The 3M Tecra Listeria Visual Immunoassay (VIA) method was previously validated in the AOAC Official Methods Program for the detection of Listeria species in raw meats, poultry, fresh produce and vegetables, processed meats, seafood, dairy foods (cultured and noncultured), fruit, and fruit juices. The method was shown to be comparable to the U.S. Food and Drug Administration’s Bacteriological Analytical Manual, the U.S. Department of Agriculture-Food Safety and Inspection Service’s Microbiology Laboratory Guidebook, and AOAC Official Method 993.12 reference culture procedures (1–3). The current study extends the validation to include ready-to-eat (RTE) meats (liver paté, hot dogs, raw fermented sausage, sliced deli turkey, and sliced deli ham) and stainless steel surfaces in comparison to the Health Canada, Health Products and Food Branch Compendium of Analytical Methods reference method (4).

Definitions

(a) Chi-square.—A Chi-square value <3.84 indicates that the proportions positive for the alternative and reference methods are not statistically different at the 5% level of significance. This criterion must be satisfied for each level of each sample type. However, a significant difference between the proportions positive for the two methods is acceptable provided that the alternative method demonstrates recovery superior to that of the reference method.

(b) Unpaired test portions.—Mantel-Haenszel:

\[ \chi^2 = (n - 1)(ad - bc)^2/[(a + b)(a + c)(b + d)(c + d)] \]

where \( n \) = total number of samples tested by the two methods, \( a \) = number of samples positive by the test method, \( b \) = number of samples positive by the reference method, \( c \) = number of samples negative by test method, and \( d \) = number of samples negative by the reference method (5).
Principle of the Method

3M Tecra Listeria VIA detects Listeria antigens from enriched foods, food ingredients, and environmental samples by an ELISA performed in “sandwich” configuration. If Listeria antigens are present, they are captured by specific high affinity polyclonal antibodies adsorbed to wells. All other materials are washed away. The sandwich is completed by addition of enzyme-labeled polyclonal antibodies (i.e., conjugate) specific for Listeria. Following washing of wells and addition of colorless substrate, development of a green color indicates a presumptive positive reaction. Determination of positive results by 3M Tecra Listeria VIA can be performed either visually or spectrophotometrically at 414 ± 10 nm for a single wavelength reader, and referenced against 490 ± 10 nm for dual wavelength readers.

General Information

Test Kit Information

(a) Kit name.—3M Tecra Listeria Visual Immunoassay.
(b) Cat. Nos.—LISVIA30, LISVIA48, LISVIA96.
(c) Ordering information.—Tel: 800-328-6553.
(d) Test kit components.—
   (1) Wash concentrate.—One vial (25 mL/vial). 1.0 g Tris, 6.0 g NaCl, 0.1 g Tween 20, and 2.0 mg thimerosal in water.
   (2) Positive control.—One vial. Lyophilized purified Listeria antigen, 0.02 g gelatin, 0.04 g borate buffer, and 0.2 mg thimerosal.
   (3) Control diluent.—One vial (6 mL/vial). 0.01% saline, 0.01 g Tris, 1.0 mg Tween 20, and 0.01 mg thimerosal.
   (4) Conjugate.—Two vials. Lyophilized anti-Listeria antibodies, 0.1 g borate buffer, 0.02 g gelatin, and 0.1 mg thimerosal. Reconstituted conjugate is stable 30 days when stored at 2–8°C.
   (5) Conjugate diluents.—Two vials (13.5 mL/vial). 0.2 g borate buffer and 2.0 mg thimerosal in water.
   (6) Substrate.—One vial. Lyophilized 0.01 g 2,2’-azino-di(3-ethylbenzthiazoline sulfonate) and 0.1 g NaH2PO4·2H2O. Substrate diluents.—One vial (26 mL/vial). 0.1% acetic acid and 0.003 g H2O2 in water.
   (8) Stop solution.—One vial (6 mL/vial). 0.15 g sodium fluoride in water.
   (9) Sample additive.—One vial (6 mL/vial). 1.0 g Tris, 0.1 g Tween, and 1.0 mg thimerosal in water.
   (10) Test (Removawell) strips (polyclonal antibodies to Listeria) and holder for securing wells or strips.
   (e) Additional supplies and reagents.—
      (1) 3M Tecra Listeria VIA broth (complete medium).—Prepare according to manufacturer’s instructions.
      (2) 3M Tecra Listeria VIA broth base.—Prepare according to manufacturer’s instructions.
      (3) 3M Tecra Listeria VIA supplement.—Use according to manufacturer’s instructions.

Apparatus

(a) Serological pipets.—Calibrated to deliver 1 mL, graduated in 0.1 mL units.
(b) Micropipets.—Accurately dispensing 0.2, 0.05, and 0.02 mL.
(c) Test tubes.—13 × 100 and 16 × 125 mm, with caps.

(d) Boiling water bath.—Alternatively, autoclave with flowing steam, set at 100°C, may be used.
(e) Incubators.—Maintaining 35–37 and 28–30°C.
(f) Plastic squeeze bottle.—500 mL, for dispensing wash solution.
(g) Package insert.
(h) Sample record sheet.
(i) Color card.—For visual interpretation of positive and negative results.
(j) Enzyme immunoassay reader.—Optional. Photometer with 414 ± 10 nm screening filter that reads through microtiter wells. Use either a single wavelength reader set to zero (blank) while reading through unreactive substrate wells or wells with water, or a dual wavelength reader, with a second reference filter set at 490 ± 10 nm set to zero (blank) on an empty cell.

Reference Test Cultures

(a) L. monocytogenes 1/2a, ATCC 49594.
(b) L. monocytogenes 1/2b, ATCC BAA-751.
(c) L. innocua, ATCC 33091 (human feces).
(d) L. monocytogenes 3b, CWD 1591 (University of Vermont).
(e) L. monocytogenes 3c, FSL-J1-049 (Cornell University).
(f) L. monocytogenes 4b, ATCC 19115 (human).
(g) L. monocytogenes 4d, ATCC 19117 (sheep).
(h) Enterococcus faecalis, ATCC 29212 (urine).

Standard Solutions

(a) Dey-Engley (D/E) neutralizing broth.—Difco product No. 0819-17, or equivalent.
(b) Fraser broth.—5.0 g protease peptone, 5.0 g tryptone, 5.0 g Lab Lemco powder (meat extract), 5.0 g yeast extract, 20.0 g NaCl, 1.35 g KH2PO4, 12.0 g Na2HPO4, 1.0 g esculin, 3.0 g LiCl, and 20 mg nalidixic acid. Suspend ingredients in 1.0 L water. Dispense 10 mL portions into 16 × 125 mm test tubes. Cap test tubes and autoclave 15 min at 121°C on slow exhaust and cool to 20–25°C. Just before use, add the following filter-sterilized reagent additives: 0.1 mL (2.5 mg/mL) acriflavine hydrochloride and 0.1 mL (5% in distilled water) ferric ammonium citrate. Use of commercially available Fraser broth is also acceptable if its formulation is the same as that described.
(c) Diagnostic reagents.—Necessary for culture confirmation of presumptive positive 3M Tecra Listeria VIA tests.

Safety Precautions

L. monocytogenes infection can cause fetal death. Pregnant women and persons who are immunocompromised because of illness, medication, or advanced age should avoid handling this organism. Sterilize contaminated equipment and media before disposal or reuse.

General Preparation

(a) Refrigerate all components in the 3M Tecra Listeria VIA kit at 2–8°C when not in use. Bring components to room temperature before use. Kit components are intended for use as an integral unit. Conjugate, positive control, and antibody-
Table 1. Sample enrichment protocols

<table>
<thead>
<tr>
<th>Sample size, g</th>
<th>Enrichment medium</th>
<th>Enrichment time, h</th>
<th>Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>N/A</td>
<td>Transfer 0.1 mL into 9.9 mL LVB</td>
<td>22–24</td>
</tr>
<tr>
<td>Environmental swab</td>
<td>N/A</td>
<td>Transfer 1 mL into 9 mL FB</td>
<td>30</td>
</tr>
<tr>
<td>Enrichment</td>
<td>20 mL UVM</td>
<td>Transfer 0.1 mL into 9.9 mL LVB</td>
<td>30</td>
</tr>
</tbody>
</table>

Secondary enrichment:

- Add 4 h in LVB base without supplement
- Incubate for 4 h, add 1 reconstituted vial of LVB base without supplement
- Incubate 20 h at 35–37°C
- Add 1 vial (2 mL) 3M Tecra Listeria VIA supplement
- Incubate 20 h at 35–37°C
- Transfer 1.0 mL 3M Tecra Listeria VIA broth culture to 9 mL fresh 3M Tecra Listeria VIA broth (complete medium)
- Incubate 24 h at 30°C
- Transfer 1.0 mL of second 3M Tecra Listeria VIA broth culture into 13 × 100 mm clean test tube

(a) Sample enrichment.—Aseptically add 25 g test portion to 475 mL 3M Tecra Listeria VIA broth base and blend 2 min at high speed in a food blender, or stomach for 2 min. Incubate 4 h at 35–37°C. Add 1 vial (2 mL) 3M Tecra Listeria VIA supplement. Incubate 20 h at 35–37°C. Transfer 1.0 mL 3M Tecra Listeria VIA broth culture to 9 mL fresh 3M Tecra Listeria VIA broth (complete medium). Incubate 24 h at 30°C. Transfer 1.0 mL of second 3M Tecra Listeria VIA broth culture into 13 × 100 mm clean test tube.

For environmental samples, hydrate sponge or swab with 10 mL D/E neutralizing broth; sample area (sponge 100 cm², swab 5 cm²) in a horizontal and vertical motion, add 200 mL University of Vermont medium (UVM) to sponge, 20 mL UVM to swabs, incubate 40–48 h at 30°C, and perform the assay.

(b) Prepare 3M Tecra Listeria VIA reagents following the product instructions for use.

(c) To the 1 mL aliquot of secondary 3M Tecra Listeria VIA broth or Fraser broth culture, add 50 µL of sample additive and mix.

(d) Heat 15 min in a boiling water bath or in an autoclave with flowing steam set at 100°C. Cool to 25–37°C. Keep the unheated broth portion for cultural confirmation.

(e) Open the pouch and break off the required number of wells from the test (Removawell) strip, using one well/sample, one well for positive control, and one for negative control. Place the unused wells back into the pouch and reseal with resealing strip.

(f) Secure the desired number of antibody-coated test strips in holder. Press firmly into place.

(g) Using a new pipet tip for each test, transfer 0.2 mL of each heated broth into an individual well. Transfer 0.2 mL negative control and 0.2 mL reconstituted positive control into individual wells. Record sample positions on the record sheet. Cover the tray with plastic film wrap and incubate 30 min at 35–37°C. Note: Tray must be covered to prevent evaporation.

(h) Wash plate as follows: Ensure that test strips are pressed firmly into the holder. Quickly invert the tray, emptying its contents into a waste container. Remove residual liquid by striking the holder firmly several times face down on a thick pile of absorbent paper towels. Hold the squeeze bottle above the plate and using a wide nozzle, squeeze, and completely fill each well. Do not trap air bubbles in the bottom of the wells. Wash and completely empty the wells three times. Make sure that the plate is empty before proceeding to the next step.

(i) Add 0.2 mL of reconstituted conjugate to each well. Cover the tray with plastic film wrap and incubate 30 min at 35–37°C.
Table 2. Test foods/surfaces and inoculating organisms

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Inoculating organism</th>
<th>Matrix</th>
<th>Inoculating organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deli turkey</td>
<td><em>L. monocytogenes</em> 3c</td>
<td>Raw fermented</td>
<td><em>L. monocytogenes</em> 3b</td>
</tr>
<tr>
<td></td>
<td>FSL-J1-049^8</td>
<td>sausage</td>
<td>CWD-1591^8</td>
</tr>
<tr>
<td>Hot dogs-1</td>
<td><em>L. monocytogenes</em> 1/2b</td>
<td>Deli ham</td>
<td>Deli ham</td>
</tr>
<tr>
<td></td>
<td>ATCC BAA-751 plus 10x<em>L. innocua</em> 4ab</td>
<td>ATCC 33091</td>
<td>ATCC 19115</td>
</tr>
<tr>
<td>Hot dogs-2</td>
<td><em>L. innocua</em> ATCC 33091 plus 10x <em>E. faecalis</em></td>
<td>ATCC 29212</td>
<td></td>
</tr>
<tr>
<td>Liver paté</td>
<td><em>L. monocytogenes</em> 1/2a</td>
<td>Stainless steel</td>
<td><em>L. monocytogenes</em> 4d</td>
</tr>
<tr>
<td></td>
<td>ATCC 49594</td>
<td></td>
<td>ATCC 19117 plus 10x <em>E. faecalis</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATCC 29212</td>
</tr>
</tbody>
</table>

^a Obtained from Cornell University culture collection.
^b Obtained from the University of Vermont culture collection.

(j) Empty the tray and wash it thoroughly four times, as in step (h).
(k) Add 0.2 mL of reconstituted substrate to each well. Incubate 15 min at room temperature (20–25°C) or until the positive control has reached an absorbance >1.0 or a color as dark as panel No. 4 on the color card. Color development tends to concentrate around the edges of the wells. Tap the sides of the plate gently to mix the contents before reading the result to obtain accurate readings. Note: If absorbance of 1.0 is not attained within 30 min, the test is invalid.
(l) Add 20 µL of stop solution to each well. Tap the sides of the plate gently to mix the contents. Read within 30 min.

Interpretation and Test Result Report

(a) **Visual determination.**—Place holder onto a white background and then compare individual test wells with the color card. Test is valid if the positive control gives a green color at least as dark as panel No. 4 on the color card and the negative control is within the negative range on the color card.

(b) **Spectrophotometric determination.**—Read test absorbance, A, at 414 ± 10 nm using the plate reader. Blank the single wavelength instrument on a well containing 200 µL substrate or water. When using dual wavelength readers, set a second reference wavelength at 490 ± 10 nm and blank the instrument on air.

(1) Test is valid if $A$ of the positive control is >1.0 and $A$ of the negative control is <0.2.

(2) Test sample is considered positive if $A$ is ≥0.2.

(3) Test is considered negative if $A$ is <0.2.

(4) If $A$ of the negative control is ≥0.2 or if $A$ of the positive control is <1.0, test is invalid.

Confirmation


Validation Study

This validation study was conducted under the AOAC Research Institute GovVal program. The study was designed to compare previously AOAC-approved rapid methods to the Health Protection Branch MFHPB-30 reference method for the detection of *L. monocytogenes* in RTE meats and *Listeria* species on stainless steel surfaces.

The specific matrices evaluated were liver paté, hot dogs, raw fermented sausage, sliced deli turkey, sliced deli ham, and stainless steel. A total of 45 samples for each of the six matrices were analyzed. Within each sample set were 20 replicate test portions, each at two contamination levels, where fractionally positive results (5–15 positive results/20 replicate portions tested) were obtained by at least one method at one level. Five uncontaminated controls were included per method. Replicates were taken from a singularly inoculated lot of material. A different serotype of *L. monocytogenes* was used for each matrix. All samples were prepared, packaged into test portions, and blind-coded before method analyses were performed. In addition, Q Laboratories performed reference method analyses on all matrices.

Methodology

All foods were prescreened using Health Protection Branch MFHPB-30 to ensure that no naturally contaminating *L. monocytogenes* were present. An aerobic plate count was conducted to evaluate the level of background flora present in each product.

Strains of *L. monocytogenes*, *L. innocua*, and *E. faecalis* were cultured by inoculating brain heart infusion (BHI) broth and incubating at 37 ± 2°C for 24 h. Following incubation, the strains to be used for inoculating the food matrices were heat-stressed by incubating at 55°C for 10 min in a water bath to achieve 50–80% injury. The degree of injury of the culture was estimated by plating an aliquot of diluted culture onto Oxford agar and tryptcase soy agar. The agars were incubated at 37°C for 24 h, and colonies counted. The degree of injury was estimated as:

$$\left(1 - \frac{n_{select}}{n_{nonselect}}\right) \times 100$$

where $n_{select}$ = number of CFU on selective agar and $n_{nonselect}$ = number of CFU on nonselective agar.

Each bulk food matrix was divided into three subsamples; (1) one subsample set aside as an uninoculated control; (2) one subsample inoculated with the heat-stressed *L. monocytogenes* culture at a target concentration of 0.2–2 CFU/25 g; and (3) one subsample inoculated with the heat-stressed *L. monocytogenes* culture at a target concentration of 2–5 CFU/25 g. In addition, two rounds of hot dog testing were conducted in which the hot dogs were inoculated with a target and competitor organism. The first set of hot dogs (Hot Dogs–1) was inoculated with *L. innocua* as the competitor, and the second set (Hot Dogs–2) with *E. faecalis*. 

**Note** - While not used in this study, *E. faecalis* (and *E. faecium*) have been shown to be present on processed meats.
such that each competitor was at 10 times the concentration of *L. monocytogenes*.

Following inoculation, the subsamples were stabilized at 4°C for approximately 48 h. Test portions (30 g) were packaged from each subsample. Each sample set consisted of five replicate test portions of uninoculated material, 20 replicate test portions of low-level inoculated material, and 20 replicate test portions of high-level inoculated material. The test portions were randomized, blind-coded, and stored at 2–8°C until the time of analysis. Analyses began approximately 72 h after inoculation of the matrix.

Stainless steel surface pieces were treated with ethanol, rinsed with distilled water, and allowed to dry. *L. monocytogenes* 4d and *E. faecalis* were cultured in BHI at 37°C for 20 h. Cultures were diluted and mixed together in 10% nonfat dry milk such that the *Listeria* was in a concentration range expected to yield fractional positives, and the *Enterococcus* was at a concentration 10 times higher than the *Listeria*. A second surface was prepared and inoculated at twice the expected fractional level. The uninoculated surface areas received 10% nonfat milk only. Surfaces were dried at room temperature for 16–24 h. Swabs were premoistened with D/E neutralizing broth before use. For each sample set, 20 replicate test areas, 1×1 in. each, were swabbed at each inoculation level, plus five replicate uninoculated test areas. The swabs were randomized, blind-coded, and held for 2 h at ambient temperature prior to analysis.

The 3M Tecra Listeria VIA method was carried out according to the product instructions for use. The Health Canada MFHPB-30 reference method was followed for the prescribed method (4). In the case of unpaired test portions, all 3M Tecra Listeria VIA enrichments, regardless of presumptive results, were streaked to selective agar for confirmation of typical colonies as described in the Health Canada MFHPB-30 method.

The most probable number (MPN) was estimated for each inoculation level and was calculated based on the probability of detection of the reference method across all laboratories using the AOAC MPN calculator (6). Enrichments, isolation, and confirmations were carried out according to the Health Canada MFHPB-30 reference method.

### Results

Results of this study are presented in Tables 1–5.

Tables 1–3 list the food matrices studied, a brief description of their properties, and the initial level of background flora as determined by aerobic plate count. Table 4 lists the specific organism inoculated per food matrix, the level at which they were inoculated, and the calculated percent injury of the inoculated organism post-heat-stress. Table 5 shows the analysis of data for all food matrices and the stainless steel surface sample. The method results were analyzed using the Mantel-Haenszel Chi-square analysis (for unmatched test portions). Fractionally positive results (5–15 positives out of 20 replicates) for all RTE meats and the stainless steel environmental surface analyzed in this study were achieved by either the test or reference method for at least one inoculum level.

### Discussion

**RTE Meats**

(a) *Deli turkey.*—A total of 16 low inoculum level (0.2–2 CFU/25 g) and 20 high inoculum level (2–5 CFU/25 g) were confirmed positive for the 3M Tecra Listeria VIA method (Table 5). For the Health Canada MFHPB-30 reference method, 15 deli turkey samples were confirmed positive for the low-level inoculum and 19 deli turkey samples were confirmed positive for the high-level inoculum. In addition, all uninoculated deli turkey samples were negative by the 3M Tecra Listeria VIA method. There was no significant difference in the number of positive results obtained at both the high and low inoculum levels between the 3M Tecra Listeria VIA method and the Health Canada MFHPB-30 reference method.

(b) *Hot dogs—Round 1.*—A total of nine low inoculum level (0.2–2 CFU/25 g) hot dogs samples and 14 high inoculum level (2–5 CFU/25 g) hot dog samples were confirmed positive by the 3M Tecra Listeria VIA method (Table 5). For the Health Canada MFHPB-30 reference method, eight hot dog samples were confirmed positive for the low-level inoculums, and 14 hot dog samples were confirmed positive for the high-level inoculum.

In addition, all uninoculated hot dog samples were negative by the 3M Tecra Listeria VIA method. There was no significant difference in the number of confirmed positive results obtained at both the high and low inoculum levels between the 3M

### Table 3. Aerobic plate counts

<table>
<thead>
<tr>
<th>Food matrix</th>
<th>Properties</th>
<th>CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver paté</td>
<td>Chicken liver, refrigerated, with onion, garlic, bay leaves, thyme, salt, and black pepper</td>
<td>6.8 × 10⁸</td>
</tr>
<tr>
<td>Hot dogs-2 Turkey</td>
<td>Turkey/chicken/pork, 16% fat, vacuum-packed, sodium diacetate, sodium diacetate, and sodium nitrate</td>
<td>8.6 × 10⁸</td>
</tr>
<tr>
<td>Raw fermented sausage</td>
<td>12% fat. Mini Sopressata pork salami with salt, nonfat dry milk, burgundy wine, lactic acid starter culture, sugar, pepper, spices, sodium erythorbate, garlic, sodium nitrite, and sodium nitrate</td>
<td>3.8 × 10³</td>
</tr>
<tr>
<td>Sliced deli turkey</td>
<td>1% fat. Vacuum-packaged, refrigerated, sodium propionate, sodium diacetate, sodium benzoate, sodium ascorbate, and sodium nitrate</td>
<td>1.2 × 10⁴</td>
</tr>
<tr>
<td>Sliced deli ham</td>
<td>2% fat. Vacuum-packaged; refrigerated, with water added</td>
<td>1.3 × 10⁵</td>
</tr>
</tbody>
</table>

### Table 4. Heat-stress injury

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Matrix</th>
<th>CFU/OXA</th>
<th>CFU/BHI</th>
<th>Degree injury, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. monocytogenes</em></td>
<td>1/2a ATCC 49594</td>
<td>2.4 × 10⁸</td>
<td>1.1 × 10³</td>
<td>78</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>1/2b ATCC BAA-751</td>
<td>4.2 × 10⁸</td>
<td>1.5 × 10³</td>
<td>72</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>3b CWD 1591</td>
<td>3.8 × 10⁸</td>
<td>1.2 × 10³</td>
<td>68</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>3c FSL-J1-049</td>
<td>6.6 × 10⁸</td>
<td>1.5 × 10³</td>
<td>56</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>4b ATCC 19115</td>
<td>7.2 × 10⁸</td>
<td>1.8 × 10³</td>
<td>60</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>ATCC 29212</td>
<td>2.8 × 10⁸</td>
<td>8.8 × 10³</td>
<td>68</td>
</tr>
</tbody>
</table>
Table 5. Method comparison results: Chi-square, unpaired test portions

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Strain</th>
<th>MPN&lt;sub&gt;1/25 g&lt;/sub&gt;</th>
<th>No. of test portions</th>
<th>3M Tecra Listeria VIA method</th>
<th>3M Tecra Listeria VIA method</th>
<th>MFHPB-30 method</th>
<th>Chi-square&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Relative sensitivity&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver paté</td>
<td>L. monocytogenes 1/2a</td>
<td>3M Tecra Listeria VIA method</td>
<td>0.693 (0.357–1.204)</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>0.10</td>
<td>115</td>
</tr>
<tr>
<td>Hot dogs-2</td>
<td>L. monocytogenes 1/2b plus 10x E. faecalis</td>
<td>0.511 (0.223–0.916)</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>0.46</td>
<td>115</td>
</tr>
<tr>
<td>Raw fermented sausage</td>
<td>L. monocytogenes 3b</td>
<td>0.7975 (0.431–1.386)</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0.10</td>
<td>110</td>
</tr>
<tr>
<td>Sliced deli turkey</td>
<td>L. monocytogenes 3c</td>
<td>0.598 (0.288–1.050)</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>15</td>
<td>0.14</td>
<td>107</td>
</tr>
<tr>
<td>Sliced deli ham</td>
<td>L. monocytogenes 4b</td>
<td>1.610 (0.932–2.775)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>19</td>
<td>1.00</td>
<td>105</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>L. monocytogenes 4d plus 10x E. faecalis</td>
<td>N/A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> MPN = Most probable number based on the probability of detection of reference method test portions across laboratories using the AOAC MPN calculator, with 95% confidence interval.

<sup>b</sup> Chi-square = Mantel-Haenszel: \( \chi^2 = (n-1)(ad-bc)^2/(a+b)(c+d)(a+c)(b+d) \), where \( n \) = total number of samples tested by the two methods; \( a \) = number of samples positive by the test method; \( b \) = number of samples negative by the test method; \( c \) = number of samples positive by the reference method; and \( d \) = number of samples negative by the reference method.

<sup>c</sup> Relative sensitivity = \( a/c \), where \( a \) = number of samples confirmed positive by the test method and \( c \) = number of samples positive by the reference method.

<sup>d</sup> N/A = Not applicable.

A total of 10 of the 19 low-level 3M Tecra Listeria VIA presumptive positives were confirmed to be negative for L. monocytogenes, resulting in a false-positive rate of 90.9\%. In addition, five of the 19 high-level 3M Tecra Listeria VIA presumptive positives were confirmed to be negative for L. monocytogenes, resulting in a false-positive rate of 83.3\%.

This is explained by the fact that the Health Canada MFHPB-30 reference method is specific to confirmation of L. monocytogenes, whereas 3M Tecra Listeria VIA is specific for Listeria species.

(c) Hot dogs—Round 2.—A total of seven low inoculum level (0.2–2 CFU/25 g) and 11 high inoculum level (2–5 CFU/25 g) hot dog samples were confirmed positive for the 3M Tecra Listeria VIA method (Table 5). For the Health Canada MFHPB-30 reference method, eight hot dog samples were confirmed positive for the low-level inoculum and 10 were confirmed positive for the high-level inoculum. In addition, all uninoculated hot dog samples were negative by the 3M Tecra Listeria VIA method. There was no significant difference in the number of positive results obtained at both the high and low inoculum levels between the 3M Tecra Listeria VIA method and the Health Canada MFHPB-30 reference method.

(d) Liver paté.—A total of 11 low inoculum level (0.2–2 CFU/25 g) and 15 high inoculum level (2–5 CFU/25 g) liver paté samples were confirmed positive for the 3M Tecra Listeria VIA method (Table 5). For the Health Canada MFHPB-30 reference method, 10 liver paté samples were confirmed positive for the low-level inoculum and 13 liver paté samples were confirmed positive for the high-level inoculum. In addition, all uninoculated liver paté samples were negative by the 3M Tecra Listeria VIA method. There was no significant difference in the number of positive results obtained at both the high and low inoculum levels between the 3M Tecra Listeria VIA method and the Health Canada MFHPB-30 reference method.

(e) Raw fermented sausage.—A total of 10 low inoculum level (0.2–2 CFU/25 g) and 20 high inoculum level (2–5 CFU/25 g) raw fermented sausage samples were confirmed positive for the 3M Tecra Listeria VIA method (Table 5). For the Health Canada MFHPB-30 reference method, 10 raw fermented sausage samples were confirmed positive for the low-level inoculum and 15 were confirmed positive for the high-level inoculum. In addition, all uninoculated raw fermented sausage samples were negative by the 3M Tecra Listeria VIA method. There was no significant difference in the number of positive results obtained at the high and low inoculum levels between the 3M Tecra Listeria VIA method and the Health Canada MFHPB-30 reference method.

(f) Deli ham.—A total of 12 low inoculum level (0.2–2 CFU/25 g) and 16 high inoculum level (2–5 CFU/25 g) deli ham samples were confirmed positive for the 3M Tecra Listeria VIA method (Table 5). For the Health Canada MFHPB-30 reference method, 13 deli ham samples were confirmed positive for the low-level inoculum and 16 were confirmed positive for the high-level inoculum. In addition, all uninoculated deli ham samples were negative by the 3M Tecra Listeria VIA method. There was no significant difference in the number of positive results obtained at both the high and low inoculum levels between the 3M Tecra Listeria VIA method and the Health Canada MFHPB-30 reference method.

Environmental Samples

Stainless steel.—A total of eight low inoculum level (0.2–2 CFU/5 cm²) and 10 high inoculum level (2–5 CFU/5 cm²) stainless steel surface samples were confirmed positive for the 3M Tecra Listeria VIA method (Table 5). For the Health Canada MFHPB-30 reference method, 11 stainless steel surface samples were confirmed positive for the low-level inoculum and 12 were confirmed positive for the high-level inoculum. In addition, all uninoculated stainless steel surface samples were negative by the 3M Tecra Listeria VIA method. There was no significant difference in the number of positive results obtained at both the high and low inoculum levels between the 3M Tecra Listeria VIA method and the Health Canada MFHPB-30 reference method.

Conclusions

Statistical analysis indicated no significant difference in the number of positive results between the 3M Tecra Listeria VIA method and the Health Canada MFHPB-30 reference method for the deli turkey, hot dogs, liver paté, deli ham, and the stainless steel surface.

The statistical analysis indicated a significant difference in the number of positive results between the 3M Tecra Listeria VIA method and reference method for the raw fermented sausage food matrix, with the 3M Tecra Listeria VIA method detecting more positives for the high inoculum level, demonstrating recovery superior to that of the reference method.

The 3M Tecra VIA method is a rapid, accurate, specific, and sensitive method for the detection of Listeria in foods and on surfaces.

References


