds), American Public Health Association, Washington, DC
- (2) Salo, S., Storgards, E., & Wirtanen, G. (1999) 30th *Nordic Contamination Control Symposium*, VTT Symposium 193, G. Wirtanen, S. Salo, & A. Mikkola (Eds), Libella Painopalvelu Oy, Espoo, Finland
- (3) *Official Methods of Analysis* (2000) 17th Ed. and suppl., AOAC INTERNATIONAL, Gaithersburg, MD
- (4) Ferreira, J.L. (2000) *J. AOAC Int.* **84**, 85–88
- (5) Potter, M.D., Meng, J., & Kimsey, P. (1993) *J. Food Prot.* **56**, 856–861
- (6) *Standard Methods for the Examination of Dairy Products* (1992) 16th Ed., R.T. Marshall (Ed.), American Public Health Association, Washington, DC
- (7) ISO Standard No. 7402—General guidance for the enumeration of *Enterobacteriaceae* without resuscitation—MPN technique and colony count technique (1991) International Organization for Standardization, Geneva, Switzerland
- (8) Hammack, T., & Andrews, W.H. (2002) *J. AOAC Int.* **85**, 262–269