

## Committee on Microbiology and Extraneous Materials

### Food Microbiology—Non-Dairy

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#### Collaborative Studies

##### *Determination of Microbial Load on Stainless Steel Surfaces Using Hygicult TPC Dipslide*

Study Director Gun L. Wirtanen (*see Recommendations*, 46). Sampling of environmental surfaces is of the utmost importance, since these surface organisms can readily be transferred to the food being processed. There are several sources of these surface organisms: workers' hands, the raw materials themselves, insects, poor sanitation practices, and niches in the equipment, allowing the growth of a variety of organisms (1). These organisms can proliferate to the extent that a biofilm is formed, especially on irregular surfaces which are difficult to clean. Moreover, these biofilms may contain pathogenic, as well as nonpathogenic, microorganisms. The sampling of environmental surfaces can be difficult because microorganisms contained in these biofilms can adhere strongly to the environmental surfaces. If these organisms are removed forcefully, they may be damaged to the extent that they would not be detectable by methods based on multiplication of microorganisms in agar.

Environmental surfaces are normally sampled by the Replicate Organism Direct Agar Contact (RODAC) plate method (1) or by swabbing. The principle of the Hygicult TPC dipslide (Orion Diagnostica, Finland) method is similar to that of the RODAC method in that both methods allow the surface of a growth medium to come in direct contact with the environmental surface to be sampled. With the swabbing method, however, it is necessary to transfer the organisms from the swab to the cultivation medium. These contact methods are also based on the gentle detachment of the surface-bound organisms, a limiting factor with the swab method (2).

A collaborative study was conducted to validate the Hygicult TPC dipslide method for enumerating total aerobic microflora on surfaces artificially contaminated with bacterial mixtures at 3 levels. The dipslide was compared with the contact plate method and the swabbing method for surface sampling. Twelve laboratories participated in the study. The total number of collaborative samples was 108. The microbial level in each sample was determined in triplicate with each of these 3 methods at each of 3 different incubation conditions (at  $25 \pm 1^\circ\text{C}$  for 48 and 72 h, as well as  $30 \pm 1^\circ\text{C}$  for 48 h). Surface sampling methods detached 25–30% at the lowest (theoretical yield  $1.4 \text{ cfu/cm}^2$ ), 18–20% at the middle (theoretical

yield  $10.7 \text{ cfu/cm}^2$ ), and 16–21% at the highest (theoretical yield  $43.6 \text{ cfu/cm}^2$ ) levels of bacteria from the stainless steel surfaces. The percentage of acceptable results after removing outliers was 89%. Repeatability standard deviations,  $\text{RSD}_r$ , ranged from 27.2–74.6%, and reproducibility standard deviations,  $\text{RSD}_R$ , ranged from 42.1–97.5%. There were no significant differences obtained either in incubation temperatures (25 and  $30^\circ\text{C}$ ) or incubation times (48 and 72 h) in all 3 methods. The effect of incubation temperature and time on precision parameters was based on an examination of the estimated precision parameters. The 3 methods gave similar results for all 3 levels tested:  $0.35\text{--}0.43 \text{ cfu/cm}^2$  at the lowest level,  $1.9\text{--}2.2 \text{ cfu/cm}^2$  at the middle level, and  $7.1\text{--}9.1 \text{ cfu/cm}^2$  at the highest level.

On the basis of these results, the Study Director has recommended that this method be adopted First Action. The General Referee has recommended that the collaborative study manuscript be revised.

##### *Detection of Botulinum Toxins A, B, E, and F from Culture Supernatants, Amplified ELISA Procedure*

Study Directors Joseph L. Ferreira, Susan Maslanka, Eric Johnson, and Michael Goodnough (*see Recommendations*, 4). The mouse bioassay method, **977.26**, is currently the only AOACI-approved method for the detection of botulinum toxins (3). Although it is a highly sensitive method, it is limited in that it requires up to 6 days to obtain final results. Moreover, this method is limited to only those laboratories that have mice available for the test. An alternative in vitro method would facilitate botulism investigations by increasing the number of laboratories capable of testing for the presence of the botulinum toxin.

A sandwich-type enzyme-linked immunosorbent assay (ELISA) has been developed for the indirect detection of types A, B, E, and F botulinum toxins. Polyclonal antibodies to the 4 toxin types are used in the amplified (amp)-ELISA procedure. Each toxin type-specific IgG is coated onto microtiter plates and used as a capture antibody for each homologous toxin. Following the addition of the culture supernatants, each of the specific IgGs is used as a secondary antibody (biotinylated form) that binds to captured toxin. The biotin residues on the secondary IgG are then detected using streptavidin conjugated to alkaline phosphatase and an amplified substrate (4, 5). Positive and negative controls are used to monitor color development. This method uses tryptone-peptone-glucose-yeast extract (TPGY) and cooked meat medium (CMM), the 2 growth media recommended in **977.26**.

A total of 11 laboratories participated in a collaborative study comparing the amp-ELISA procedure with Method **977.26** for the detection of botulinum toxins in culture supernatants. Results from one of the laboratories was not used due to high background and erratic absorbance values. Supernatants from nonbotulinum clostridia were also included in the study.

The toxicity of each botulinal culture was determined in the Study Directors' laboratories by Method 977.26, and the cultures were diluted to high (approximately 10 000 minimal lethal dose units [MLD]/mL) and low (approximately 100 MLD/mL) levels in the test samples. The overall sensitivity of the amp-ELISA procedure was 97.8% for all test samples with >100 MLD/mL toxicity. The detection sensitivity for high toxin samples by type of growth medium was 98.8% in TPGY and 99.4% in CMM. The false-positive rate ranged from 1.5% for Type A to 19.7% for Type F. Most of the cross-reactivity was due to detection of other botulinal types, especially in the high-toxin samples.

On the basis of these results, the Study Directors have recommended that this method be adopted First Action, and the General Referee concurs.

#### **Enumeration of Total Aerobic Microorganisms in Foods, SimPlate Total Plate Count Color Indicator Method**

Study Director Philip T. Feldsine (*see Recommendations*, 48). Current methods of the AOACI and of the International Organization for Standardization (ISO) for the enumeration of total aerobic microorganisms involve the use of pour plates that are incubated for 48 h (AOACI) to 72 h (ISO) before they are examined and counted. The SimPlate Total Plate Count-Color Indicator (TPC-CI, BioControl Systems, Inc., Bellevue, WA) system uses binary detection technology (BDT) to enumerate these same organisms in foods in only 24–28 h of incubation. In the SimPlate multiple test format, prepared food test portions are placed onto the center of the test device followed by the addition of the TPC-CI liquid medium. In the single test format, a premixed test portion/medium homogenate is dispensed into the test device. The test portion/medium homogenate is distributed into a fixed number of individual incubating wells.

With this test system, foodborne microorganisms are suspended in a nutritionally-defined growth medium. Discrete aliquots are compartmentalized in the incubating wells where the biochemical activities of the microorganisms are monitored in the liquid medium. A fewer number of organisms are required to produce a detectable signal in the test device well than the number of organisms required to produce a visible colony with the AOACI and ISO pour plate procedures. Each well is either positive or negative. Any color change in the well is interpreted as a positive reaction. Enumeration is done by counting the number of wells giving a positive reaction. The final count is derived from the test device conversion table that is based on the Poisson Distribution.

A collaborative study was performed in which 4 methods for enumerating total aerobic microorganisms in foods were compared: (a) AOAC Method 996.23 using pour plates incubated at 35°C (AOAC 35); (b) ISO method 4883 using pour plates incubated at 30°C (ISO 30); (c) test device incubated at 30°C (SimPlate 30); and (d) test device incubated at 35°C (SimPlate 35). Nineteen laboratories participated and analyzed one or more of 6 naturally-contaminated food types used in the study. In general, there was less than a 0.3 mean log difference in recovery between the AOACI 35 method and

the SimPlate 35 method, the ISO method and the SimPlate 30 method, and the AOACI 35 method and the ISO 30 method. The repeatability ( $s_r$ ) and reproducibility ( $s_R$ ) standard deviations were similar in the 3 method comparisons mentioned above.

On the basis of this study, the Study Director recommends that the SimPlate 35 method for enumerating total aerobic microorganisms be adopted First Action, and the General Referee concurs.

#### **Enumeration of Enterobacteriaceae in Foods, Dry Rehydratable Film (Petrifilm) Method**

Study Director Karen Silbernagel (*see Recommendations*, 43). The determination of coliforms has traditionally been used by the food industry as an indicator of unsanitary conditions or inadequate processing. Coliforms, by definition, are those organisms capable of fermenting lactose in a nutritionally-defined medium under specified conditions of incubation. However, not all the members of the *Enterobacteriaceae* are capable of fermenting lactose.

A practice widely used in Europe, and one that represents a rapidly-growing trend in this country, is the enumeration of all oxidase-negative, glucose-fermenting, gram-negative rods. This description essentially defines the members of the *Enterobacteriaceae*, a broader taxonomic grouping that may encompass many organisms which do not utilize lactose, yet have possible sanitary significance.

The Petrifilm Enterobacteriaceae Count (EB) plate (3M, St. Paul, MN) is designed for the enumeration of *Enterobacteriaceae* in foods. The product consists of a medium optimized for the growth of the members of the *Enterobacteriaceae*, while also being inhibitory to the gram-positive bacteria. The plate also contains a pH indicator, a dye to enhance the visualization of growth, and a cold water-soluble gelling agent. Diluted 1.0 mL test portions of the food are added to the plate. A plastic spreader is placed on the overlay film and gentle pressure is applied to the spreader so as to spread the test portion over a growth area of approximately 20 cm<sup>2</sup>. The gelling agent is allowed to solidify. The plates are incubated for 24 h at 37°C and then counted.

A collaborative study was performed in which the test method was compared with reference methods described in *Compendium of Methods for the Microbiological Examination of Foods* (Compendium, 1) and in *Standard Methods for the Examination of Dairy Products* (SMEDP; 6). In addition, ISO Standard #7402 (7) was consulted for guidance on the enumeration of *Enterobacteriaceae*. Twelve laboratories analyzed 6 food types by the 3 methods. The mean log counts and the repeatability/reproducibility precision estimates of the Petrifilm method were similar to those for the SMEDP method and higher than those for the Compendium method.

On the basis of the results from the collaborative study, the Study Director recommends that this method be adopted First Action for the enumeration of the *Enterobacteriaceae* in 6 specific food types: pasteurized fluid milk, cheddar cheese, frozen broccoli, frozen prepared meals, nut meat pieces, and flour. The General Referee concurs.

## Recommendations

(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 Immunoprecipitate 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). Method **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). Method **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: dmigibson@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/noncultured 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 Method **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 Method **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 Laboratories, 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 Karen Jarvis, Microbiology International, 97 H Monocracy Blvd, Frederick, MD 21701, Tel: +1-301-662-6835, Fax: +1-301-662-8096, E-mail: karen.jarvis@sygene.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<sup>4</sup> 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<sup>4</sup> 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 & 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
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