FOOD BIOLOGICAL CONTAMINANTS

# TECRA® Unique™ Test for Rapid Detection of *Salmonella* in Food: Collaborative Study

**DENISE HUGHES, ANGELA E. DAILIANIS, and LOUISE HILL**
TECRA International, 13 Rodborough Rd, French's Forest, NSW 2086, Australia
**DEBORAH A. MCINTYRE and AIMEE ANDERSON**
R-TECH Laboratories, Lexington Ave, N. Arden Hills, Minneapolis, MN 55126

Collaborators: A. Anderson, C. Barrat, K. Battista, S. Beatty, T. Blanch, D. Briones, R. Brooks, J. Brown, A. Burnie, T. Clark, A. Dailianis, C. Destro, B. Dijs, P. Dombroski, R. Fyfe, J. Gebler, A. Gerry, T. Graham, D. Hadjimichael, S. Heaton, S. Hetrick, E. High, L. Hill, B. Hirt, B. Hulett, Z. Hussain, R. Kalinowski, D. Kerr, N. Matouchi, L. Maycock, S. Munyard, M. Murphy, A. Remes, K. Ristov, R. Robbins, R. Rodriquez, J. Shebuski, V. Simes, K. Smith, S. Vanderpoel, D. Wang

**The TECRA® Unique™ *Salmonella* test uses the principle of immunoenrichment to allow rapid detection of *Salmonellae* in food. A collaborative study was conducted to compare the TECRA *Salmonella* Unique test with the reference culture method given in the U.S. Food and Drug Administration's *Bacteriological Analytical Manual*. Three food types (milk powder, pepper, and soy flour) were analyzed in Australia and 2 food types (milk chocolate and dried egg) were analyzed in the United States. Forty-one collaborators participated in the study. For each of the 5 foods at each of the 3 levels, a comparison showed no significant differences ($p \geq 0.05$) in the proportion of positive test samples for Unique and that for the reference method using the Chi-square test for independence with continuity correction.**

The TECRA® Unique™ *Salmonella* test provides convenient and rapid detection of *Salmonella* in food and food-related test samples. Presumptive positive results can be obtained in <22 h and are then confirmed by standard cultural methods. The Unique *Salmonella* test provides the user with all necessary reagents in a single-test module. An antibody-coated dipstick performs both the immunoenrichment and detection steps (*see* Figure **2000.07**). Prior to the collaborative study, inclusivity and exclusivity studies were undertaken; a precollaborative study including 20 food types was performed to validate the TECRA Unique *Salmonella* test by comparison to the reference method in the U.S. Food and Drug Administration (FDA)'s *Bacteriological Analytical Manual* (BAM; 1).

Submitted for publication July 2000.
The recommendation was approved by the Methods Committee on Microbiology and Extraneous Materials and was adopted by the Official Methods Board of AOAC INTERNATIONAL. *See* "Official Methods Board Actions," (1999) *Inside Laboratory Management*, November/December issue.

## Collaborative Study

The collaborative study was conducted in 2 parts. Milk powder, ground black pepper, and soy flour were analyzed in Australia and New Zealand, and chocolate and dried egg were analyzed in the United States. For the collaborative study all foods were artificially contaminated, although some naturally contaminated foods were included in the precollaborative study.

To obtain the required number of collaborators in a small country such as Australia, some modifications were included in the collaborative study protocol. Two or more analysts working at the same institution were regarded as separate collaborators, provided that they worked independently and used separate media and reagents. In addition, a small number of collaborators sent their isolates to the organizing laboratory for confirmation.

### *Preparation of Inoculum*

The cultures to be used as inocula were grown for 24 h at 35°C in brain heart infusion (BHI) broth. Cultures were centrifuged to pellet cells, and washed twice with 0.1M phosphate buffer, pH 7. The cell pellets were then resuspended in sterile nonfat milk and lyophilized at room temperature (20–25°C) for 24 h. Freeze-dried inocula were ground to a fine powder before use.

### *Inoculation of Test Samples*

For dried products (milk powder, pepper, soy flour, dried egg) a concentrated "seed" was made by adding freeze-dried inoculum to 200–500 g test product. This seeded product was mixed well and stored at room temperature (20–25°C) for 1–2 weeks to allow cell levels to stabilize, before estimation of cell count by serial dilution and plating on xylose lysine desoxycholate agar. According to the estimated *Salmonella* count, an appropriate amount of concentrated seed was added

to the test product for a high level (10–50 cells/25 g) and a low level (1–5 cells/25 g). A most probable number (MPN) determination was made before samples were shipped, and levels of *Salmonella* were adjusted by adding more seed or product if necessary.

For milk chocolate the appropriate dilution of a broth culture was mixed thoroughly into chocolate that had been tempered at 42°C in a plastic bag. The chocolate was allowed to set in a thin layer; it was then pounded to produce small pieces and mixed again by shaking vigorously. The chocolate was stored in the refrigerator (2–8°C) for 1–2 weeks before shipment.

### *Test Sample Shipment*

Dried foods and chocolate were shipped at ambient temperature (20–25°C). Each collaborator received 15 test samples of the food to be analyzed (5 high, 5 low, and 5 uninoculated). The appropriate test samples were shipped to participating laboratories in the week before analysis and stored at room temperature (20–25°C) upon arrival at the laboratory.

### *Analysis*

A different food product was scheduled for testing each week. On the day test sample analysis was initiated by collaborators, an MPN determination was also performed by the organizing laboratory. A triplicate tube MPN was performed using 3 dilutions of the retained test samples for each level according to methods described (3). The MPN test samples were enriched according to the cultural reference method (1). Each test sample was analyzed by the BAM method and the Unique test, using the manufacturer's recommended enrichment. The immunoassay was confirmed by streaking from the M broth culture in tube 3 of the module onto selective agar as specified in the BAM reference culture method (1).

### *Data Analysis*

Data from each food type were collated and the number of positive test samples for each method was calculated. Different enrichment broths and different subsamples of the test food were analyzed with each method. To calculate the performance parameters it was assumed that all inoculated food (i.e., all the low and high level samples) contained *Salmonella*. The problems with statistical analysis of this type of data were discussed in more detail by Gibson et al. (2). Tables **2000.07A** and **B** show 50% end points of each method as well as performance parameters as an indication of method performance. False negative and false positive rates, sensitivity, specificity, and percentage agreement were calculated according to the definitions in Table **2000.07A**.

A false positive result was determined at the first assay or decision point for the procedure, i.e., by reading the color reaction on the paddle for the Unique test and at the plate reading stage for the cultural method. Using culturally confirmed positive dipstick results, the proportion of positive test samples for the Unique method was compared with that for the reference method using the Chi-square test for independence with continuity correction ($p \geq 0.05$) to determine whether the methods were significantly different.

$$\lambda^2 = N\,(|AD - BC| - N/2)^2 / (A + B)\,(C + D)\,(A + C)\,(B + D)$$

where A is the number of positive test samples using the test method, B is the number of positive test samples using the reference method, C is the number of negative test samples using the test method, and D is the number of negative test samples using the reference method. N = A + B + C + D.

## AOAC Official Method 2000.07
## *Salmonella* in Foods
### Rapid Colorimetric Immunoenrichment-Based Screening Method (TECRA Unique *Salmonella* Test)
### First Action 2000

(Applicable to determine presence of *Salmonella* in all foods except raw flesh foods.)

*See* Tables **2000.07A** and **B** for the results of the interlaboratory study supporting acceptance of the method.

### *A. Principle*

The Unique *Salmonella* test provides all necessary reagents in a single-test module. An antibody-coated dipstick performs both the immunoenrichment and detection steps (Figure **2000.07**).

The Unique test begins by adding a preenriched test suspension to tube 1, together with the dipstick. Highly specific, purified antibodies on the surface of the dipstick selectively capture any *Salmonella* present. After washing in tube 2, the dipstick is transferred to tube 3 and incubated in an enrichment broth. Any *Salmonella* captured on the dipstick will then replicate to detectable levels. The dipstick is transferred to tube 4 which contains enzyme-linked antibodies (conjugate) specific for *Salmonella*. This conjugate will bind to any *Salmonella* on the dipstick.

Excess conjugate is removed by washing the dipstick in tube 5. The dipstick is transferred to tube 6 which contains substrate for the enzyme. If *Salmonella* are present, a purple color on the lower half of the dipstick is produced. The upper half is the negative control and should remain white. If there are no *Salmonella* in the suspension the dipstick should remain white except for the positive control, which appears as a purple cross at the base of the dipstick. Determination of positive results is performed visually by aid of color comparator card where a result is valid when positive and negative control areas on the dipstick give the specified color reactions.

The method is a screening procedure for the presence of *Salmonella* in all foods except raw flesh foods; it is not a confirmatory test because polyclonal antibodies used in the test may cross-react with a small percentage of non-*Salmonella* organisms. M broth cultures from test samples positive by the Unique method must be streaked on selective media as in AOAC **967.26B** (*see* 17.9.02), and typical or suspicious colonies must be identified as in AOAC **967.26C**, **967.27** (*see* 17.9.03), and **967.28** (*see* 17.9.07).

**Table 2000.07A. Statistical analyses of collaborative study results for TECRA Unique assay compared to FDA BAM 8th Ed. method**

| Food | MPN, cfu/g[a] | Method agreement, %[b] | Total samples | Samples positive: Unique Pres. | Samples positive: Unique Conf. | Samples positive: Culture Pres. | Samples positive: Culture Conf. | $\chi^{2c}$ | Incidence of false negatives among total positive samples, %[d]: Unique | Incidence of false negatives among total positive samples, %[d]: Culture | Sensitivity rate, %[d]: Unique | Sensitivity rate, %[d]: Culture | Incidence of false positives among total negative samples, %[e]: Unique | Incidence of false positives among total negative samples, %[e]: Culture | Specificity rate, %[e]: Unique | Specificity rate, %[e]: Culture |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nonfat dry milk | <0.003 | 100 | 85 | 1 | 0 | 1 | 0 | — | — | — | — | — | 1.2 | 1.2 | 98.8 | 98.8 |
| | 0.072 | 94.1[f] | 85 | 81 | 81 | 84 | 84 | 0.824 | 4.8[f] | 1.2[f] | 95.2[f] | 98.8[f] | — | — | — | — |
| | 0.748 | 100 | 85 | 85 | 85 | 85 | 85 | — | 0 | 0 | 100[f] | 100 | — | — | — | — |
| Black pepper | <0.003 | 100 | 85 | 0 | 0 | 0 | 0 | — | — | — | — | — | 0 | 0 | 100 | 100 |
| | 0.036 | 68.2[f] | 85 | 58 | 58 | 54 | 54 | 0.118 | 31.8[f] | 35.3[f] | 68.2[f] | 64.7[f] | — | — | — | — |
| | 0.932 | 97.6[f] | 85 | 84 | 84 | 85 | 85 | 0 | 2.4[f] | 0 | 97.6[f] | 100 | — | — | — | — |
| Soy flour | <0.003 | 100 | 75 | 0 | 0 | 0 | 0 | — | — | — | — | — | 0 | 0 | 100 | 100 |
| | 0.110 | 43.0[f] | 75 | 36 | 36 | 40 | 40 | 0.240 | 52.0[f] | 46.7[f] | 48.0[f] | 53.3[f] | — | — | — | — |
| | 0.430 | 100 | 75 | 75 | 75 | 75 | 75 | — | 0 | 0 | 100 | 100 | — | — | — | — |
| Milk chocolate | <0.003 | 100 | 75 | 2 | 0 | 3 | 0 | — | — | — | — | — | 2.7 | 4.0 | 97.3 | 96.0 |
| | 0.043 | 50.6[f] | 75 | 35 | 35 | 42 | 40 | 0.427 | 53.3[f] | 46.7[f] | 46.7[f] | 53.3[f] | — | — | — | — |
| | 0.240 | 98.6[f] | 75 | 50 | 48 | 50 | 50 | 0.029 | 36.0[f] | 33.3[f] | 64.0[f] | 66.7[f] | — | — | — | — |
| Dried whole egg | <0.003 | 100 | 80 | 0 | 0 | 0 | 0 | — | — | — | — | — | 0 | 0 | 100 | 100 |
| | 0.043 | 47.5[f] | 80 | 28 | 28[g] | 31 | 30 | 0.027 | 58.7[f] | 62.5[f] | 41.3[f] | 37.5[f] | — | — | — | — |
| | 0.427 | 51.25[f] | 80 | 51 | 51[g] | 51 | 49 | 0.027 | 27.5[f] | 38.8[f] | 72.5[f] | 61.3[f] | — | — | — | — |

[a] Most probable number of colony forming units per gram of food.

[b] Percentage agreement is defined as 100 times the number of samples for which confirmed results were in agreement divided by total number of samples. Samples were not paired.

[c] A $\chi^2$ value >3.84 indicates significance at $p \geq 0.05$.

[d] Incidence of false negatives is 100 – sensitivity rate. Sensitivity rate is defined as 100 times total number of confirmed positive test portions divided by total number of inoculated samples.

[e] Incidence of false positives is 100 – specificity rate. Specificity rate is defined as 100 times total number of presumptive negative test portions divided by number of uninoculated control samples at uninoculated level.

[f] Different enrichment media and different subsamples were used for each method. To calculate performance parameters it was assumed that all inoculated samples contained salmonellae (Table **2000.07B**).

[g] For dried egg powder 5 dipstick negative samples were reported as culture positive when streaked from tube 3 of Unique module at the low level and simarly 7 samples at the high level. It was difficult to determine whether this was due to powder contamination problems. Because negative dipstick results are not normally confirmed, the data presented here and used for calculations is for positive dipsticks that confirmed positive by subculture only.

**Table 2000.07B. 50% Detection end points, cfu/g, and 95% confidence limits (Spearmann-Karber)**

| Food matrix (N)[a] | Unique | Culture |
|---|---|---|
| NFDM (85) | 0.017 (0.015–0.019) | 0.015 (0.014–0.016) |
| BP (85) | 0.027 (0.021–0.034)[b] | 0.030 (0.021–0.041) |
| SF (75) | 0.066 (0.057–0.077) | 0.058 (0.050–0.068) |
| MC (75) | 0.075 (0.052–0.108)[b] | 0.062 (0.043–0.088)[b] |
| DWE (80) | 0.131 (0.093–0.185)[b] | 0.130 (0.092–0.185)[b] |

[a] NFDM = nonfat dry milk; BP = black pepper; SF = soy flour; MC = milk chocolate; DWE = dried whole egg.

[b] The method needs data points for 100% recovery so when these were not available it was assumed, conservatively, that 100% recovery would have been attained at a level 10 times the highest level tested. The lowest level tested was assumed to be 0.003 cfu/g in all cases rather than < 0.003 cfu/g.

### B. Safety Precautions

Any positive dipsticks will carry live *Salmonella*. Therefore, standard laboratory safety practices should be followed. *See* Appendix B, Safe Handling of Microorganisms.

### C. Reagents

Items (**a**)–(**d**) are available as TECRA Unique *Salmonella* test from TECRA International, 13 Rodborough Rd, French's Forest, NSW 2086, Australia and International BioProducts, Inc., PO Box 0746, Bothell, WA 98041-0746. Substitutions must be pretested for equivalency.

(**a**) *Unique test modules*.—(Supplied by TECRA, or equivalent.) Containing:

*Tube 1: Buffer (200 μL)*.—pH 8.5 283.1 g Tris/L $H_2O$.

*Tube 2: Wash (3.5 mL)*.—Modified buffered peptone water. Formulation is as follows: Peptone 10 g, NaCl 5 g, anhydrous $Na_2HPO_4$ 7 g, $KH_2PO_4$ 3 g. Suspend ingredients in 1 L water. Mix well and dispense. Sterilize in autoclave 15 min at 121°C.

*Tube 3: M Broth (1 mL)*.—Ingredients per L water: 5.0 g yeast extract, 12.5 g tryptone, 2.0 g D-mannose, 5.0 g sodium citrate·$2H_2O$, 5.0 g NaCl, 5.0 g $K_2HPO_4$, 0.14 g $MnCl_2·4H_2O$, 0.8 g anhydrous $MgSO_4$, 0.04 g $FeSO_4·7H_2O$, 0.75 g Tween 80. Final pH should be 7.0 ± 0.2.

*Tube 4: Conjugate (1 mL)*.—Contains anti-*Salmonella* antibodies (from sheep) conjugated to alkaline phosphatase in stabilizer.

*Tube 5: Wash (3.5 mL)*.—Contains 0.006 g Tris [tris(hydroxymethyl) aminomethane], 0.044 g NaCl, 0.0025 g Tween 20 and 0.005 g thimerosal in 3.5 mL $H_2O$.

*Tube 6: Substrate (1 mL)*.—5-bromo-4-chloro-3-indolyl-phosphate *p*-nitro blue tetrazolium chloride (Moss, Inc., PO Box 189, Pasadena, MD 21122).

(**b**) *Antibody-coated dipsticks in resealable pouch with resealing strip*.—(Supplied by TECRA, or equivalent.)

(**c**) *Caps for resealing tubes*.—(Supplied by TECRA, or equivalent.)

(**d**) *Color comparison card*.—(Supplied by TECRA, or equivalent.)

(**e**) *Modified buffered peptone water*.—*See* formulation given in **C**(**a**), for tube 2 of Unique module.

### D. Apparatus

(**a**) *Incubators*.—35–37°C and 41–43°C.

(**b**) *Pipet*.—Delivering 4 mL for Unique test.

(**c**) *Absorbent paper*.

### E. General Instructions

Unique modules must be used within 2 months of opening the pouch of dipsticks and before the use-by date on the outside label of the box. Write the date of opening the pouch (date of first use) on the outside label of the box. Refrigerate all components (2–8°C) when not in use. Do not freeze. Components in the kit are intended for use as an integral unit. The modules and antibody-coated dipsticks carrying the same batch number are matched and should not be used with tests of a different batch number. Bring all components to 20–25°C before use.

### F. Preparation of Test Sample

*Sample preparation*.—Refer to Unique package insert.

*Pre-enrichment*.—Refer to the manufacturer's directions to determine if product tested has any special requirements. If product does not have special requirements, enrich product in modified buffered peptone water, **C**(**e**), for formulation. Incubate enrichment broth at 35–37°C for 16–20 h unless otherwise specified.

### G. Performing the Unique Assay

(**a**) Bring modules to 20–25°C before use.

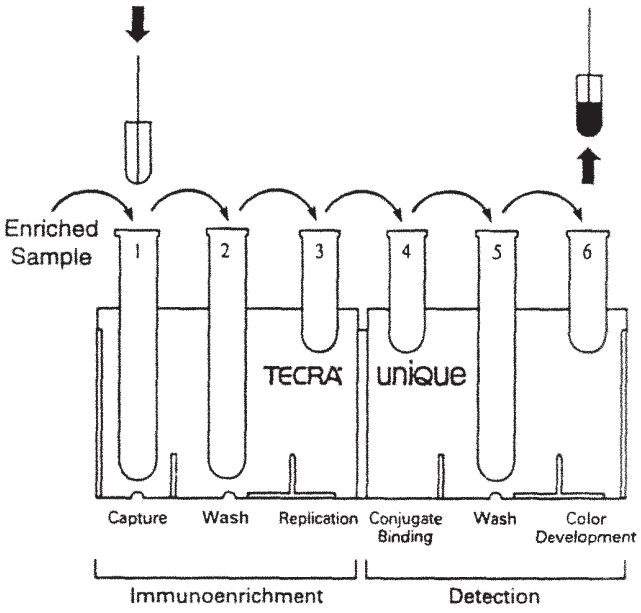


**Figure 2000.07—The module used in the *Salmonella* test.**

(**b**) One module is required for each test sample. Label end of each module with test sample number ID.

(**c**) *Step 1: Capture.*—(*1*) Open pouch of dipsticks and remove one dipstick for each test sample. Replace unused dipsticks in foil pouch with silica gel and reseal with resealing strip provided. (*2*) Open packet of caps and place a sufficient number in a container. Six caps are required for each module. (*3*) Remove foil from tube 1 (Figure **2000.07**). Mix enriched test suspension well. Add enriched test suspension to 4 mL mark on this tube in correspondingly labeled module. Remove dipstick from storage tube and screw firmly into tube 1. Retain empty dipstick storage tube for later use. Gently mix contents by inversion of module. (*4*) Incubate module at 35–37°C for a minimum of 20 min, but no longer than 40 min.

(**d**) *Step 2: Wash.*—(*1*) Remove foil from tube 2, transfer dipstick to this tube and screw it down firmly. Tightly seal tube 1 with cap. (*2*) Invert module twice to wash dipstick. Ensure that trapped air bubble travels completely past dipstick when inverting.

(**e**) *Step 3: Replication*.—(*1*) Remove foil from tube 3, transfer dipstick to this tube and screw it down firmly. Tightly seal tube 2 with cap. (*2*) Incubate module at 35–37°C for 4–5 h.

(**f**) *Step 4: Conjugate binding*.—(*1*) Remove foil from tube 4; transfer dipstick to this tube and screw firmly down. Tightly seal tube 3 with a cap. (*2*) Incubate module at 35–37°C for 30–40 min.

(**g**) *Step 5: Wash.*—(*1*) Remove foil from tube 5; transfer dipstick to this tube and screw firmly down. Tightly seal tube 4 with cap. (*2*) Invert tube 5 twice to wash dipstick. Ensure that trapped air bubble travels completely past dipstick when inverting.

(**h**) *Step 6: Color development*.—(*1*) Remove foil from tube 6, transfer dipstick to this tube and screw firmly down. Tightly seal tube 5 with cap. (*2*) Incubate entire module at 20–25°C for 10 min, and then read result.

(**i**) *Step 7: Reading the result*.—(*1*) Place double layer of absorbent paper onto piece of foil. Remove dipstick from tube 6, and press the end lightly onto absorbent paper to remove excess substrate. (*2*) Read the result visually using color card provided. Seal tube 6 with cap. Leave dipstick to dry on absorbent paper. When it is dry return it to its original storage tube for future reference if required. The foil and absorbent paper can now be disposed of after autoclaving. (*3*) *Using the color card*.—A test sample is considered positive when a purple color covers the lower half of the dipstick and a white band covers the upper half. If color appears weak or non-uniform, the result should be considered positive. If the entire dipstick is purple, the result cannot be used. A test sample is considered negative when the entire dipstick is white except for a small purple cross on the base. This small cross is a positive control. Its absence indicates a step may have been omitted, and the result cannot be used.

### *H. Confirmation of Positive Unique Test Samples*

A positive result on the Unique dipstick indicates that *Salmonella* may be present. However, since antibodies may cross-react with a few other organisms, culture confirmations must be performed by streaking hektoen enteric, xylose lysine desoxycholate, and bismuth sulfite plates from the broth in tube 3.

Refs.: (1) *Official Methods of Analysis of AOAC INTERNATIONAL* (2000), 17th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, **967.26**, **967.27**, **967.28**

(2) *J. AOAC Int.* **84**, 417–420(2001)

## Results and Discussion

Table 1 shows collaborator participation in the study by product type for both Australia and the United States. In Australia 18 collaborators participated in the study that included nonfat dry milk, pepper, and soy flour. In the United States, 21 collaborators participated in the study that included milk chocolate and dry egg. However, not all collaborators analyzed all foods, and data from some laboratories were excluded because of method deviations or because uninoculated control test samples were reported as giving culturally confirmed positive results. Some collaborators also failed to set up test samples or to return data.

Results for analysis of each food type at each inoculation level are given in Tables 2–6. Table **2000.07B** has results of 50% end point analysis; results for other performance parameters are given in Table **2000.07A**. In comparing the results for the Unique test and the BAM method different subsamples were examined by each method. Because the distribution of inoculum, particularly at the low level, would not be completely homogeneous, the presence or absence of salmonellae in particular subsamples would increase the variation in results between the 2 methods. To calculate the false negative rates and sensitivity for the high and low level materials it was assumed that all these "inoculated" test samples contained *Salmonella*. However, the study design required that test samples be fractionally positive at the low level, i.e., it was not acceptable for all 20 replicates to be positive for *Salmonella*. For this type of study, the calculated false negative rate may sometimes appear abnormally higher than that in studies where the same subsample is examined by both methods. Similarly, the sensitivity rate and the percentage agreement may be abnormally low (Table **2000.07A**). For this reason, Table **2000.07B** with 50% detection end points has also been included. Table **2000.07B** and the 95% confidence limits show that the performance of the Unique test and the reference method correlated very closely for all foods tested.

### *Nonfat Dry Milk*

Table 2 gives results for determination of *S. typhimurium* in nonfat dry milk by 17 collaborators. Of the 255 test samples analyzed, 166 were confirmed positive for the Unique method and 169 were confirmed positive for the cultural method. For each inoculation level of nonfat dry milk, a comparison showed no significant differences ($p \geq 0.05$) in the proportion of positive test samples for the Unique method and that for the reference method using the Chi-square test for independence with continuity correction (Table **2000.07A**).

**Table 1. Collaborator participation in study by product type**

| Collaborator (Australia) | Nonfat dry milk | Black pepper | Soy flour | Collaborator (United States) | Milk chocolate | Dried egg |
|---|---|---|---|---|---|---|
| 1 | Y[a] | Y | Y | 1 | Y | Y |
| 2 | Y | Y | Y | 2 | Y | Y |
| 3 | Y | N[b] | N | 3 | Y[c] | Y |
| 4 | Y | Y | Y | 4 | Y | Y |
| 5 | Y | Y | Y | 5 | Y[e] | Y[e] |
| 6 | Y | Y | Y | 6 | Y | Y |
| 7 | Y | Y | Y | 7 | Y[c] | Y[c] |
| 8 | Y | Y | Y | 8 | Y | Y |
| 9 | Y | Y | Y | 9 | Y[e] | Y[e] |
| 10 | Y | Y | Y | 10 | Y | Y |
| 11 | Y | Y | Y | 11 | Y | Y |
| 12 | Y[d] | Y | Y | 12 | Y | Y |
| 13 | Y | Y | Y | 13 | N | Y |
| 14 | Y | Y | N | 14 | Y | Y[d] |
| 15 | Y | Y | Y | 15 | Y | Y |
| 16 | Y | Y | Y | 16 | Y | Y |
| 17 | Y | Y | Y | 17 | Y | Y |
| 18 | Y | Y | Y[d] | 18 | Y[d] | Y |
| | | | | 19 | Y | Y |
| | | | | 20 | Y | Y |
| | | | | 21 | Y | N |

[a] Y = Participated in trial.
[b] N = Did not participate.
[c] Results not used in analysis because of method error.
[d] Results not used in analysis because one or more control samples tested positive for *Salmonella*.
[e] Received samples but did not set up or did not return data.

**Table 2. Collaborative study results for determination of *Salmonella typhimurium* in milk powder using TECRA Unique assay**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| | TECRA assay (presumptive result) | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 2 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 3 | + | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 4 | – | – | – | – | – | + | + | – | + | + | + | + | + | + | + |
| 5 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 6 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 7 | – | – | – | – | – | + | + | + | + | + | – | + | + | + | + |
| 8 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 9 | – | – | – | – | – | + | + | – | + | + | + | + | + | + | + |
| 10 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 11 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 13 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |

**Table 2. (*continued*)**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 14 | – | – | – | – | – | + | – | + | + | – | + | + | + | + | + |
| 15 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 16 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 17 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 18 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| TECRA assay (confirmed result) | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 2 | – | – | – | – | – | + | – | + | + | + | + | + | + | + | + |
| 3 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | – |
| 4 | – | – | – | – | – | + | + | – | + | + | + | + | + | + | + |
| 5 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 6 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 7 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 8 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 9 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 10 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 11 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 13 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 14 | – | – | – | – | – | + | – | + | + | – | + | + | + | + | + |
| 15 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 16 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 17 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 18 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| FDA BAM | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 2 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 3 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 4 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 5 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 6 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 7 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 8 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 9 | – | –[a] | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 10 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 11 | – | – | – | – | – | + | + | – | + | + | + | + | + | + | + |
| 13 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 14 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 15 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 16 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 17 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 18 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |

[a] Suspect colonies on plate, i.e., presumptive positive result confirmed negative.

**Table 3. Collaborative study results for detection of *Salmonella virchow* in pepper using TECRA Unique assay**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 1 | 2 | 3 | 4 | 5 | 11 | 12 | 13 | 14 | 15 | 6 | 7 | 8 | 9 | 10 |
| TECRA assay (presumptive result) | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | + | + | – | + | – | + | + | + | + | + |
| 2 | – | – | – | – | – | + | + | – | – | + | + | + | + | + | + |
| 4 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 5 | – | – | – | – | – | – | – | + | + | – | + | + | + | – | + |
| 6 | – | – | – | – | – | – | + | – | + | + | + | + | + | + | + |
| 7 | – | – | – | – | – | + | – | – | + | + | + | + | + | + | + |
| 8 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 9 | – | – | – | – | – | + | + | + | + | – | + | + | + | + | + |
| 10 | – | – | – | – | – | – | + | + | + | – | + | + | + | + | + |
| 11 | – | – | – | – | – | + | – | + | + | – | + | + | + | + | + |
| 12 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 13 | – | – | – | – | – | + | – | – | + | – | + | + | + | + | + |
| 14 | – | – | – | – | – | – | – | + | – | + | + | + | + | + | + |
| 15 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 16 | – | – | – | – | – | + | + | – | + | + | + | + | + | + | + |
| 17 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + |
| 18 | | | | | | + | – | – | – | + | + | + | + | + | + |
| TECRA assay (confirmed result) | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | + | + | – | + | – | + | + | + | + | + |
| 2 | – | – | – | – | – | + | + | – | – | + | + | + | + | + | + |
| 4 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 5 | – | – | – | – | – | – | – | + | + | – | + | + | + | – | + |
| 6 | – | – | – | – | – | – | + | – | + | + | + | + | + | + | + |
| 7 | – | – | – | – | – | + | – | – | + | + | + | + | + | + | + |
| 8 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 9 | – | – | – | – | – | + | + | + | + | – | + | + | + | + | + |
| 10 | – | – | – | – | – | – | + | + | + | – | + | + | + | + | + |
| 11 | – | – | – | – | – | + | – | + | + | – | + | + | + | + | + |
| 12 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 13 | – | – | – | – | – | + | – | – | + | – | + | + | + | + | + |
| 14 | – | – | – | – | – | – | – | + | – | + | + | + | + | + | + |
| 15 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 16 | – | – | – | – | – | + | + | – | + | + | + | + | + | + | + |
| 17 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + |
| 18 | – | – | – | – | – | + | – | – | – | + | + | + | + | + | + |
| FDA BAM | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | – | – | – | – | – | + | + | + | + | + |
| 2 | – | – | – | – | – | + | + | – | – | + | + | + | + | + | + |
| 4 | – | – | – | – | – | – | + | + | – | + | + | + | + | + | + |
| 5 | – | – | – | – | – | – | + | + | + | – | + | + | + | + | + |

**Table 3. (*continued*)**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 1 | 2 | 3 | 4 | 5 | 11 | 12 | 13 | 14 | 15 | 6 | 7 | 8 | 9 | 10 |
| 6 | – | – | – | – | – | – | + | – | – | + | + | + | + | + | + |
| 7 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 8 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 9 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 10 | – | – | – | – | – | + | – | + | – | + | + | + | + | + | + |
| 11 | – | – | – | – | – | – | + | – | + | + | + | + | + | + | + |
| 12 | – | – | – | – | – | – | – | – | + | + | + | + | + | + | + |
| 13 | – | – | – | – | – | – | – | + | + | – | + | + | + | + | + |
| 14 | – | – | – | – | – | – | + | + | + | – | + | + | + | + | + |
| 15 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 16 | – | – | – | – | – | + | + | – | + | + | + | + | + | + | + |
| 17 | – | – | – | – | – | – | + | – | – | – | + | + | + | + | + |
| 18 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |

[a] Suspect colonies on plates.

**Table 4. Collaborative study results for detection of *Salmonella mississippi* in soy flour using TECRA Unique assay**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| TECRA assay (presumptive result) | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | – | – | + | + | – | + | + | + | + | + |
| 2 | – | – | – | – | – | – | – | – | – | – | + | + | + | + | + |
| 4 | – | – | – | – | – | – | – | – | – | – | + | + | + | + | + |
| 5 | – | – | – | – | – | – | + | + | – | + | + | + | + | + | + |
| 6 | – | – | – | – | – | – | + | + | – | + | + | + | + | + | + |
| 7 | – | – | – | – | – | + | – | – | + | – | + | + | + | + | + |
| 8 | – | – | – | – | – | + | – | – | – | + | + | + | + | + | + |
| 9 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 10 | – | – | – | – | – | – | + | + | – | – | + | + | + | + | + |
| 11 | – | – | – | – | – | + | + | – | + | – | + | + | + | + | + |
| 12 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + |
| 13 | – | – | – | – | – | + | + | – | – | + | + | + | + | + | + |
| 15 | – | – | – | – | – | – | – | + | – | + | + | + | + | + | + |
| 16 | – | – | – | – | – | – | + | + | – | + | + | + | + | + | + |
| 17 | – | – | – | – | – | – | + | – | + | – | + | + | + | + | + |
| TECRA assay (confirmed result) | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | – | – | + | + | – | + | + | + | + | + |
| 2 | – | – | – | – | – | – | – | – | – | – | + | + | + | + | + |
| 4 | – | – | – | – | – | – | – | – | – | – | + | + | + | + | + |
| 5 | – | – | – | – | – | – | + | + | – | + | + | + | + | + | + |

**Table 4. (*continued*)**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 6 | – | – | – | – | – | – | + | + | – | + | + | + | + | + | + |
| 7 | – | – | – | – | – | + | – | – | + | – | + | + | + | + | + |
| 8 | – | – | – | – | – | + | – | – | – | + | + | + | + | + | + |
| 9 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 10 | – | – | – | – | – | – | + | + | – | – | + | + | + | + | + |
| 11 | – | – | – | – | – | + | + | – | + | – | + | + | + | + | + |
| 12 | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + |
| 13 | – | – | – | – | – | + | + | – | – | + | + | + | + | + | + |
| 15 | – | – | – | – | – | – | – | + | – | + | + | + | + | + | + |
| 16 | – | – | – | – | – | – | + | + | – | + | + | + | + | + | + |
| 17 | – | – | – | – | – | – | + | – | + | – | + | + | + | + | + |
| | | | | | | | FDA BAM | | | | | | | | |
| 1 | – | – | – | – | – | – | – | – | – | + | + | + | + | + | + |
| 2 | – | – | – | – | – | + | – | – | + | + | + | + | + | + | + |
| 4 | – | – | – | – | – | + | – | – | + | – | + | + | + | + | + |
| 5 | – | – | – | – | – | + | + | – | + | – | + | + | + | + | + |
| 6 | – | – | – | – | – | + | + | + | – | – | + | + | + | + | + |
| 7 | – | – | – | – | – | – | – | + | – | + | + | + | + | + | + |
| 8 | – | – | – | – | – | – | + | + | – | – | + | + | + | + | + |
| 9 | – | – | – | – | – | + | – | – | + | – | + | + | + | + | + |
| 10 | – | – | – | – | – | + | – | – | + | – | + | + | + | + | + |
| 11 | – | – | – | – | – | + | + | – | + | + | + | + | + | + | + |
| 12 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 13 | – | – | – | – | – | – | + | + | + | – | + | + | + | + | + |
| 15 | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + |
| 16 | – | – | – | – | – | + | + | – | – | – | + | + | + | + | + |
| 17 | – | – | – | – | – | – | + | – | – | – | + | + | + | + | + |

[a] Suspect colonies on plates.

### *Ground Black Pepper*

Table 3 gives the results for determination of *S. virchow* in ground black pepper by 17 collaborators. Of the 255 test samples analyzed, 142 were confirmed positive using the Unique method, and 139 were confirmed positives using the BAM method. For each inoculation level of pepper, a comparison showed no significant differences ($p \geq 0.05$) in the proportion of positive test samples for the Unique method and that for the reference method using the Chi-square test for independence with continuity correction (Table **2000.07A**).

### *Soy Flour*

Table 4 shows the results for determination of *S. mississippi* in soy flour by 15 collaborators. Of the 225 test samples analyzed, 111 were confirmed positive with the Unique method, and 115 were confirmed positive with the BAM method. For each inoculation level of soy flour, a comparison showed no significant differences ($p \geq 0.05$) in positive test samples for the Unique method with that for the reference method using the Chi-square test for independence with continuity correction (Table **2000.07A**).

### *Milk Chocolate*

Table 5 shows the results for determination of *S. anatum* in milk chocolate by 15 collaborators. For the 225 test samples analyzed, 83 were confirmed positive using the Unique method, and 90 were confirmed positive using the BAM method. For each inoculation level of milk chocolate, a comparison showed no significant differences ($p \geq 0.05$) in the proportion of positive test samples for the Unique method and that for the reference method using the Chi-square test for independence with continuity correction (Table **2000.07A**).

**Table 5. Collaborative study results for detection of *Salmonella anatum* in milk chocolate using TECRA Unique assay**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 2 | 3 | 7 | 9 | 14 | 5 | 11 | 10 | 12 | 15 | 1 | 4 | 6 | 8 | 13 |
| TECRA assay (presumptive result) | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | + | + | – | + | – | – | + | + | + | + |
| 2 | – | – | – | – | – | – | + | – | – | + | + | + | – | – | + |
| 4 | – | – | – | – | – | + | + | + | + | + | – | + | + | + | + |
| 6 | – | – | – | – | – | – | – | – | – | – | + | – | – | – | – |
| 8 | – | – | – | – | – | – | – | – | + | – | + | + | – | + | + |
| 10 | – | – | – | – | – | + | – | + | + | – | + | + | + | – | + |
| 11 | – | – | – | – | – | – | – | + | + | + | + | + | + | + | + |
| 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | + | + |
| 14 | – | + | – | – | – | – | – | + | – | – | + | + | – | + | – |
| 15 | – | – | – | – | – | – | + | + | + | + | – | + | + | – | + |
| 16 | – | – | – | – | – | – | + | + | + | – | + | + | – | – | + |
| 17 | – | – | – | – | – | + | – | – | + | – | + | + | + | – | – |
| 19 | – | + | – | – | – | + | – | + | + | – | + | – | + | + | + |
| 20 | – | – | – | – | – | – | + | + | – | – | + | + | + | – | + |
| 21 | – | – | – | – | – | – | – | + | + | + | + | – | + | + | – |
| TECRA assay (confirmed result) | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | + | + | – | + | – | – | + | + | + | + |
| 2 | – | – | – | – | – | – | + | – | – | +[a] | + | + | – | + | + |
| 4 | – | – | – | – | – | + | + | + | + | + | – | + | + | + | + |
| 6 | – | – | – | – | – | – | – | – | – | – | + | – | – | – | – |
| 8 | – | – | – | – | – | – | – | – | + | – | + | + | – | + | + |
| 10 | – | – | – | – | – | + | – | + | + | – | + | + | + | – | + |
| 11 | – | – | – | – | – | – | – | + | + | + | + | + | + | + | + |
| 12 | – | – | – | – | – | – | – | – | – | – | – | – | – | + | + |
| 14 | – | – | – | – | – | – | – | + | – | – | – | – | – | + | – |
| 15 | – | – | – | – | – | – | + | + | + | + | – | + | + | – | + |
| 16 | – | – | – | – | – | – | + | + | + | – | + | + | – | – | + |
| 17 | – | – | – | – | – | + | – | – | + | – | + | + | + | – | – |
| 19 | – | – | – | – | – | + | – | + | + | – | + | – | + | + | – |
| 20 | – | – | – | – | – | + | – | + | – | – | + | + | + | – | + |
| 21 | – | – | – | – | – | – | – | + | + | + | + | – | + | + | – |
| FDA BAM | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | – | + | + | + | – | + | – | – | + | – |
| 2 | – | – | – | – | – | – | – | – | – | + | + | + | + | – | + |
| 4 | –[b] | – | – | – | – | + | + | – | – | – | + | + | – | + | + |
| 6 | – | – | – | – | – | – | + | – | – | – | + | + | + | + | + |
| 8 | – | – | – | – | – | + | + | + | – | + | – | + | – | + | + |
| 10 | – | – | – | –[b] | – | + | + | + | + | + | + | + | + | + | + |
| 11 | – | – | – | –[b] | – | + | –[b] | – | + | –[b] | + | + | + | + | – |

**Table 5. (*continued*)**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 2 | 3 | 7 | 9 | 14 | 5 | 11 | 10 | 12 | 15 | 1 | 4 | 6 | 8 | 13 |
| 12 | – | – | – | – | – | + | + | – | – | – | – | – | – | + | – |
| 14 | – | – | – | – | – | + | – | + | – | – | – | + | + | + | + |
| 15 | – | – | – | – | – | + | + | + | + | – | – | – | + | + | + |
| 16 | – | – | – | – | – | + | – | – | + | + | – | + | – | + | + |
| 17 | – | – | – | – | – | – | – | + | + | + | – | – | – | + | + |
| 19 | – | – | – | – | – | – | – | + | + | + | + | + | + | + | – |
| 20 | – | – | – | – | – | + | – | – | + | – | + | – | – | + | – |
| 21 | – | – | – | – | – | – | + | + | + | – | – | + | + | + | + |

[a] Confirmed by antisera, not by API 20E.
[b] Suspect colonies on plates.

**Table 6. Collaborative study results for detection of *Salmonella enteritidis* in dried egg using TECRA Unique assay**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 6 | 7 | 10 | 11 | 15 | 3 | 4 | 9 | 12 | 14 | 2 | 1 | 5 | 8 | 13 |
| TECRA assay (presumptive result) | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | + | + | + | – | + | – | + | + | – | + |
| 2 | – | – | – | – | – | + | – | – | + | + | + | + | – | + | + |
| 3 | – | – | – | – | – | – | + | – | – | + | + | + | + | – | + |
| 4 | – | – | – | – | – | – | + | – | – | + | + | + | + | + | – |
| 6 | – | – | – | – | – | + | – | – | – | – | + | – | + | – | + |
| 8 | – | – | – | – | – | – | + | – | + | – | + | – | – | + | – |
| 10 | – | – | – | – | – | – | – | – | – | + | + | – | + | + | – |
| 11 | – | – | – | – | – | – | – | – | – | + | – | + | – | + | – |
| 12 | – | – | – | – | – | – | – | – | – | + | + | – | – | + | + |
| 13 | – | – | – | – | – | + | – | + | – | – | + | – | + | + | + |
| 15 | – | – | – | – | – | + | – | – | – | – | + | + | + | – | – |
| 16 | – | – | – | – | – | – | – | + | + | – | + | + | + | – | + |
| 17 | – | – | – | – | – | – | + | + | – | + | + | + | + | + | + |
| 18 | – | – | – | – | – | – | – | – | – | + | + | – | + | – | – |
| 19 | – | – | – | – | – | – | – | – | – | – | + | + | – | – | – |
| 20 | – | – | – | – | – | + | – | – | – | + | + | – | + | + | – |
| TECRA assay (confirmed result) | | | | | | | | | | | | | | | |
| 1 | – | – | – | – | – | + | + | + | – | + | – | + | + | – | + |
| 2 | – | – | – | – | – | + | – | – | + | + | + | + | – | + | + |
| 3 | – | – | – | – | – | – | + | – | – | + | + | + | + | – | + |
| 4 | – | – | – | – | – | – | + | – | – | + | + | + | + | + | – |
| 6 | – | – | – | – | – | + | – | – | – | – | + | – | + | – | + |
| 8 | – | – | – | – | – | – | + | + | + | – | + | – | – | + | – |
| 10 | – | – | – | – | – | – | + | – | – | + | + | + | + | + | – |
| 11 | – | – | – | – | – | – | – | – | – | + | – | + | – | + | + |

**Table 6. (*continued*)**

| | Uninoculated samples | | | | | Low inoculum samples | | | | | High inoculum samples | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Collaborator | 6 | 7 | 10 | 11 | 15 | 3 | 4 | 9 | 12 | 14 | 2 | 1 | 5 | 8 | 13 |
| 12 | – | – | – | – | – | – | – | – | – | + | + | – | – | + | + |
| 13 | – | – | – | – | – | + | – | + | + | – | + | + | + | + | + |
| 15 | – | – | – | – | – | + | – | – | – | – | + | + | + | + | + |
| 16 | – | – | – | – | – | – | – | + | + | – | + | + | + | – | + |
| 17 | – | – | – | – | – | – | + | + | – | + | + | + | + | + | + |
| 18 | – | – | – | – | – | – | – | – | – | + | + | – | – | – | – |
| 19 | – | – | – | – | – | + | – | – | + | – | + | + | – | + | + |
| 20 | – | – | – | – | – | + | – | – | + | – | + | + | + | + | – |
| | | | | | | | | FDA BAM | | | | | | | |
| 1 | – | – | – | – | – | – | – | – | – | + | – | – | + | – | + |
| 2 | – | – | – | – | – | – | – | + | – | + | – | – | + | + | + |
| 3 | – | – | – | – | – | + | + | – | + | – | + | + | + | + | + |
| 4 | – | – | – | – | – | + | + | – | + | – | + | + | – | – | + |
| 6 | – | – | – | – | – | + | – | + | + | – | + | + | + | + | + |
| 8 | – | – | – | – | – | + | – | – | – | + | + | + | + | – | – |
| 10 | – | – | – | – | – | + | – | – | + | – | + | + | + | – | + |
| 11 | – | – | – | – | – | + | + | + | – | + | + | + | + | – | + |
| 12 | – | – | – | – | – | + | – | – | – | + | + | + | – | + | + |
| 13 | – | – | – | – | – | – | + | – | + | – | + | – | + | – | – |
| 15 | – | – | – | – | – | – | –[a] | – | – | + | –[a] | –[a] | + | – | – |
| 16 | – | – | – | – | – | – | – | – | + | – | + | – | + | + | – |
| 17 | – | – | – | – | – | – | – | – | – | – | + | + | – | + | + |
| 18 | – | – | – | – | – | – | – | – | – | – | + | + | – | – | – |
| 19 | – | – | – | – | – | – | – | + | – | + | + | – | – | + | – |
| 20 | – | – | – | – | – | + | + | – | – | – | + | – | – | – | + |

[a] Suspect colonies on plates.

### *Dried Whole Egg*

Table 6 shows results from 16 collaborators for determination of *S. enteritidis* in dry egg. For the 240 test samples analyzed, 79 were confirmed positive using the Unique method, 79 were confirmed positive with the BAM method. At the low level, 5 of 80 test samples were reported as negative on the Unique dipstick and positive when subcultured from tube 3 of the Unique module, and 7 of 80 test samples at the high level for which this was reported. It was difficult to determine whether this abnormality was caused by powder contamination problems.

Using Unique results which were both dipstick positive and culturally confirmed, for each inoculation level of dried whole egg, a comparison showed no significant differences ($p \geq 0.05$) in the proportion of positive test samples for the Unique method and that for the reference method using the Chi-square test for independence with continuity correction (Table **2000.07A**).

## Recommendation

No significant differences ($p \geq 0.05$) were observed for any of the 5 foods at any inoculation level, in a comparison of the proportion of positive test samples for the Unique method and that for the reference method. A sixth food, raw poultry, was originally intended to be included in the study; however, the collaborative study for raw poultry has been delayed. We therefore recommend that the Unique method be adopted Official First Action with applicability to all foods except raw flesh foods.

## Acknowledgments

We thank the following collaborators for their participation in the study:

Don Arnold and Adrienne Burnie, Graysons & Associates, Penrose, New Zealand

Christine Barrat, Ministry of Agriculture and Fisheries, Auckland, New Zealand

Veronica Simes and Nasrin Matouchi, Microtech Laboratories, Greenacre, NSW, Australia

Karen Smith, Microtech Laboratories, Blackburn, Vic, Australia

Angela Dailianis, Louise Hill, and Di Kerr, Biotech Australia, Roseville, NSW, Australia

Ron Fyfe, Cawthron Institute, Nelson, New Zealand

Jill Gebler and Bettine Dijs, Murray Goulburn Co-op, Yarram, Vic, Australia

Ann Gerry and Sharon Vanderpoel, Analchem Bioassay, Lilyfield, NSW, Australia

Trudy Graham, Queensland Health Scientific Services, Coopers Plains, Qld, Australia

Des Hadjimichael, EML Consulting Services, Parramatta, NSW, Australia

Selina Heaton, Microbiological Diagnostic Unit, University of Melbourne, Parkville, Vic, Australia

Zaffar Hussain, Stanford Consulting Laboratories, Rydalmere, NSW, Australia

Cherie Le, Australian Government Analytical Laboratories, Pymble, NSW, Australia

Steven Munyard, Food Hygiene Laboratory, Nedlands, WA, Australia

Katerina Ristov, Pacific Analysis, Chippendale, NSW, Australia

Aimee Anderson, Jill Brown and Amy Remes, R-TECH Laboratories, Arden Hills, MN

Karen Battista, Nabisco Foods, East Hannover, NJ

Sandy Beatty, MAK-BEA Laboratories, Blue Earth, MN

Tony Blanch, National Foods, Arlington, WA

David Briones, American Dehydrated Foods Inc., Social Circle, GA

Robert Brooks, Woodson Tenent Laboratories, Gainsville, GA

Therese Clark, Department of Agriculture, Reynoldsberg, OH

Chris Destro, International Bioproducts Inc., Redmond, WA

Pete Dombroski, State Department of Health, Springfield, IL

Susan Hetrick, Hershey Foods, Hershey, PA

Eileen High, Department of Agriculture, Nashville, TN

Bill Hirt, Woodson Tenent Laboratories, Dayton, OH

Bill Hullet, J.L. Leek, Albany, GA

Robin Kalinowski, Armour Swift Eckrich, Downers Grove, IL

Lisa Maycock, Miller Laboratories, Phoenix, AZ

Mary Ann Murphy, USDA, AMS, Science Division, Gastonia, NC

Rob Robbins, BSK Food and Dairy Laboratories, Fresno, CA

Rick Rodriquez, Leprino Foods, Denver CO

Joe Shebuski, Pillsbury Company, St Paul, MN

David Wang, Skaklee Corporation, Hayward, CA

## References

(1) Andrews, W.H., June, G.A., Sherrod, P.S., Hammack, T.S., & Amaguana, R.M. (1995) *Bacteriological Analytical Manual, 8th Ed.*, AOAC INTERNATIONAL, Gaithersburg, MD

(2) Gibson, D.M., Coombs, P., & Pimbley, D.W. (1992) *J. AOAC Int.* **70**, 931–935

(3) Garthright, W.E. (1995) *FDA Bacteriological Analytical Manual, 8th Ed.*, Appendix 2, AOAC INTERNATIONAL Gaithersburg, MD