

METHODS COMMITTEE REPORTS

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Committee Actions

Food Microbiology—Non-Dairy, General Referee,
Wallace H. Andrews

(1) *BAX System for Detection of Listeria monocytogenes in Foods.*—Study Director, Karen Silbernagel, rtech laboratories, MS 0075, PO Box 64101, St. Paul, MN 55164-0101, Tel: +1-651-766-1303, Fax: +1-651-486-0837, E-mail: kmsilbernagel@landolakes.com. The BAX system (DuPont Qualicon, Inc., Wilmington, DE) uses the polymerase chain reaction (PCR) to amplify a specific fragment of bacterial DNA. To perform the BAX assay, the user begins with a 2-stage enrichment of the food sample. An aliquot of the secondary enrichment is combined with a buffer containing a proteolytic enzyme, and then heated to rupture the walls of the bacterial cells and to release their DNA. The lysed mixture is used to hydrate a BAX assay tablet. The PCR tablet contains single-stranded DNA oligonucleotide primers that selectively bind to a unique sequence in *L. monocytogenes*. DNA polymerase then amplifies the DNA sequence generating a double-stranded product. This is done through repeated cycles of annealing and extending, resulting in an exponential increase in the number of target DNA fragments, creating millions of copies within 2.5 h. If the target sequence is not present, no detectable amplification takes place. A DNA-binding dye present in the tablets binds to the amplified product, resulting in an increase in test portion fluorescence. During the detection phase of the assay, the test portion is slowly heated while the fluorescence is monitored to determine the melting temperature of the amplified DNA fragment. When the melting temperature is reached, the double-stranded fragment denatures, resulting in a drop in fluorescence. The system software uses the melting temperature data to determine the presence or absence of *L. monocytogenes* in the test portion.

A collaborative study was conducted to compare the automated BAX assay and the standard culture methods (1–3) for the detection of *L. monocytogenes* in foods. Six food types (cheese, fresh radishes, frozen peas, smoked salmon, frankfurters, and raw, ground beef) were analyzed by each method. A total of 25 laboratories representing government and industry participated. In this study 2335 test portions were analyzed of which 1109 were positive by the BAX assay and 1115 were positive by the standard method. For all the foods, with the exception of radishes, the BAX assay performed as well as or better than the standard reference methods on the basis of the Chi square results.

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

(2) *Modification of Enrichment Protocols for the TECRA Listeria Visual Immunoassay Method 995.22.*—Study Director, Ian Garthwaite, TECRA International Pty Ltd, 13 Rodborough Rd, Frenchs Forest, NSW, 2086, Australia, Tel:

+61-2-9877-3000, Fax: +61-2-9972-7869. Method **995.22**, "Listeria in Foods, Colorimetric Polyclonal Enzyme Immunoassay Screening Method (TECRA Listeria Visual Immunoassay [TLVIA])" (TECRA International Pty Ltd, Frenchs Forest, NSW, Australia) was adopted First Action in 1995 and Final Action in 1998. TECRA has developed a new enrichment formulation, TECRA Listeria Enrichment Broth (TLEB), which does not include the toxic antifungal agent, cycloheximide, which is present in other *Listeria* enrichment media.

The objective of this study was to validate the new enrichment procedures and media formulations for use with the TLVIA. A collaborative study was conducted involving 16 laboratories in Australia and 14 laboratories in the United States. Two food types (cooked fish filets and cooked turkey) were examined in Australia and 3 food types (ice cream, lettuce, and raw, ground beef) were analyzed in the United States. With the exception of one lot of raw, ground beef, there were no significant differences in numbers of positive test portions between the TLVIA and the reference method for the 5 foods at 3 inoculation levels. For one lot of naturally contaminated raw, ground beef, TLVIA gave significantly more confirmed positive results than did the reference method.

The Methods Committee has adopted First Action this modification of enrichment protocols for the TLVIA.

(3) *SimPlate Yeast and Mold Color Indicator (Y&M-CI) Method for Enumeration of Yeasts and Molds in Foods*.—Study Director, Philip T. Feldsine, BioControl Systems, Inc., 12822 SE 32nd St, Bellevue, WA 98005-4318, Tel: +1-425-603-1123, Fax: +1-425-603-0070, E-mail: ptf@biocontrolsys.com. With the SimPlate method (BioControl Systems, Inc., Bellevue, WA), foodborne microorganisms are suspended in a nutritionally defined growth medium. To enhance performance, medium supplements are provided for use with certain food matrixes. These supplements balance certain inherent attributes of some matrixes such as increased acidity found in some foods. Discrete aliquots are separately compartmentalized and isolated from each other in the incubating wells where biochemical activities of viable organisms are monitored in a liquid environment. Detection by this biochemical process requires fewer microorganisms to produce a detectable signal in a SimPlate well than the number required to form a clearly visible colony on an agar plate. Enumeration is measured by a simple binary reaction; each well is either positive or negative. Any color change from the original background color in each well is interpreted as a positive reaction. Yeasts and molds are enumerated by counting the numbers of wells in each plate that exhibit a color change (positive wells) after incubation. The final count per plate is derived from a conversion table that is based on the Poisson distribution.

A collaborative study was conducted in which the relative effectiveness of the SimPlate Yeast and Mold-Color Indicator (Y&M-CI) method was compared to the U. S. Food and Drug Administration's (FDA) *Bacteriological Analytical Manual* (BAM) method (2) and the proposed International Organization for Standardization (ISO) method, ISO/CD 21527 (4), for

enumerating yeasts and molds in foods. Test portions were prepared and incubated according to the conditions stated in both the BAM and ISO methods. Six food types were analyzed: frozen corn dogs, nut meats, frozen fruits, cake mix, cereal, and fresh cheese. Nut meats, frozen fruits, and fresh cheese were naturally contaminated. All other foods were artificially contaminated with either a yeast or mold. Seventeen laboratories throughout North America and Europe participated in the study. In general, there was <0.3 mean log difference in recovery between the SimPlate method and the 2 corresponding reference methods. Moreover, mean log counts between the 2 reference methods were also very similar. The repeatability and reproducibility standard deviations were comparable between the 3 method comparisons.

This method has been adopted First Action as Official Method **2002.11**.

(4) *ISO vs AOAC for the Detection of Motile and Non-Motile Salmonella in Foods*.—Study Director Philip T. Feldsine. Over a period of several decades, standardized methods for the detection of *Salmonella* organisms in foods and food ingredients have been independently developed in the United States and Europe. Although the basic procedures are essentially similar, there are differences in the specified media and in incubation conditions. The rapidly increasing development of international commerce and the need for worldwide cooperation, particularly during the occurrence of a foodborne outbreak, necessitates harmonized testing methods worldwide for the detection of *Salmonella* organisms. Thus, an international collaborative was conducted comparing AOAC reference culture methods **965.25–965.68**, **995.20**, and **2000.06** (1) with the ISO reference culture method 6579:2002 (5).

Three food types were analyzed for the presence of *Salmonella* organisms by the AOAC and ISO methods. Paired test portions of each food type were simultaneously analyzed by both methods. A total of 21 laboratories representing federal government agencies and private industry, both in the United States and in Europe, participated in the collaborative study. The 3 food types were artificially contaminated with *Salmonella* organisms and competing microflora. No statistical differences ($p < 0.05$) were observed between the AOAC and ISO culture methods for fresh cheese and dried egg products. A statistically significant difference, in favor of the ISO method, was observed for one of the 2 lots of poultry in the first trial. The poultry meat used in this trial was radiation sterilized, artificially contaminated with *Salmonella* organisms and competitive microflora, and then lyophilized. The results from a second trial, using 2 separate lots of naturally contaminated raw, ground chicken, showed no statistical difference between the 2 culture methods. A third trial involving 4 laboratories was conducted on 2 separate lots of naturally contaminated raw poultry. Again, there were no statistically significant differences between the 2 reference culture methods. The ISO 6579:2002 method was adopted First Action as Official Method **2002.10** for the analysis of fresh cheese, dried egg, and raw, ground poultry.

(5) *VIDAS SLM Method for Detection of Salmonella in Foods*.—Study Directors, Wendy Lepper McMahon, Silliker Laboratories Group, 160 Armory Dr, South Holland, IL 60473, Tel: +1-708-225-1435, Fax: +1-708-225-1536, E-mail: wendy.mcmahon@silliker.com and Ronald L. Johnson, bioMerieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042-2320, Tel: +1-314-506-8182, Fax: +1-314-731-8678, E-mail: ron.johnson@na.biomerieux.com. The VIDAS Salmonella (SLM) assay (bioMerieux, Inc., Hazelwood, MO) is an automated enzyme-linked fluorescent immunoassay for the detection of *Salmonella* organisms in foods and food ingredients. The SLM assay is an already-approved Official Method (996.08). A collaborative study was conducted to obtain approval of the VIDAS SLM assay using the selective enrichment, tetrathionate broth, in combination with Rappaport-Vassiliadis (RV) medium in place of selenite cystine (SC) broth. The replacement of SC broth with RV medium eliminates an Environmental Protection Agency hazardous waste issue for laboratories. Eight food types (nonfat dried milk, casein, dried whole egg, milk chocolate, soy flour, raw, peeled shrimp, raw, ground turkey, and raw, ground pork) were analyzed by the VIDAS SLM assay and the reference culture methods (1, 2). A total of 25 laboratories participated in the study. A total of 1746 test portions were analyzed of which 771 were positive by the VIDAS SLM assay and 775 were positive by the reference culture method. There were no significant differences in the numbers of positive test portions given by the 2 methods.

On the basis of these results, the Study Director has recommended that the VIDAS SLM method, Official Method 996.08, be modified by replacing the SC broth with RV medium. The General Referee concurs.

(6) *3M Staph Express Method for Detection of Staphylococcus aureus in Meat, Seafood, and Poultry*.—Study Director, Wendy McMahon. The 3M Petrifilm Staph Express Count plate and disk system (3M Microbiology Products, St. Paul, MN) are used for enumerating *Staphylococcus aureus* in foods. The Petrifilm Staph Express Count plate contains a plating medium and a water-soluble gelling agent optimized for the growth of staphylococcal bacteria, yet inhibitory to the growth of most nonstaphylococcal bacteria.

A 1.0 mL test portion is added per plate. Pressure applied to a plastic spreader placed on the top film spreads the test portion over a growth area of approximately 30 cm². The gelling agent is allowed to solidify, and the plates are then incubated for 24 h at 35° or 37 C. Red-violet colonies on the plate are *S. aureus*. When only red-violet colonies are present, these colonies are counted as *S. aureus* colonies, and the test is complete.

If background flora are encountered, the 3M Petrifilm Staph Express disk may be used to identify *S. aureus* from all suspect colonies. The Petrifilm Staph Express disk should be used whenever colonies other than red-violet are present on the plate. The Petrifilm Staph Express disk contains a dye and deoxyribonucleic acid. *S. aureus* produces deoxyribonuclease (DNase), and the DNase reacts with the dye to form pink zones. When the disk is inserted into the plate, the plate and disk are then incubated for a minimum of 1 h and a maximum

of 3 h at 35 or 37 C. *S. aureus* (and occasionally *S. hyicus* and *S. intermedius* which can produce enterotoxins) produce a pink zone. The pink zones are counted as *S. aureus* colonies, regardless of the size of the zones.

In a collaborative study, the 3M Petrifilm Staph Express Count plate method was compared with AOAC Official Method 975.55 (1) for the enumeration of *Staphylococcus aureus* in selected foods. Four foods (cooked, diced chicken, cured ham, smoked salmon, and pepperoni) were analyzed for *S. aureus* by 12 laboratories. The mean log₁₀ counts for the methods were comparable for all 4 foods. The repeatability and reproducibility variances of the 24 h Petrifilm Staph Count plate method were similar to those of the 72 h standard method.

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

Selected Study Director Topics

Detection of Botulinal Toxins A, Proteolytic B, E, and F from Culture Supernatants, Amplified ELISA Procedure

Study Director, Joseph 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. The amplified enzyme-linked immunoabsorbent assay (ELISA) is a rapid procedure for the determination of botulinal toxin type from 2 culture media used in the mouse bioassay for culture toxin production (6). The amplified ELISA method utilizes an antibody-coated plate to capture specific toxin followed by specific biotin-labeled antibody. The biotin residues are bound by alkaline phosphatase-labeled streptavidin, and the enzyme is then detected using an amplified substrate. The substrate is used in a 2-step process. The absorbance of the reaction is read at 490 nm. A positive test result is one which has an absorbance value >0.20 above that of the negative control. The ELISA results are obtained in 6–7 h using a previously-prepared microtiter plate.

This method, now referred to as the digoxigenin-labeled IgG (DIG)-ELISA has been subsequently modified in the following ways: (a) digoxigenin-labeled IgG was substituted for the biotin-labeled IgG, (b) anti-digoxigenin IgG horseradish peroxidase conjugate was substituted for streptavidin-alkaline phosphatase conjugate, and (c) a peroxidase substrate was substituted for the amplified substrate that is needed for the alkaline phosphatase conjugate. The modified method (7) has been tested for botulinal toxins in a variety of foods using cooked meat medium and Trypticase-peptone-glucose-yeast extract medium. The DIG-ELISA has been successfully evaluated in laboratories of FDA and of the Centers for Disease Control and Prevention for the detection of botulinal toxins A, B, E, and F. Moreover, the DIG-ELISA is being considered by FDA for a validation study for the detection of botulinal toxins in dairy products.

Other Activities

The AOAC Research Institute (RI) Advisory Board formed a task force to determine how to complete the harmonization of the *Performance Tested Methods* (PTM) and *Official Methods of Analysis* (OMA) programs that had been initiated in 1997. Although the AOAC RI adopted the technical guidelines of the OMA program in 1997, there still remained differences in the review procedures of the 2 programs. These differences in review procedures led to some differences in data requirements, e.g., which types of foods should be studied. As a result, some of the methods in the PTM program were not accepted by the OMA program. Moreover, the OMA program maintained a policy of requiring the submission of these methods through the complete OMA review process, beginning with the precollaborative study.

A task group was formed to resolve the problem and met by conference call several times in 2002–2003. A draft set of recommendations was presented to the AOAC RI Board and to the Official Methods Board, and to the AOAC INTERNATIONAL Board of Directors, both of which agreed with the recommendations in May, 2003. These recommendations were:

Changes in PTM procedures for microbiological methods intended to be subsequently submitted to the OMA program: These changes pertain only to microbiology test kit methods that intend to proceed to the OMA program.

The AOAC RI will create an “OMA Track” program for these particular test kit methods.

The AOAC RI Consulting Application form will be revised to indicate if test kit method is “OMA Track” or other track.

“OMA Track” methods will be required to use the AOAC RI consultation services to develop Validation Outline equivalent to Precollaborative Study Protocol. Validation Outline for OMA Track methods will be submitted to the General Referee and Statistical Advisor for review.

OMA Track Validation Study Reports (Precollaborative Study Report) will be reviewed by the General Referee, Statistical Advisor, and Methods Committee in addition to Expert Reviewers.

Study Directors for OMA Track method reviews will prepare a Validation Study Report written in the OMA style.

Study Directors for OMA Track method reviews will be required to submit a Validation Study Report to the *J. AOAC INTERNATIONAL* for publication.

Expert Reviewers participating in OMA Track PTM reviews will not receive an honorarium.

Data of approved non-OMA Track PTM test kits are resubmitted for OMA precollaborative review if seeking OMA status.

Changes in the OMA program:

Permit simultaneous review of Validation Study Reports by General Referee, Statistical Advisor, and Methods Committee.

Chairperson of Methods Committee on Microbiology may delegate review of the Validation Study Reports to one Committee member. This review by one Committee member will

be the sole Committee involvement at the precollaborative study level.

Approved OMA Track PTM test kit method fulfills OMA precollaborative study requirements and may be submitted directly to OMA program which involves submission of collaborative study protocol.

Recommendations

(1) **998.08** *Confirmed Escherichia coli Counts in Poultry, Meats, and Seafoods, Dry Rehydratable Film Method (Petri-film E. coli/Coliform Count Plate Method).*—Study Director, 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-Lenarz@mmm.com. Discontinue topic, since any activities can be reported in topic which follows immediately.

(2) **998.08** *Enumeration of Escherichia coli in Poultry, Meat, and Seafood Products.*—Study Directors, Sonya A. Gambrel-Lenarz. Continue study.

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

(4) *LOCATE-ELISA Immunoassay for Identification of Salmonella in Foods.*—Study Director, Michael S. Curiale. Discontinue topic, since the method is now in Final Action status.

(5) *Detection of Botulinum Toxins A, B, E, and F from Culture Supernatants, Amplified ELISA Procedure.*—Study Directors, Joseph L. Ferreira; Susan Maslanka, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30333, 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: +1-608-263-6949, Fax: +1-608-263-1114, E-mail: eajohnso@facstaff.wisc.edu; Michael Goodnough, University of Wisconsin, 1925 Willow Dr, Madison, WI 53706, Tel: +1-608-263-6949, Fax: +1-608-263-1114, E-mail: moodnou@facstaff.wisc.edu. Continue study.

(6) **999.09** *Visual Immunoprecipitate Assay for the Detection of Motile and Non-Motile Salmonella in Foods.*—Study Director, Philip T. Feldsine. Discontinue topic, since the method is now in Final Action status.

(7) *H-5, ISO vs AOAC Reference Culture Methods for the Detection of Motile and Non-Motile Salmonella in Selected Foods.*—Study Director, Philip T. Feldsine. Continue study.

(8) **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 was adopted Final Action in 1998. A

method applicability modification to include the monitoring of environmental surfaces was validated and approved Revised First Action in 2001. The Study Director reports that the performance of this method in the field remains satisfactory and recommends that this method be adopted Final Action, and the General Referee concurs. Continue study.

(9) **H6, *Probelia* PCR Method for *Salmonella*.**—Study Director, Philip T. Feldsine. Discontinue topic.

(10) **999.08 Assurance Gold *Salmonella* EIA for the Visual or Instrumental Detection of *Salmonella* in Foods.**—Study Director, Philip T. Feldsine. Discontinue topic, since the method is now in Final Action status.

(11) **996.09 *Escherichia coli* O157:H7, Visual Immunoprecipitate Assay.**—Study Director, Philip T. Feldsine. 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.

(12) **996.10 MOD 9/21/00 Assurance Polyclonal Enzyme Immunoassay for the Detection of *Escherichia coli* O157:H7 in Ground Beef.**—Study Director, Philip T. Feldsine. This method 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 only raw and cooked beef products 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. Continue study.

(13) **992.11 MOD 12/00 Assurance Enzyme Immunoassay for the Detection of Motile and Non-motile *Salmonella* in Foods.**—Study Director, Philip T. Feldsine. This method 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 was widely used with favorable results and adopted Final Action in 2002. Discontinue topic.

(14) **H68, SimPlate CEC Quantitative Method for Total Coliforms and *Escherichia coli* in Foods.**—Study Director, Philip T. Feldsine. Continue study.

(15) **997.03 Visual Immunoprecipitate (VIP) Assay for *Listeria monocytogenes* and Related *Listeria* Species in Selected Foods.**—Study Director, Philip T. Feldsine. This assay was adopted First Action in 1997 and Final Action in 1999. A method applicability statement modification to include the monitoring of environmental surfaces was validated and approved Revised First Action in 2001. The Study Director reports that the performance of this method in the field remains satisfactory and recommends that this method be adopted Final Action, and the General Referee concurs.

(16) **SimPlate Total Plate Count: Indicator (TPC-CI) Method for the Enumeration of Total Aerobic Microorganisms in Foods.**—Study Director, Philip T. Feldsine. This method was adopted First Action in 2002. Continue study.

(17) **SimPlate Yeast and Mold Color Indicator (Y&M-CI) Method for Enumeration of Yeasts and Molds in Foods.**—This method was adopted First Action in 2002. Continue study.

(18) **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. This method was adopted First Action in 2000. The Study Director reports that this method has been used favorably and recommends that the method be adopted Final Action, and the General Referee concurs. Continue study.

(19) **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. Continue study.

(20) **2001.07 *Salmonella* in Selected Foods by Immuno-Concentration *Salmonella* (ICS) and Selective Plate (BS, HE, SMID) Procedure.**—Study Directors, Wendy McMahon and Ronald L. Johnson. A new method for detection of *Salmonella* in selected foods in 48 h has been developed which uses both the VIDAS immuno-concentration *Salmonella* (ICS) assay and a combination of 3 selective agar plates: bismuth sulfite (BS), Hektoen enteric (HE), and *Salmonella* Identification (SMID). After overnight preenrichment of the test portion, immunological capture of the *Salmonella* organisms is accomplished by the VIDAS ICS assay. Following the VIDAS ICS assay, the released *Salmonella* cells are streaked to BS, HE, and SMID agars. The Study Director recommends that this First Action method, recommended for specific foods, be adopted Final Action, and the General Referee concurs. A manuscript, supporting the approval of this method for the analysis of all foods, has been approved by the General Referee, and was reviewed by the Committee. Committee Chair approved modified method for First Action approval.

(21) **2001.08 *Salmonella* in Selected Foods by Immuno-Concentration *Salmonella* (ICS) and Selective Plate (BS, HE, XLD) Procedure.**—Study Directors, Wendy McMahon and Ronald L. Johnson. A new method for detection of *Salmonella* in selected foods in 48 h has been developed which uses both the VIDAS ICS assay and a combination of 3 selective agar plates: BS, HE, and xylose lysine desoxycholate (XLD). After overnight preenrichment of the test portion, immunological capture of the *Salmonella* organisms is accomplished by the VIDAS ICS assay. Following the VIDAS ICS assay, the released *Salmonella* cells are streaked to BS, HE, and XLD agars. The Study Director recommends that this First Action method, recommended for specific foods, be adopted for Final Action, and the General Referee

concurr. A manuscript, supporting the approval of this method for the analysis of all foods, has been approved by the General Referee, and was reviewed by the Committee. Committee Chair approved modified method for First Action approval.

(22) **2001.09** *Salmonella in Selected Foods by Immuno-Concentration Salmonella (ICS) and Enzyme-Linked Immunofluorescent Assay (ELFA)*.—Study Directors, Wendy McMahon and Ronald L. Johnson. A new method for detection of *Salmonella* in selected foods in a minimum of 24 h has been developed which uses both the VIDAS ICS assay and the VIDAS *Salmonella* (SLM) assay, the latter being Official Method **996.08**. After overnight preenrichment of the test portion, immunological capture of the *Salmonella* organisms is accomplished by the VIDAS ICS assay. Following the VIDAS ICS assay and release of the *Salmonella* cells, preenrichment is accomplished in a nonselective growth medium for 5–6 h (6–7 h for nonfat dry milk) followed by detection of *Salmonella* in the test portion by the VIDAS SLM assay. The Study Director recommends that this First Action method, recommended for specific foods, be adopted Final Action, and the General Referee concurs. A manuscript, supporting the approval of this method for the analysis of all foods, has been approved by the General Referee, and was reviewed by the Committee. Committee Chair approved modified method for First Action approval.

(23) **3M Staph Express for Detection of Staphylococcus aureus in Meat, Seafood, and Poultry**.—Study Director, Wendy McMahon. Continue study.

(24) **996.08 VIDAS SLM Method for Detection of Salmonella in Foods**.—Study Directors, Wendy McMahon and Ronald L. Johnson. Continue study.

(25) **2000.13 Escherichia coli O157:H7 in Foods, 8-Hour Reveal Screening Test**.—Study Director, Mark A. Mozola, Neogen Corp., 620 Leshler Pl, Lansing, MI 48912, Tel: +1-517-372-9200, Fax: +1-517-372-0108, E-mail: mmozola@neogen.com. Continue study.

(26) **H78, Salmonella in Foods, Reveal for Salmonella Test System**.—Study Director, Mark Mozola. Discontinue topic.

(27) **2000.14 Escherichia coli O157:H7 in Foods, 20-Hour Reveal Screening Test**.—Study Director, Mark Mozola. Continue study.

(28) **H-9, Salmonella in Foods, Alert for Salmonella Test System**.—Study Director, Mark Mozola. Discontinue topic.

(29) **Mod. 999.06 Precollaborative Study Protocol Evaluation of VIDAS Listeria (LIS) Immunoassay**.—Study Director, Ronald L. Johnson. Rename topic as *VIDAS Listeria (LIS) Immunoassay for the Detection of Listeria species in Foods*. Continue study.

(30) **995.22 MOD 2/6/01 TECRA Immunoassay for Detecting Listeria Species on Environmental Surfaces**.—Study Director, Ian Garthwaite. Continue study.

(31) **H17, Listeria in Selected Foods by TECRA Unique 2000 Listeria Method**.—Study Director, Ian Garthwaite. Continue study.

(32) **H71, Staphylococcus aureus in Foods, TECRA STAPH AUREUS Visual Immunoassay**.—Study Director, Ian Garthwaite. Continue study.

(33) **995.22 MOD 5/23/01 TECRA Enrichment for Listeria in Foods**.—Study Director, Ian Garthwaite. A collaborative study was conducted to validate a new enrichment medium for use with the TECRA *Listeria* VIA. Continue study.

(34) **H66, Determination of Escherichia coli in Flesh Foods Using Visual Immunoassay with a Modified Culture Procedure**.—Study Director, Ian Garthwaite.

(35) **2000.07 MOD 5/9/01 Salmonella in Foods, Rapid Colorimetric TECRA Unique Test**.—Study Director, Ian Garthwaite. Continue study.

(36) **2000.07 MOD 2/15/01 TECRA Unique Salmonella Test**.—Study Director, Ian Garthwaite. A validation study was conducted to obtain approval of a specific method modification of the already approved UNIQUE *Salmonella* test, Method **2000.07**, to extend the applicability of the method to fruit juices. In the modified method, the UNIQUE module is incubated at 42°C rather than at 37°C. In the validation study 60 replicate test portions of orange juice (20 test portions each at high inoculum, low inoculum, and uninoculated levels) were analyzed with the modified TECRA UNIQUE method and the reference culture method. No significant differences were observed in the numbers of positive test portions with the 2 methods. This modification of the TECRA UNIQUE assay, Method **2000.07**, was approved for Revised First Action status by the Official Methods Board in 2003.

(37) **998.09 Salmonella in Foods, TECRA Salmonella Visual Immunoassay, Validation Study to Demonstrate Equivalence of a Minor Modification in 998.09 with Reference Culture Method**.—Study Director, Ian Garthwaite. A study was conducted to validate the inclusion of a test portion additive which allows the direct analysis of an aliquot from the RV medium, without subsequent post enrichment in M broth. A study manuscript has been approved by the General Referee and forwarded to the Methods Committee for approval. Continue study.

(38) **2000.15 Coliform Counts in Foods, Dry Rehydratable Film Method**.—Study Director, Karen Silbernagel. Since this method received First Action status in 2001, no adverse comments have been received by the Study Director, who now recommends that this method be adopted Final Action. The General Referee concurs. Continue study.

(39) **2001.05 Staphylococcus aureus in Foods, Dry Rehydratable Film Method**.—Study Director Karen Silbernagel. Since this method received First Action status in 2001, no adverse comments have been received by the Study Director, who now recommends that this method be adopted Final Action. The General Referee concurs. Continue study.

(40) **H-51, Enterobacteriaceae in Foods, Dry Rehydratable Film Method**.—Study Director, Karen Silbernagel. This system was approved First Action Method **2003.01** in 2003. Continue study.

(41) **Hg125, BAX System for Detection of Listeria monocytogenes in Foods**.—Study Director, Karen Silbernagel. A report of a precollaborative study has been ap-

proved by the General Referee and submitted to the Methods Committee. Continue study.

(42) *Hg128, 3M Petrifilm Staph Express Count Plate Method for the Enumeration of Staphylococcus aureus in Selected Processed and Prepared Foods.*—Study Director, Karen Silbernagel. This system was approved First Action Method **2003.07** in 2003. Continue study.

(43) *Hg129, BAX System with Automated Detection of Salmonella in Foods.*—Study Director Karen Silbernagel. This system was approved First Action Method **2003.09** in 2003. Continue study.

(44) *Hg130, VIDAS (LM02) Immunoassay Method for Detection of Listeria monocytogenes in Foods.*—Study Director, Karen Silbernagel. The protocol for a collaborative study has been approved, and this study is in progress. Continue study.

(45) **999.06 MOD VIDAS (LIS) Immunoassay Method for Detection of Listeria species in Foods.**—Study Director, Karen Silbernagel. This topic represents a validation of a modification in the enrichment of the previously approved Method **996.06**. Continue study.

(46) *ALOA Medium for Detection of Listeria Species in Foods.*—Study Director, Karen Jarvis, Microbiology International, 97 H Monocacy Blvd, Frederick, MD 21701, Tel: +1-301-662-6835, Fax: +1-301-662-8096, E-mail: karen.jarvis@sygene.com. Discontinue topic.

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- (2) *Bacteriological Analytical Manual* (1995) 8th Ed., AOAC INTERNATIONAL, Gaithersburg, MD
- (3) U.S. Department of Agriculture, Food Safety and Inspection Service, *Microbiology Laboratory Guidebook* (1999) 3rd Ed. (Revision 2), U.S. Department of Agriculture, Washington, DC
- (4) International Organization for Standardization (2001) *Horizontal Method for the Enumeration of Yeasts and Molds—Colony Count Technique, ISO/CD 21527*, International Organization for Standardization, Geneva, Switzerland
- (5) International Organization for Standardization (2002) *Horizontal Method for the Detection of Salmonella spp., ISO 6579*, International Organization for Standardization, Geneva, Switzerland
- (6) Ferreira, J.L., Maslanka, S., Johnson, E., & Goodnough, M. (2003) *J. AOAC Int.* **86**, 314–331
- (7) Ferreira, J.L., Maslanka, S., & Andreadis, J. (2002) *Laboratory Information Bulletin* **4292**, 1–10, U.S. Food and Drug Administration, Rockville, MD

Food Microbiology—Dairy, General Referee, Melissa C. Newman

2003.08 3M Petrifilm Staph Express Count Plate Method for the Enumeration of Staphylococcus aureus in Selected Dairy Foods.—Study Director, Karen Silbernagel. This method was adopted First Action June 13, 2003.

Microbiological Efficacy Testing of Disinfectants, General Referee, Steve Tomasino

Study Director Topics

Currently, no official studies are being conducted.

EPA-Sponsored Research: Evaluation and Development of Laboratory Methods for Determining Efficacy of Liquid Sporicides on Hard Surfaces

Background

The EPA Office of Pesticide Programs (OPP) has responsibility for regulating antimicrobial products used to control pathogenic bacteria (including spores of bacteria), viruses, and other microorganisms on porous and nonporous surfaces. Following an intentional release of anthrax spores into office buildings in 2001, the agency was inundated with requests to use various decontamination chemicals against *Bacillus anthracis*. Currently, no sporicidal products are registered for *B. anthracis*. Numerous test methodologies and surrogates were used in generating the efficacy data necessary to support these requests. Currently, the AOAC Sporicidal Activity Test (SAT; AOAC Method **966.04**) is accepted by the EPA for performance (efficacy) testing of sporicides. The AOAC method provides a qualitative measure of the efficacy of chemicals (liquids and gases) against *B. subtilis* and *Clostridium sporogenes*. The AOAC SAT method has limitations, primarily its range of application (i.e., only 2 types of surfaces and liquid and gaseous compounds) and lacks standardization in several key steps. Issues were raised with regards to the suitability of using the AOAC SAT and *Bacillus subtilis* as the test system for evaluating sporicides used to remediate anthrax contaminated sites.

To aid in addressing these issues, the EPA sponsored the formation and meeting of a panel of experts from the federal government, academia, and industry. As a result of the meeting, an interagency government panel (Interagency Expert Panel on Efficacy Test Methods and Surrogates for Anthrax Spores) was formed with participants from EPA, FDA, CDC, Air Force, Army, Navy, and DOE as well as a list of efficacy test attributes which could be used to rank methods and surrogates for various use scenarios. The Interagency Panel formed subgroups (e.g., Test Methods, Surrogates, Gases/Fumigants) to address specific topics of interest to the various government agencies and stakeholders. The Test Methods subgroup was tasked with the review of available efficacy methods for sporicides and to make recommendations for research and method development.

Proposed Research

In conjunction with the priorities outlined in an EPA-OPP Research Proposal submitted to EPA's Office of Research Development (ORD)-Safe Buildings Program, the recommendations provided by the Test Methods subgroup were used in the

development of a collaborative research project. It is likely that recommendations made by the Surrogates subgroup will also be used in the development of research projects under the ORD-Safe Buildings Program in the future. The purpose of this research is to evaluate efficacy test methods for sporicidal chemicals and to provide data, analyses, and study conclusions to EPA, FDA, and other government agencies necessary to establish regulatory guidelines for sporicidal products to be used in the treatment of buildings and contents contaminated with spores of *Bacillus anthracis*. This research may lead to a replacement of the agency-accepted AOAC SAT. Studies on *B. anthracis* have been prioritized. Using funds received by OPP under the ORD-Safe Buildings Program, the OPP Microbiology Laboratory will coordinate and manage this project. Stephen Tomasino is the Principal Investigator (PI) and the Work Assignment Manager and will have overall responsibility for the technical conduct of the project. Portions of this project will involve validation support from AOAC INTERNATIONAL. Discussions are currently underway with AOAC officials to determine the appropriate mechanism for formal study review; Tomasino is currently the GR for disinfectant efficacy test methods and will be the Study Director for projects described below. In addition, discussions with the AOAC Task Force on Disinfectant Test Methods regarding this research plan took place during the 2003 Annual Meeting.

Test Method Research for Liquid Sporicides on Hard Surfaces

The initial investigation involves the evaluation of quantitative methods of liquids on hard surfaces. Three methods have been selected for comparative investigation: (1) AOAC SAT, (2) Standard Quantitative Carrier Test (QCT-1) Method (ASTM E-2111), and (3) Three-Step Recovery Method (TSM; a DOD-sponsored method). The QCT-1 and TSM are quantitative methods (i.e., designed to generate estimates of \log_{10} reduction in viable spores). Each method will be evaluated against spores of *Bacillus subtilis* (ATCC 19659) and a set of reference chemicals and test conditions. Multiple laboratories will conduct the initial research; however, precollaborative studies and research in support of this project may be conducted by a single laboratory. Conducting the tests side-by-side in a comparative, standardized fashion will provide information on various method attributes such as throughput, technical expertise, inter- and intralaboratory variability, and the estimates of chemical efficacy. The data will be subjected to statistical analysis to determine if the methods are suitable for regulatory/enforcement purposes. In future studies, existing and/or new efficacy methods suitable for other product formulations such as gases, foams, gels, and sprays will be evaluated on hard and porous surfaces.

The AOAC method is a qualitative test which generates positive (tubes with growth) or negative (tubes without growth) results. The method calls for treating 60 inoculated carriers with the sporicidal agent for a specified exposure time, neutralizing the germicide and recovering the surviving spores in growth media. Each of the 60 carrier sets (2 tubes of recovery media per carrier) is evaluated visually for growth

following incubation. A data transformation procedure (i.e., transformation of qualitative data to an estimate of \log_{10} reduction) will be performed to allow for comparison between the AOAC method and the quantitative methods.

TSM to Determine Sporicidal Efficacy of Disinfectants on Carrier Surfaces is a quantitative method utilizing inoculated coupons to deliver spores into liquid sporicides. Following exposure, spores are removed from the coupons by sonication and shaking and plating on recovery medium to recover spores. Enumeration of surviving spores is performed by counting the colonies on agar plates following incubation. The efficacy data is presented as: Log_{10} spore killing = Log spore survival in the water control minus log spore surviving disinfection.

QCT-1-Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal and Sporicide Potencies of Liquid Chemical Germicides-ASTM Method 2111 is a quantitative method utilizing glass vials as carriers; surviving spores are enumerated on filters. Efficacy is determined by calculating Log_{10} reduction (LR) of the test organism. To better standardize the source and quality of the spore preparation across all 3 methods, the spores will be produced using the AOAC soil extract medium identified in the AOAC SAT.

General Objectives for Test Method Research for Liquid Sporicides on Hard Surfaces

Develop expertise in conducting multiple sporicidal tests, specifically quantitative tests for liquid chemicals on hard surfaces.

Identify precollaborative studies (e.g., use of new carrier types and associated inoculum recovery); perform research prior to the collaborative testing.

Conduct and compare the results of 3 efficacy test methods, including the AOAC method, using selected liquid sporicidal chemicals against spores of *B. subtilis*.

Perform appropriate statistical analyses to determine means and variances, method reproducibility, within-laboratory variation (repeatability), total variation (reproducibility), variability in carrier counts, LR and the generation of LR estimates for the AOAC SAT.

Select and/or modify 1 of the 2 quantitative methods for further investigation and validation testing. (If either of the 2 quantitative test methods exhibit excessive variability, other quantitative methods may be sought for further testing.) In this project, studies will be conducted which compare the current AOAC method (or a modified version approved by the PI) to the selected alternative methods. In addition, the relative differences in the response of selected *Bacillus* species (surrogates for *B. anthracis*) to chemical treatment will be evaluated.

The most promising method (per product type) and surrogate (e.g., virulent and/or avirulent *B. anthracis*) will be subjected to validation testing across multiple independent laboratories under identical conditions. The purpose of the validation is to establish the characteristics of the methods with respect to accuracy, precision (repeatability and

reproducibility), sensitivity, ruggedness, and practicality under typical laboratory conditions. Options for conducting validation testing will be discussed with AOAC INTERNATIONAL.

Modifications to the AOAC SAT

In a separate, yet closely related project, research and method development will be conducted to modify the existing AOAC SAT. The AOAC Sporicidal Activity Test (**966.04**; AOAC SAT), a qualitative method, is currently accepted by the EPA for performance testing of sporicides; however, as it currently exists, the AOAC SAT has limitations and lacks standardization in several key steps. Several authors have proposed modifications to the test method to eliminate or reduce variability associated with important steps. The overall goal of this project is to provide a short-term improvement to sporicidal efficacy testing by modifying the AOAC SAT. Initial research has already been conducted on the modifications by other scientists. This investigation will generate comparative data necessary to proceed with validation testing of the selected modifications to the AOAC SAT and will be limited to liquid chemicals tested against *Bacillus subtilis* on hard and porous surfaces (porcelain and suture loop carriers). Modifications to other components of the AOAC SAT (e.g., gaseous chemicals and *Clostridium sporogenes*) will not be investigated at this time.

General Research Objectives

Develop expertise in conducting the specific steps identified as modifications to the standard method.

Conduct and compare the results of modified components to the standard method using a selected test matrix (e.g., liquid

sporicidal chemicals, organic burden) against the test organism, spores of *Bacillus subtilis*.

Perform appropriate statistical analyses to determine means and variances, method reproducibility, variability in carrier counts, LR, and the generation of LR estimates for the AOAC SAT.

Proceed with the *Peer-Verified* approach to AOAC method validation.

The following modifications to AOAC SAT will be pursued:

- Use of a chemically defined medium for spore production
- Use of stainless steel carriers

- Addition of a carrier count procedure for enumeration of spore inoculum

- Establishment of a minimum spore challenge

- Addition of a neutralization confirmation procedure

- Application of a data transformation procedure

Both research projects were scheduled to begin in October 2003. Interagency Agreements have been established between the EPA, FDA, and DOD (U.S. Army and Air Force) for technical support. A Quality Assurance Project Plan has also been prepared to ensure proper quality control, record keeping, and reporting necessary to defend, reconstruct, or re-analyze each study conducted.

*Genetically Modified Organisms, General Referee,
Markus Lipp*

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