

GENERAL REFEREE REPORTS

Committee on Microbiology and Extraneous Materials

Food Microbiology—Non-Dairy

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Summary

Several important studies have taken place over the last year. First, a collaborative study to harmonize the *Salmonella* reference methods, recommended by AOAC INTERNATIONAL (Methods **995.20** and **2000.06**) and the International Organization for Standardization (ISO; Method 6579), has been completed. Three foods (fresh cheese, dried egg powder, and raw chicken) were included in the study. No significant differences ($p < 0.05$) were observed between the methods.

Second, a collaborative study has been completed by Study Directors Joseph Ferreira, Susan Maslanka, Eric Johnson, and Mike Goodnough comparing an amplified enzyme immunoassay (EIA) system for the detection of botulinum toxins A, B, E, and F with Method **977.26**. If successful, this method would reduce the time necessary to detect botulinum toxins in foods from 6 days to 6 or 7 h and would eliminate the need for a mouse bioassay. The data are being analyzed and will be submitted to the Committee for its evaluation.

Third, there was an evaluation of an alternative enrichment system in the GENE-TRAK method for the detection of *Salmonella* in foods (**990.13**) in which the enrichment pair, tetrathionate (TT) broth/selenite cystine (SC) broth, was replaced with TT broth/Rappaport-Vassiliadis (RV) medium. A precollaborative study has been completed and a collaborative study is planned. This change would allow Method **990.13** to use the same selective enrichments as the reference methods, **995.20** (*Salmonella* in Raw, Highly Contaminated Foods and Poultry Feed) and **2000.06** (*Salmonella* in Foods with a Low Microbial Load).

Fourth, Tecra *Listeria* VIA (Method **995.22**) has eliminated the use of cycloheximide, a highly toxic antifungal agent, from its enrichment broth, without reducing the sensi-

tivity of its assay. The elimination of cycloheximide will enhance safety for laboratory personnel as well as reduce hazardous waste disposal costs. Finally, the applicability of BioControl Systems' Assurance® Enzyme Immunoassay and Visual Immunoprecipitate Assay kits for *Listeria monocytogenes* and related species have been extended to environmental surfaces. These are the first methods to be validated for use on environmental surfaces. The validation format established with these methods will be used by AOAC to validate future methods for environmental surfaces.

Selected Study Director Topics

bioMérieux Methods

Salmonella in Selected Foods by Immunoconcentration *Salmonella* and Selective Plate Procedure

Study Directors Wendy Lepper, Silliker Laboratories, South Holland, IL, and Ronald Johnson, bioMérieux, Inc., Hazelwood, MO, evaluated 2 new 48 h methods for detection of *Salmonella* in selected foods that use both the VIDAS Immunoconcentration *Salmonella* (ICS) Assay and a combination of 3 selective plates (Hektoen enteric [HE], bismuth sulfite [BS], and *Salmonella* Identification agars for Method **2001.07**; HE, BS, and xylose lysine desoxycholate agars for Method **2001.08**). After overnight pre-enrichment of the test portions, immunological capture of *Salmonella* is accomplished with the VIDAS ICS *Salmonella* assay. Following the VIDAS ICS assay, released *Salmonella* cells are streaked onto 3 selective plates.

Precollaborative and collaborative studies have been completed. The methods have been approved for First Action in selected foods. Studies are currently underway for obtaining approval for analysis of all foods.

Salmonella in Selected Foods by Immuno-concentration *Salmonella* (ICS) and Enzyme-Linked Immunofluorescent Assay (ELFA)

Study Directors Wendy Lepper and Ronald Johnson. A new method (**2001.09**) for detection of *Salmonella* in selected foods in a minimum of 24 h has been developed by bioMérieux, Inc., using both the VIDAS ICS *Salmonella* assay and VIDAS *Salmonella* (SLM) assay. The VIDAS SLM assay is AOAC Official Method **996.08**. After overnight pre-enrichment of the test portion, immunological capture of *Salmonella* is accomplished with the VIDAS ICS *Salmonella* assay. Following the VIDAS ICS and release of *Salmonella* cells, post-enrichment 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 with the VIDAS SLM assay.

Precollaborative and collaborative studies have been completed. The method is approved for First Action in selected foods. Studies are currently underway for obtaining approval for the analysis of all foods.

GENE-TRAK Methods

Salmonella in Foods

Study Director Mark Mozzola, GENE-TRAK Systems, Hopkinton, MA, reported that a precollaborative study was completed to validate an alternative enrichment protocol for use with Final Action Method **990.13** (*Salmonella* in Foods, Colorimetric Deoxyribonucleic Acid Hybridization Method [GENE-TRAK]) and the GENE-TRAK *Salmonella* Direct Labeled Probe Assay (AOAC Research Institute Performance Tested Method 961101; DLP). The alternative enrichment protocol uses the combination of TT and RV broths for selective enrichment rather than the combination of TT and SC broths. Results of testing 21 foods totaling 1050 test portions showed no statistically significant differences in method performance for any food comparing the GENE-TRAK *Salmonella* assay, the GENE-TRAK *Salmonella* DLP assay, and the reference culture method. However, several foods yielded data sets without fractionally positive results. Thus, additional trials were performed with these foods. This study has been recently completed, and a revised manuscript is in preparation.

A microtiter-format DNA hybridization assay for *Salmonella* in foods (Sequepoint™ *Salmonella*) has been developed using the GENE-TRAK DLP chemistry. Following sample pre-enrichment, selective enrichment in TT and RV broths, and a post-enrichment in Gram negative broth (GN), aliquots of the final GN cultures are transferred to test tubes. The test portions are lysed and transferred to a poly dT-coated microtiter well. A 4:1 mixture of hybridization solution and probe solution (containing horseradish peroxidase-labeled *Salmonella*-specific oligonucleotide, and poly dA-tailed *Salmonella*-specific oligonucleotide) is added. After this hybridization and capture step, the microtiter wells are washed with wash solution. Substrate-chromogen reagent, followed by stop solution, is added and the absorbance determined using a microtiter well strip or plate reader at 450 nm. The assay can be performed manually, or in an automated fashion using an off-the-shelf laboratory instrument. The manual and automated assay protocols are identical with respect to all assay parameters (incubation times and temperatures, reagent volumes, etc.). In the automated version, the system can analyze 186 test portions, plus controls, in approximately 2 1/2 h following test portion enrichment.

An in-house validation study was performed in which 20 foods, inoculated with low levels of *Salmonella* (target levels 0.04–0.2 and 0.4–2.0 CFU/g), were tested in parallel by both the manual and automated microtiter assays, as well as by the dipstick-format *Salmonella* DLP assay and the AOAC ref-

erence culture method (1). All methods tested showed a sensitivity of 99%. A data submission has recently been made to the AOAC Research Institute Performance Tested Program. A collaborative study is planned for later this year. Additional microtiter-format DNA hybridization assays are in development, including assays for *Listeria monocytogenes* and other *Listeria* spp.

TECRA Methods

Modified Enrichment Procedures for the TECRA Listeria Visual Immunoassay (AOAC Method 995.22)

Study Director Denise Hughes of TECRA International, Willoughby, New South Wales, Australia, developed a modified version of Method **995.22**, *Listeria* in Foods, Colorimetric Polyclonal Enzyme Immunoassay Screening Method (TECRA® *Listeria* Visual Immunoassay [TLVIA]). Method **995.22** was adopted as First Action by the Official Methods Board in September 1995, and was recommended for Final Action status in 1998. Following the original collaborative study, there has been a number of developments in enrichment procedures for *Listeria*, some of which resulted in revision of the U.S. Food and Drug Administration's (FDA) *Bacteriological Analytical Manual* (BAM) recommended methodology (2).

TECRA has developed new enrichment procedures for use with the TECRA *Listeria* Visual Immunoassay. These procedures use a new medium, TECRA *Listeria* Enrichment broth, which does not contain the highly toxic antifungal agent, cycloheximide. A precollaborative study that included 20 foods was conducted. For 17 of 20 foods, the study showed no significant differences at the 5% level between the TECRA method and the reference culture method using Chi square analysis with Yates correction for unpaired data. For the other 3 foods (salami, smoked salmon, and frozen egg yolk), the TECRA method identified a significantly higher number of positive test portions than did the reference culture method at one of the inoculation levels.

Of the 1200 test portions tested, 735 were confirmed positive with the TECRA assay and 665 were confirmed positive with the reference culture method. No problems with mold growth were observed, despite the absence of cycloheximide from the enrichment broth. A report on this study has been submitted, and the alfalfa sprouts (low level) are being retested at the request of the General Referee (GR).

A collaborative study with 16 collaborators in Australia and 14 collaborators from the United States has been conducted. A report, with results for 5 foods (cooked turkey, cooked fish, raw ground beef, ice cream, and lettuce), has been submitted and the new enrichment methods are recommended for First Action approval. It is intended that the original AOAC Method **995.22** be retained and that new enrichment methods be designated as a separate method with applicability to raw meats, fresh produce/vegetables, processed meats, seafood, dairy cultured/noncultured, fruit/fruit juices.

In addition, a study designed to extend the applicability of the TECRA *Listeria* Visual Immunoassay (AOAC Method 995.22) to environmental samples is contemplated. A protocol has been submitted for a precollaborative study to validate the TECRA *Listeria* Visual Immunoassay for the detection of *Listeria* spp. on environmental surfaces.

TECRA *Staphylococcus aureus* Visual Immunoassay

Study Director Denise Hughes. The TECRA *Staphylococcus aureus* Visual Immunoassay is an enzyme-linked immunosorbent assay (EIA) performed in the "sandwich" configuration, which is intended for use as a rapid method for detection of low numbers of *S. aureus* in foods.

The food sample is enriched in TECRA *Staphylococcus* Growth Medium for 24 h at 37°C prior to performing the assay. Presumptive positive immunoassay results are confirmed by streaking the enrichment broth onto selective agar, such as Baird Parker agar, and testing typical colonies for the presence of coagulase.

In addition, the TECRA Staphylococcal Enterotoxin Visual Immunoassay may be used to identify the presence of enterotoxin in the enrichment broth of samples which give positive results for the presence of *S. aureus*.

Protocols have been approved for precollaborative and collaborative studies to validate the use of the TECRA *S. aureus* Visual Immunoassay to detect *S. aureus* in food and the TECRA Staphylococcal Enterotoxin Visual Immunoassay to determine whether the isolate is enterotoxigenic.

A revised protocol, requesting a change to the sample size and the reference method, is presently being reviewed by the Methods Committee. The precollaborative study will commence as soon as the Committee's response is received.

Minor Modification of TECRA UNIQUE *Salmonella* Test (AOAC Method 2000.07)

Study Director Denise Hughes. The Official Methods Board approved the TECRA® UNIQUE™ *Salmonella* Test as First Action AOAC Method 2000.07 in January 2000. Recently, TECRA International has changed the format of the test and developed an instrument called UNIQUE Plus that can perform the test and read the results automatically. The modification to the UNIQUE Test represents a design change only. The immunochemistry of the test is unchanged and does not alter test performance. Automation is optional.

Based on directions from the GR, a protocol for an in-house study to validate performance of the new format of UNIQUE has been submitted. The study is also intended to extend the AOAC approval of Method 2000.07 to include automated UNIQUE reading. All enrichment procedures would remain unchanged. As this is a minor change, it is intended that the UNIQUE Plus modification retain First Action status.

TECRA UNIQUE *Listeria* Test

Study Director Denise Hughes. A precollaborative study protocol has been submitted to validate a rapid method for the detection of *Listeria*, the TECRA UNIQUE *Listeria* Test.

This test is similar in principle to AOAC Method 2000.07, TECRA UNIQUE *Salmonella* Test. For detection of *Listeria*, test portions are enriched in TECRA Buffered *Listeria* Enrichment broth for 24 h at 35–37°C before being tested in the UNIQUE *Listeria* module. The UNIQUE Test provides the user with all the necessary reagents in a single-test module. The UNIQUE dipstick is used for both immunoenrichment and detection steps. A presumptive positive result for *Listeria* can be obtained in less than 32 h after sample enrichment is started and should be confirmed by standard culture methods. With the optional UNIQUE Plus instrument, the test can also be performed and read automatically.

It is intended that an inclusivity and exclusivity study would be followed by a precollaborative study for 16 foods and a collaborative study, with a view to gaining approval for the new method to be used for the following food groups: raw meats, fresh produce/vegetables, processed meats, seafood, dairy cultured/noncultured, fruit and fruit juices.

Hydrophobic Grid Membrane Filter (ISO-GRID) Methods

Study Director Phyllis Entis, QA Life Sciences, Inc., San Diego, CA, developed a method that uses a hydrophobic grid filter. The hydrophobic grid membrane filter comprises a 0.45 µm membrane filter on which has been imprinted a water-repellent material in a 40 × 40 grid matrix. The hydrophobic nature of the material largely restrains through its physical properties the spread of bacterial, yeast, and mold colonies beyond the initial grid square in which they develop. This restriction of spread enables a 3-log₁₀ counting range on a single membrane filter. In addition, the grid lines enhance colony separation, facilitating the use of a variety of dyes in the culture media. Finally, the filtration process separates the microorganisms from the sample matrix, eliminating any interactions between the sample and the culture medium which might otherwise produce a false result. Several method applications based on this technology have already been accorded First Action or Final Action status (2), and others are under development and validation.

Twenty-Four Hour Presumptive Enumeration of *Listeria monocytogenes* Using LM-137 Agar

Study Director Phyllis Entis. A new culture medium, LM-137 agar, was designed to enable selective and differential direct enumeration of presumptive *L. monocytogenes* from foods and environmental samples. The method consists of direct filtration of up to 5 mL of a 10⁻¹ dilution of food homogenate or swab rinse liquid and incubation of the filter on LM-137 agar for 24 h at 35–37°C. Presumptive colonies are pink. Following enumeration of presumptive colonies, a representative number of them are subcultured and submitted to a panel of screening tests to determine whether each is *L. monocytogenes*. The proportion of confirming colonies is multiplied by the presumptive count to obtain a confirmed count. This count is then converted to a most probable number (MPN) value and multiplied by the appropriate dilution factor

to obtain the confirmed *L. monocytogenes* MPN per g or mL. A precollaborative study was completed in 1999 and the report was reviewed and accepted by the GR, Statistician, and Committee. The results of the precollaborative study have been published (3).

A collaborative study was initiated in 1999 and performed in the first quarter of 2000. The collaborative study report has been reviewed by the GR and Statistician and is presently at the Committee level.

***L. monocytogenes* Presence/Absence Method Using LM-137 Agar**

Study Director Phyllis Entis. A method was developed which combines an enrichment procedure followed by filtration and incubation of the filter on LM-137 agar to obtain sensitivity of one *L. monocytogenes* organism in a 25 g test portion. The precollaborative study protocol for this method has been accepted by the GR, Statistician, and Committee, but the study has not yet been initiated. This precollaborative study should be conducted in the current year.

Twenty-Four Hour Salmonella Detection Using SCCRAM Enrichment with EF-18 Agar

Study Director Phyllis Entis. AOAC Final Action Method **991.12** describes a 2-stage enrichment in lactose broth (18–24 h) and TT broth (6–8 h) followed by filtration and incubation of the hydrophobic grid membrane filter on EF-18 agar, a selective and differential culture medium for *Salmonella*, for 18–24 h (1). Overall, a “negative screen” result can be obtained in as little as 42 h following initiation of the analysis. A major advantage of the method is that the presumptive positive reaction comprises viable, isolated colonies which can be subcultured directly for confirmation. This obviates the need to return to one or more of the enrichment broths and to isolate the presumptive microorganisms conventionally before being able to perform confirmation tests. This method has worked well over the years and is in use in many laboratories.

The possibility of reducing the total analytical time of this method to approximately 24 h by simplifying and reducing the enrichment process was investigated. To that end, a new single-stage enrichment medium, SCCRAM broth, was developed to enhance rapid repair and growth of *Salmonella* from food samples. This medium, when used already warm and incubated at 42°C, enables the enrichment step to be reduced to a single 6–7 h enrichment. Following this short enrichment, a 1 mL portion is filtered and the filter incubated on EF-18 agar for 18–24 h at 42°C, as is the case in the current method. Moreover, an investigation was made of direct somatic antigen screening of presumptive positive colonies directly from the EF-18 filter using a commercial (Oxoid, Inc. Basingstoke, Hampshire, UK) latex agglutination test kit to maximize the specificity of the presumptive test. As is the case for the existing 42 h method, presumptive positive colonies on EF-18 agar are viable, isolated colonies and can be subcultured directly for confirmation.

This method was developed during 1999 and the precollaborative study protocol was accepted by the GR, Statistician, and Committee in the first quarter of 2000. The precollaborative study is approximately 50% completed. Accumulated results to date indicate that the test method is performing equivalently to the reference method.

BioControl Systems Methods

ISO 6579 Salmonella Culture Method

Study Director Philip Feldsine, BioControl Systems, Inc., Bellevue, WA, reported on the evaluation of the relative efficiencies of the ISO 6579 *Salmonella* culture method and AOAC culture methods **995.20** and **2000.06**. A collaborative study report, consisting of inclusivity data and a collaborative study, has been submitted. This effort is the result of a cooperative effort between AOAC and the organizers of the Comité Européen de Normalisation (CEN) project to develop performance data on ISO 6579. Three foods (fresh cheese, dried egg powder, and raw poultry meat) were included in this trial. Twenty-one laboratories from Europe and the United States participated in this trial. It is the first time that such a worldwide study of 2 internationally recognized reference procedures has been conducted. The objective was to demonstrate the equivalence of ISO 6579 and AOAC **995.20** and **2000.06** reference culture methods for the detection of *Salmonella* and to promote international method harmonization. Two food types (dried egg and poultry) were evaluated twice. Although there were differences between the methods for every food, the methods were statistically equivalent for each of the food types.

SimPlate Total Plate Count Color Indicator Method for Quantitative Enumeration of Total Aerobic Bacteria

Study Director Philip Feldsine. SimPlate Total Plate Count Color Indicator (TPC-CI) uses a color indicator reaction that correlates enzyme activity to the presence of foodborne bacteria. Total viable bacteria are detected in each well of the SimPlate device by the biochemical reduction of the substrate, which is blue, to a pink or clear byproduct. Variations in the color of positive wells also occur and are considered positive results. Enumeration of total aerobic bacteria is achieved by counting the numbers of wells in each plate with a color change after 24 h incubation. A method comparison study has been completed comparing SimPlate TPC-CI to the AOAC reference culture procedure for all food types, except dairy products, which were compared against the *Standard Methods for the Examination of Dairy Products* (4) procedure. The study manuscript has been submitted for review, and the collaborative study is pending.

Listeria monocytogenes and Related Species, Assurance® Enzyme Immunoassay

Study Director Philip Feldsine. The Assurance *Listeria* Enzyme Immunoassay (EIA) is a colorimetric EIA configured in a 96-test well format and uses a formulation of polyclonal antibodies to *L. monocytogenes* and related species.

Precollaborative and inclusivity studies were conducted comparing the Assurance system to methods contained in the BAM and/or in use by the U.S. Department of Agriculture (USDA; 5), depending upon the food type being analyzed. A collaborative study was completed. The method was adopted First Action in 1996 and Final Action in 1998.

Recently, considerable interest has been expressed for monitoring the production environment for the presence of *Listeria* spp. A validation methodology consisting of both methods comparison and collaborative studies has been published by AOAC as a guideline. Using this guideline, a method applicability statement modification to include monitoring environmental surfaces was validated and approved in February, 2001.

Listeria monocytogenes and Related Species, Visual Immunoprecipitate Assay

Study Director Philip Feldsine. The Visual Immunoprecipitate (VIP) method for *L. monocytogenes* and related species uses highly specific antibodies directed against antigens produced by *L. monocytogenes* and related species. The assay is configured in a single-use device, which produces a visually determined reaction on a solid support in the presence of *L. monocytogenes* and related species. Following a 2-step enrichment protocol, 0.1 mL of the secondary enrichment broth is transferred into the sample addition well of the VIP device. If *Listeria* is present in the sample, a detection line will form in the viewing window of the device. Additionally, a procedural control line will form, indicating proper test completion. After enrichment, total assay time is about 10 min from the time of broth addition to the device. Methods comparison and collaborative studies were conducted. The method was adopted Final Action by the membership in 1999.

Salmonella, Assurance® Enzyme Immunoassay (EIA)

Study Director Philip Feldsine. The Assurance *Salmonella* EIA is a colorimetric EIA configured in a microwell format. This assay incorporates polyclonal antibodies specific to *Salmonella*. The Method, **992.11**, was adopted Official First Action in January 1992, and Final Action by the membership in 1996. The method has also been reviewed and approved by USDA Agricultural Marketing Service for testing egg and powdered milk products and Food Safety Inspection Service for meat and poultry products. A method modification protocol to examine alternative enrichments is currently under review.

Other Methods

Clostridium botulinum Toxins A, Proteolytic B, and E, ELCA Enzyme Immunoassay

Study Director Wendy Lepper tested an EIA procedure, developed by Elcotech, that determines presence or absence of toxin within 5 h when applied directly to a culture filtrate or a food sample extract. Moreover, if the sample is positive, the type is identified as toxin type A, B, or E. Positive results are confirmed by the mouse bioassay. For routine screening of

samples for botulinal toxin, the procedure offers a considerable time savings and minimizes the need for live animals.

In a precollaborative comparative evaluation of the EIA test and the mouse bioassay, 960 test portions, representing 16 food types, were prepared without toxin and with low and high levels of toxin. The tests were in agreement 93% of the time. The false-negative level for the EIA was 0% compared to 11% for the mouse bioassay. Among the wide variety of foods tested, no interferences among the 960 test portions were noted.

The precollaborative study has been submitted and approved by AOAC. A collaborative study protocol is under review by the Committee. A collaborative study is planned.

Detection of Botulinal Neurotoxins A, B, E, and F Using an Amplified EIA System

Study Directors Joseph L. Ferreira, U.S. FDA, Atlanta, GA; Susan Maslanka, Centers for Disease Control and Prevention, Atlanta, GA; Eric Johnson and Mike Goodnough, University of Wisconsin, Madison, WI. There are 4 *Clostridium botulinum* toxins commonly implicated in human botulism that may be manifested as wound, infant, or foodborne disease (6). The standard test for toxin, either performed in foods or from culture, is the mouse bioassay (1). Mice are inoculated and observed for botulism symptoms in a 3-stage process. This process begins with a screening assay followed by a titration of toxicity (using end point death) and finally toxin typing using an antibody neutralization assay. The entire process requires at least 6 days. The amplified EIA is a rapid procedure for the determination of botulinal toxin type from culture media used in the mouse bioassay for cultural toxin production (7). The EIA method uses an antibody-coated plate to capture specific toxin followed by specific biotin-labeled antibody. The biotin residues are bound by alkaline phosphatase-labeled avidin 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. The positive test is an absorbance value >0.015 above that of the negative control. The EIA results are obtained in 6–7 h using a previously prepared microtiter plate.

In the past 6 months several important accomplishments have been achieved. First, the collaborative study protocol was approved by the Committee. Second, the assay has been improved slightly by changing the buffering system to reduce background interference while maintaining the sensitivity and specificity. Third, the test portions were prepared and sent out to the collaborators. The test portions have been analyzed and collaborative study data are being analyzed.

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 Michael Curiale, Silliker Laboratories Group, Inc., 160 West Armory Dr, South Holland, IL 60473, Tel: +1-708-225-1435, Fax: +1-708-225-1536, E-mail: michael.curiale@silliker.com. Method adopted First Action in

1998. No adverse reports about the method. Method is recommended for Final Action. Editorial correction: Change incubation temperature noted in section D, Analysis, from 35°C to 35 ± 1°C.

(2) **999.06** *Listeria in Foods Products, VIDAS Listeria Assay*: Study Directors Ronald Johnson, bioMérieux, Inc., 595 Anglum Rd, Hazelwood, MO 63042-2320, Tel: +1-314-506-8182, Fax: +1-314-731-8678, E-mail: ron.johnson@biomerieux.com; Michael Curiale (*see* 1). Method adopted First Action in 1999. No adverse reports about the method. Method is recommended for Final Action.

(3) **996.08** *Salmonella in Foods, VIDAS SLM Method*: Study Director Michael Curiale (*see* 1). Method adopted First Action in 1996. Editorial correction: In Table **996.08A**, the test portions in column 1 are not properly positioned. Each food type should be raised 2 lines, so that the food type will be on the same line as "Control" in column 2. No adverse reports about the method. Method is recommended for Final Action.

(4) **997.16** *LOCATE® Enzyme-Linked Immunosorbent Assay for Identification of Salmonella in Foods*: Study Director Michael Curiale (*see* 1). Method adopted First Action in 1997. Continue study.

(5) *Clostridium botulinum* Toxins A, Proteolytic B, and E, ELCA Enzyme Immunoassay: Study Director Wendy Lepper, Silliker Laboratories Group, Inc., 160 Armory Dr, South Holland, IL 60430, Tel: +1-708-225-1435, Fax: +1-708-225-1536, E-mail: wendy.lepper@silliker.com. Precollaborative study report has been approved by Methods Committee, and a collaborative study is planned. Continue study.

(6) **2001.07** *Salmonella in Selected Foods by Immunoconcentration: Salmonella (ICS) Selective Plate (HE, BS, SMID) Procedure*: Study Directors Wendy Lepper, (*see* 5); Ronald Johnson (*see* 2). Method adopted First Action in selected foods 2001. Studies currently underway to extend method applicability to all foods. Continue study.

(7) **2001.08** *Salmonella in Selected Foods by Immunoconcentration: Salmonella (ICS) Selective Plate (HE, XLD, BS) Procedure*: Study Directors Wendy Lepper and Ronald Johnson (*see* 5 and 2, respectively). Method adopted First Action in selected foods in 2001. Studies are currently underway to extend method applicability to all foods. Continue study.

(8) **2001.09** *Salmonella in Selected Foods by Immunoconcentration: Salmonella (ICS) and Enzyme-Linked Immunofluorescent assay (ELFA)*: Study Directors Wendy Lepper and Ronald Johnson (*see* 5 and 2, respectively). Method adopted First Action in selected foods in 2001. Studies are currently underway to extend method applicability to all foods. Continue study.

(9) *Evaluation of VIDAS® Salmonella (SLM) Immunoassay Method with Rappaport-Vassiliadis Medium for Detection of Salmonella in Foods*: Study Directors Wendy Lepper and Ronald Johnson (*see* 5 and 2, respectively). Precollaborative and collaborative study protocols, investigating the replacement of SC broth with RV medium in Method **996.08**, have been submitted to the GR.

(10) *Detection of Botulinal Toxins A, B, E, and F from Culture Supernatants, Amplified EIA Procedure*: Study Directors Joseph L. Ferreira, U.S. Food and Drug Administration, 60 8th St NE, Atlanta, GA 30309, Tel: +1-404-253-2216, Fax: +1-404-253-1210, E-mail: jferreira@ora.fda.gov; Susan Maslanka, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA, Tel: +1-404-639-3867, Fax: +1-404-639-3333, E-mail: sht5@cdc.gov; Eric Johnson and Mike Goodnough, 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 and mgoodnou@facstaff.wisc.edu. Precollaborative manuscript approved by Methods Committee. Collaborative study protocol approved by GR. Collaborative study is expected to be completed in August 2001. Continue study.

(11) **997.11** *Escherichia coli O157:H7 Counts in Foods, Hydrophobic Grid Membrane Filter (ISO-GRID) Method Using SD-39 Agar and Serological Confirmation*: Study Director Phyllis Entis, QA Life Sciences, Inc., 6645 Nancy Ridge Dr, San Diego, CA 92121, Tel: +1-858-622-0560, Fax: +1-858-622-0564, E-mail: phyllis@qalifesciences.com. Method adopted First Action in 1997. Continue study.

(12) *Rapid Presence/Absence Screen for Listeria monocytogenes in Foods Using HGMP with LM-137 Agar*: Study Director Phyllis Entis (*see* 11). The precollaborative study protocol has been accepted by the Methods Committee, and the study is planned for this year. Continue study.

(13) *Twenty-Four Hour Rapid Presence/Absence Screen for Salmonella in Foods Using HGMP*: Study Director Phyllis Entis (*see* 11). The precollaborative study has been initiated. Continue study.

(14) *Twenty-Four Hour Presumptive Enumeration of Listeria monocytogenes Using HGMP Procedure with LM-137 Agar*: Study Director Phyllis Entis (*see* 11). Collaborative study report approved by GR and submitted to Committee for possible First Action status. Continue study.

(15) **999.08** *Assurance® Gold Salmonella EIA for the Visual or Instrumental Identification of Motile and Non-Motile Salmonella in All Foods*: Study Director Philip 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. Method adopted First Action in 1999. The Study Director indicates that the method is widely used and that analysts have reported favorably. Method is recommended for Final Action.

(16) **997.03** *Listeria monocytogenes and Related Listeria spp. in Selected Foods, Visual Immunoprecipitate Assay*: Study Director Philip Feldsine (*see* 15). Method adopted First Action in 1997. Method was validated for its effectiveness in detecting *Listeria* species on environmental surfaces and was approved Revised First Action for environmental surfaces in 2001. The method applicability statement has been modified to include environmental surfaces. Continue study.

(17) **999.09** *VIP for Salmonella for the Detection of Motile and Non-Motile Salmonella in All Foods*: Study Director Philip Feldsine (*see* 15). Method adopted First Action in 1999. The Study Director indicates that the method is widely used

and that analysts have reported favorably. Method is recommended for Final Action.

(18) *ISO versus AOAC Reference Culture Methods for the Detection of Motile and Non-Motile Salmonella in Selected Foods*: Study Director Philip Feldsine (see 15). Collaborative study has been completed and no statistically significant difference for the recovery of *Salmonella* from cheese, dried egg, and poultry by the 2 methods was identified. Continue study.

(19) **996.14** *Listeria monocytogenes and Related Listeria Species Detection in Selected Foods, Assurance Polyclonal Enzyme Immunoassay Method*: Study Director Philip Feldsine (see 15). Method adopted First Action in 1996, Revised First Action in 1999, and Final Action in 1999. Method was validated for its effectiveness in detecting *Listeria* species on environmental surfaces and was approved Revised First Action for environmental surfaces in 2001. The method applicability statement has been modified to include environmental surfaces. Continue study.

(20) *Probelia PCR Method for Salmonella*: Study Director Philip Feldsine (see 15). Precollaborative study manuscript has been reviewed by GR. Continue study.

(21) **992.11** *Motile and Nonmotile Salmonella in Foods, Polyclonal Enzyme Immunoassay Method*: Study Director Philip Feldsine (see 15). Method adopted First Action in 1992, Final Action in 1996, and Revised First Action in 1999. Method modification protocol submitted to the GR to validate the replacement of SC broth with RV medium. Study Director was asked to revise the protocol. Continue study.

(22) *SimPlate CEs Quantitative Method for Total Coliforms and E. coli*: Study Director Philip Feldsine (see 15). Precollaborative study protocol approved by the Committee. Continue study.

(23) **2000.06** *Detection of Salmonella in Foods with a Low Microbial Load, Rappaport-Vassiliadis Medium Method*: Study Director Thomas Hammack, U.S. Food and Drug Administration, 200 C St SW, Washington, DC 20204, Tel: +1-202-205-4753, Fax: +1-202-401-7740, E-mail: thomas.hammack@cfsan.fda.gov. Method adopted First Action in 2000. Editorial correction: When published in the OMA, Method **2000.06** should directly follow Method **995.20**. Continue study.

(24) *Staphylococcus aureus in Foods, TECRA S. aureus Visual Immunoassay*: Study Director Denise Hughes, TECRA International, PO Box 788, Willoughby, NSW, 2068, Australia, Tel: +61-2-9928-8827, Fax: +61-2-9928-8861, E-mail: denise.hughes@tecrea.net. Precollaborative and collaborative study protocols have been approved by the Committee. The Study Director has requested: (a) the use of a 3 g sample with the TECRA method; (b) the use of surface plating method **975.55** rather than **987.09** as the reference culture method. These requests have been approved by the GR and have been forwarded to the Committee.

(25) *Determination of E. coli in Flesh Foods Using Visual Immunoassay (TECRA Immunoassay) with a Modified Culture Procedure*: Study Director Denise Hughes (see 24). A collaborative study protocol has been approved by the Methods Committee. Continue study.

(26) **2000.07** *Salmonella in Foods, Rapid Colorimetric Immuno-enrichment-Based Screening Method (TECRA Unique Salmonella Test)*: Study Director Denise Hughes (see 24). Method adopted First Action in 2000. Study report to validate the method for fruit juices approved by the GR. Study protocol approved by the GR to validate a new format for the test module, which will allow the test to be conducted and read automatically. Recommend Revised First Action to include fruit juices in the applicability statement and recommend Revised First Action for format changes to module to be included in the method, subject to review of validation data. Continue study.

(27) **2000.07** *Modified TECRA Unique Salmonella Test*: Study Director Denise Hughes (see 24). Even though the TECRA Unique *Salmonella* Test, **2000.07**, was recently adopted First Action (see 26), TECRA is planning to modify the enrichment methods and module format. The Committee has approved a precollaborative study protocol to validate changes to the enrichment protocols, as well as the manual and automated reading of results. Continue study.

(28) **995.22** *Listeria in Foods, Colorimetric Polyclonal Enzyme Immunoassay Screening Method (TECRA® Listeria Visual Immunoassay [TLVIA])*: Study Director Denise Hughes (see 24). Method adopted First Action in 1995 and Final Action in 1999. A collaborative study has been conducted to extend this method to validate new enrichment media for the method. A precollaborative study protocol has been submitted to extend the method to environmental surfaces. Continue study.

(29) *Salmonella in Foods, REVEAL for Salmonella Test System*: Study Director Charles Bird, Neogen Corp., 620 Leshar Pl, Lansing, MI 48912, Tel: +1-517-372-9200, Fax: +1-517-372-0108, E-mail: Cbird@neogen.com. No report submitted. Continue study.

(30) *REVEAL for Escherichia coli O157:H7, Twenty-Hour Screening Test*: Study Director Charles Bird (see 29). No report submitted. Continue study.

(31) *REVEAL for Escherichia coli O157:H7, Eight-Hour Screening Test*: Study Director Charles Bird (see 29). No report submitted. Continue study.

(32) *Salmonella in Food, REVEAL Alert Test System*: Study Director Charles Bird (see 29). No report submitted. Continue study.

(33) **990.13** *Salmonella in Foods, Colorimetric Deoxyribonucleic Acid Hybridization Method (GENE-TRAK)*: Study Director Mark Mozola, GENE-TRAK Systems, 94 South St, Hopkinton, MA 01748, Tel: +1-508-435-7401, Fax: +1-508-435-0025, E-mail: mmozola@vysis.com. Method adopted First Action in 1990, Revised First Action in 1992, and Final Action in 1996. A precollaborative study was completed to replace SC broth with RV medium in the method. Several food types yielded nonfractional results. The analysis of those foods will be repeated. Continue study.

(34) *Detection of Salmonella, GENE-TRAK Salmonella DLP (Direct-Labeled Probe) Assay*: Study Director Mark Mozola (see 33). Precollaborative study is in progress. A collaborative study is planned. Continue study.

(35) **2000.15** *Coliform Counts in Foods, Dry Rehydratable Film Method*: Study Director Karen

Silbernagel, R-Tech Laboratories, MS 0075, PO Box 64101, St. Paul, MN 55164, Tel: +1-651-766-1303, Fax: +1-651-486-0837, E-mail: ksilb@landolakes.com. Method adopted First Action in 2000. Continue study.

(36) **2001.05 Rapid Enumeration of *Staphylococcus aureus* in Selected Foods, Dry Rehydratable Film Method:** Study Director Karen Silbernagel (see 35). Method adopted First Action in 2001. Continue study.

(37) **Enumeration of Enterobacteriaceae in Foods, Dry Rehydratable Film Method:** Study Directors Karen Silbernagel (see 35). A collaborative study has been completed and a report is being prepared. Continue study.

(38) **VIDAS *Listeria monocytogenes* (LMO) Immunoassay Method for Detection of Listeria:** Study Director Karen Silbernagel (see 35); Ronald Johnson (see 2). A precollaborative study protocol has been submitted. Continue study.

(39) **Salmonella in Foods, Automated Conductance Method:** Study Director Donald Gibson, Biodon, 43 Brighton Pl, Aberdeen AB10-6RT, Untied Kingdom, Tel: +44-1224-322-777, Fax: +44-1224-322-777. Continue study.

(40) **Aloa Listeria Detection Medium:** Study Director Karen Jarvis, Microbiology International, 97 H. Monocracy Blvd, Frederick, MD 21701, Tel: +1-301-662-6835, Fax: +1-301-662-8096. Continue study.

(41) **SimPlate Total Plate Count Color Indicator Method:** Study Director Philip Feldsine (see 15). Continue study.

(42) **988.18 Aerobic Plate Count, Pectin Gel Method:** Study Director Sonya Gambrel-Lenarz, 3M Health Care, 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. Method adopted First Action in 1988 and Final Action in 1990. Editorial correction: Insert “™” following first use of “Redigel” (see A. Principle, first line).

(43) **990.12 Aerobic Plate Count in Foods, Dry Rehydratable Film (Petrifilm Aerobic Count Plate) Method:** Study Director Sonya Gambrel-Lenarz (see 42). Method adopted First Action in 1990 and Final Action in 1994. Editorial correction: (a) Place “™” after “Petrifilm” in method title and the first time the product name is mentioned in the method description; (b) Change address in Method **990.12** to Microbiology Products, 3M Center, Bldg 275-5W-05, St. Paul, MN 55144 [see B. Apparatus and Reagent (a) and (b)].

(44) **991.14 Coliform and *Escherichia coli* Counts in Foods, Dry Rehydratable Film (Petrifilm *E. coli* Count Plate) and (Petrifilm Coliform Count Plate) Methods:** Study Director Sonya Gambrel-Lenarz (see 42). Method adopted First Action in 1991 and Final Action in 1994. Editorial correction: (a) Replace “®” with “™” in method title and the first time the product name is mentioned in the method description; (b) Change address in Method **991.14** to Microbiology Products, 3M Center, Bldg 275-5W-05, St. Paul, MN 55144 [see B. Apparatus and Reagent (a) and (b)]; (c) Change product

name from “Petrifilm™ *E. coli* Count Plate” to “Petrifilm™ *E. coli* / Coliform Count Plate.” Do not italicize “*E. coli*” within the product name.

(45) **997.02 Yeast and Mold Counts in Foods, Dry Rehydratable Film (Petrifilm) Method:** Study Director Sonya Gambrel-Lenarz (see 42). Method adopted First Action in 1997 and Final Action in 2000. Editorial correction: (a) Insert “Final Action 2000”; (b) Change address to Microbiology Products, 3M Center, Bldg 275-5W-05, St. Paul, MN 55144 [see, B. Apparatus (a)].

(46) **995.20 Salmonella in Raw, Highly Contaminated Foods and Poultry Feed:** Study Director Thomas Hammack (see 23). Method adopted First Action in 1995 and Final Action in 1999. Editorial correction: (a) Insert “The analyst should be aware that the selective enrichment combination tetrathionate broth/Rappaport-Vassiliadis medium may not be effective for the recovery of *Salmonella* Typhi and Paratyphi from foods.” after “...selective agars.” (see A. Principle); (b) The Method **995.20** is misplaced and should directly follow Method **967.26**; (c) In Table **995.20A**, change footnote a, “Sensitivity rate is test positive to known positive ratio [McClure, F.D. (1990) *J. Assoc. Off. Anal. Chem.* **73**, 953–960]. Known positive is defined as a test portion that was positive from any one or more of the 4 selective enrichments.” to “The sensitivity rate is the ratio of positive test portions to known positive test portions [McClure, F.D. (1990) *J. Assoc. Off. Anal. Chem.* **73**, 953–960]. A known positive test portion is defined as a test portion that was positive from any one or more of the 4 selective enrichments.”

(47) **966.24 Coliform Group and *Escherichia coli* in Tree Nut Meats:** Study Director Vacant. Adopted First Action in 1966 and Final Action in 1971. Editorial correction: “in Tree Nut Meats” appears to have been incorrectly added to the method title after the method was adopted First Action. The collaborative study, referring to tree nuts, was not published until 1968 (*J. AOAC* **51**, 867) and was used to revise Method **966.23**. Thus, “in Tree Nut Meats” should be removed from the method title.

References

- (1) *Official Methods of Analysis* (2000) 17th Ed. and suppl., AOAC INTERNATIONAL, Gaithersburg, MD
- (2) *Bacteriological Analytical Manual* (1998) 8th Ed., Rev. A., AOAC INTERNATIONAL, Gaithersburg, MD
- (3) Entis, P., & Lerner, I. (2000) *J. Food Prot.* **63**, 354–363
- (4) *Standard Methods for the Examination of Dairy Products* (1993) 16th Ed., R.T. Marshall (Ed.), American Public Health Association, Washington, DC
- (5) *Microbiology Laboratory Guidebook* (1998), 3rd Ed., U.S. Government Printing Office, Washington, DC
- (6) Hatheway, C.L., & Ferreira, J.L. (1996) *Natural Toxins II: Structure, Mechanism of Action, and Detection*, B.R. Singh and A.T. Tu (Eds.), Plenum Publishing Corp., New York, NY
- (7) Ferreira, J.L. (2001) *J. AOAC Int.* **84**, 85–88