

that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h.

(d) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H₂O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (see 967.26B, 967.26C, 967.27 and 967.28). Cool heated M broths to 25°–37°C prior to analysis.

G. EIA

(a) The following reagents must be prepared prior to commencing assay: (1) Prepare working-strength wash solution by diluting contents of one vial of wash solution concentrate to 2 L with distilled or deionized H₂O into plastic reagent bottle. Plastic squeeze bottle is ideal for washing trays manually. (2) Prepare reconstituted positive control by transferring 3 mL control diluent to vial of lyophilized positive control antigen; mix thoroughly. The remaining control diluent acts as negative control. (3) Prepare reconstituted conjugate by adding vial of conjugate diluent to vial of lyophilized conjugate. Let conjugate rehydrate

at room temperature. Gently mix reconstituted conjugate. (4) Prepare reconstituted substrate by adding vial of substrate diluent to lyophilized substrate. Be sure substrate has dissolved and mixture is at room temperature prior to use. Reconstituted substrate will appear pale green. (5) Use stop solution as received. No reconstitution is required.

(b) Secure desired number of test (Removawell) strips in tray, allowing one well per food sample plus 2 wells for controls. *Press wells firmly into place.* Remove sealing film from top of wells to be used. Transfer 0.2 mL of each heated M broth sample to single well. Transfer 0.2 mL of reconstituted positive control and negative control into individual wells. Record sample position on sample record sheet provided.

(c) Cover tray with plastic film and incubate 30 min at 35°–37°C in standard laboratory incubator. Tray must be covered to prevent evaporation.

(d) After incubation, wash plate by hand with plastic squeeze bottle containing working-strength wash solution or use automatic washer charged with working-strength wash solution as follows: (1) Quickly invert tray, emptying its contents into container. (2) Remove any residual liquid by *firmly* tapping tray facedown on paper towel. (3) Completely fill each well with working-strength wash solution. (4) Repeat (1)–(3) another 2 times.

(e) Empty tray according to (d)(1) and (d)(2); then add 0.2 mL reconstituted conjugate to each well. Cover tray and incubate 30 min at 35°–37°C.

(f) Empty contents of tray and wash tray thoroughly 4 times according to (d)(1) and (d)(3); then empty tray according to (d)(1) and (d)(2).

Table 1. Test products, organisms, and levels

Product	<i>Salmonella</i> serovar	Inoculation level	MPN/g
Milk chocolate		Control	<0.003
	<i>Senftenberg</i> (H ₂ S neg)	Low	0.009
	<i>Senftenberg</i> (H ₂ S neg)	High	0.093
Dried egg		Control	<0.003
	B:r:1 complex	Low	0.009
	B:r:1 complex	High	0.430
Nonfat dry milk		Control	<0.003
	<i>Bovis moribificans</i>	Low	0.240
	<i>Bovis moribificans</i>	High	2.40
Black pepper		Control	<0.003
	<i>Anatum</i>	Low	0.014
	<i>Anatum</i>	High	0.088
Soy flour		Control	<0.003
	<i>Heidelberg</i>	Low	0.240
	<i>Heidelberg</i>	High	2.40
Raw ground turkey		Control	<0.003
	B:G, B:E h1 complex	Low	0.043
	C ₁ :G complex	High	0.46

(g) Add 0.2 mL reconstituted substrate to each well. Incubate at room temperature (20°–25°C) for a minimum of 10 min until positive control has reached color equivalent to positive control on color comparator card or to $A \geq 1.0$. Because color development tends to concentrate around edges of wells, it is important to tap sides of plate gently to mix contents prior to reading result. In this way, accurate readings will be obtained.

(h) Add 0.02 mL stop solution to each well. Incubation time should be ca 10–20 min. If >30 min has elapsed and A of 1.0 has not been attained, test is invalid.

H. Reading

Results of tests can be determined (1) visually or (2) with a microtiter tray reader. (1) Place tray on white background, and then compare individual test wells with color comparator. Positive control should give strong blue-green color indicating that all reagents are functional. If positive control is lighter than "Positive" on color comparator card, test is invalid; refer to "Troubleshooting Guide" in package insert, C(k). If negative control is darker than "Negative" on color comparator card, it is probable that the tray was inadequately washed and assay must be repeated.

(2) A maximum absorbance of the blue-green end product occurs at 414 nm; therefore, tray can be read at 414 ± 10 nm. For dual-wavelength readers, set reader to zero (blank) on air and set second reference wavelength at 490 ± 10 nm. For single-wavelength readers, set reader to zero (blank) with a well containing 200 μ L water or substrate. $A \geq 0.3$ indicates positive

results. Positive control should give $A \geq 1.0$, negative control should give $A < 0.2$.

Ref.: *J. AOAC Int.* **82**, 635–639(1999)

Results and Discussion

Table 1 gives the product types, serotypes, and levels of salmonellae present at the time of analysis. Table 2 shows product analysis by collaborator number for both Australia and the United States. In Australia, 18 collaborators participated in the study, which included nonfat dry milk, ground black pepper, and soy flour. In the United States, 20 laboratories participated in the study, which included milk chocolate, dry egg, and raw ground turkey. However, not all collaborators analyzed all foods, and data from some laboratories were excluded because of method deviations or because uninoculated control samples gave positive results. Some collaborators also failed to set up samples or to return data.

Results for sample analysis of each food type are given in Tables 3 to 8.

Nonfat Dry Milk

Table 3 gives results of analysis of nonfat dry milk by 16 collaborators. Of the 240 samples analyzed, 239 gave identical results for the TECRA method and the reference method. The remaining sample gave a false-negative result with the TECRA method, as well as an unconfirmed positive result for the TECRA method with the plate reader only. No significant dif-

Table 2. Collaborator participation in study by product type

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

^a Y, participated in trial.

^b N, did not participate.

^c Results not used in analysis because one or more control samples tested positive for *Salmonella*.

^d Results not used in analysis because of method error.

^e Received samples but did not set up or did not return data.

Table 3. Collaborative study results for detection of *Salmonella* spp. in milk powder

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

^a r+, positive with reader only.

Table 4. Collaborative study results for detection of *Salmonella* spp. in black pepper

Collaborator	Uninoculated samples					Low inoculum samples					High inoculum samples				
	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	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
12	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
14	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+
16	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+
17	-	-	-	-	-	+	+	-	-	+	+	-	+(r-) ^b	+	+
TECRA assay (confirmed result)															
1	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+
3	-	-	-	-	-	+	+	-	+	+	+	+	-	-	+
4	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+
5	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+
6	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+
8	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+
9	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
12	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
14	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+
16	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+
17	-	-	-	-	-	+	+	-	-	+	+	-	+	+	+
BAM															
1	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+
3	-	-	-	-	-	+	+	-	+	+	+	+	-	-	+
4	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+
5	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+
6	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+
8	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+
9	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
12	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
14	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+
16	- ^c	-	-	-	-	-	-	+	-	-	+	+	+	-	+
17	-	- ^c	-	- ^c	-	+	+	- ^c	-	+	+	- ^c	+	+	+

^a No reader result.^b r-, negative with reader only.^c Suspect colonies on plates.

Table 5. Collaborative study results for detection of *Salmonella* spp. in soy flour

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

^a No reader result.^b Suspect colonies on plates.

Table 6. Collaborative study results for detection of *Salmonella* spp. in milk chocolate

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

^a Borderline reading classified as negative because positive control was greater than 1.5.^b Suspect colonies on plates.

Table 7. Collaborative study results for detection of *Salmonella* spp. in dried egg

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

^a Borderline reader result (positive control was 2.24).

Table 8. Collaborative study results for detection of *Salmonella* spp. in raw turkey

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

^a r+, positive with plate reader only.^b Visual reading positive.^c Suspect colonies on plates.

ferences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Ground Black Pepper

Table 4 gives the results for analysis of ground black pepper by 15 collaborators. This was a raw product with high levels of competitive flora and very low inoculation levels. For the 225 samples analyzed, there was exact agreement between the confirmed results with the TECRA and the cultural method. There were 5 false positives for the cultural method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Soy Flour

Table 5 shows the results of analysis for soy flour by 15 collaborators. For the 225 samples analyzed, there was exact agreement between the confirmed data for the TECRA method and the cultural method. One false positive occurred for the cultural method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Milk Chocolate

Table 6 shows the results of analysis for milk chocolate by 14 collaborators. For the 210 samples analyzed, there was exact agreement between confirmed results for the TECRA method and the cultural method. There were 11 false positives for the cultural method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples with that for the reference method.

Dried Whole Egg

Table 7 shows results from 13 collaborators for dry egg. For the 195 samples analyzed, using confirmed results, there were no false negatives for the TECRA method and one false negative for the cultural method. There was one false positive for the TECRA method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Raw Turkey

Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant

differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Recommendation

No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the new enrichment procedure for the TECRA method with that for the reference method.

Therefore, it is recommended that the new enrichment procedure for the TECRA method be adopted official First Action. This method is not intended to replace AOAC Official Method 989.14 but to be available as an alternative procedure for enrichment.

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Anonymous (by request)

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Exhibit 1048
Mullins Decl. Appendix D-2
(Hughes 1999 from OUP)

Salmonella in Foods—A New Enrichment Procedure for Use with the TECRA *Salmonella* Visual Immunoassay: Collaborative Study

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A collaborative study was conducted to compare a new enrichment procedure for the TECRA *Salmonella* Visual Immunoassay with the reference method given in the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (BAM 7th Ed.). Three food types (milk powder, black pepper, and soy flour) were analyzed in Australia, and 3 food types (milk chocolate, dried egg, and raw turkey) were analyzed in the United States. Thirty-eight collaborators participated in the study. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method. The new enrichment procedure for the TECRA method has been adopted First Action by AOAC INTERNATIONAL.

The TECRA *Salmonella* Visual Immunoassay (TSVIA) is an enzyme-linked immunosorbent assay performed in the sandwich configuration. High-affinity "capture" antibodies specific to *Salmonella* spp. are adsorbed onto the surface of Removawell strips. If *Salmonella* antigens are present in the sample, they are captured by the antibodies. All other material in the sample is washed away. The sandwich is completed by the addition of enzyme-labeled antibodies (conjugate) specific for *Salmonella* spp. The presence of salmonellae is indicated when the bound conjugate converts the substrate to a green color.

Submitted for publication May 6, 1997.

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" (1998) *Inside Laboratory Management*, July issue.

Method 989.14, *Salmonella* in Foods (Using the TSVIA), was granted Association of Official Analytical Chemists (AOAC) First Action approval in 1989 and Final Action approval in 1990. In 1996, a modified version of the TSVIA, which had been supplemented with antibodies to *S. pullorum* and *S. gallinarum*, was adopted First Action (1).

For the previous AOAC studies on TSVIA, a cultural protocol using selective enrichment in selenite cystine (SC) and tetrathionate (TT) broths at 35°C was used. However, there has been a recent trend to use elevated incubation temperatures and to replace SC broth with the Rappaport-Vassiliadis (RV) medium (2).

This study was undertaken to validate a new enrichment procedure for use with the latest version of the TSVIA (approved in 1996). The new enrichment procedure for the assay incorporates Rappaport-Vassiliadis broth (R10) and TT broth at 42°C for selective enrichment. The reference method for the study was the 7th edition of the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (BAM; 3), because this method was current at commencement of the precollaborative study. Since then, an 8th edition of BAM has been published, in which the method for raw flesh foods has been changed.

Collaborative Study

The collaborative study was conducted in 2 parts: with milk powder, ground black pepper, and soy flour analyzed in Australia and New Zealand, and with milk chocolate, dried egg, and raw turkey analyzed in the United States. The 6 food types chosen were the same as those in the original collaborative study of the TSVIA (4). All foods were artificially contaminated with the exception of raw turkey, which was naturally contaminated.

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 cell pellets 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 for 24 h. Freeze-dried inocula were ground to a fine powder prior to use.

Inoculation of Samples

For dried products (milk powder, ground pepper, soy flour, and dried egg), a concentrated "seed" was made by addition of freeze-dried inoculum to ca 500 g of the test product. This seeded product was mixed well and stored at room temperature for at least 2 weeks to allow cell levels to stabilize, prior to estimation of cell count by serial dilution and plating on Xylose Lysine Desoxycholate Agar. According to the estimated *Salmonella* count, an appropriate amount of the concentrated seed was added to the test product with the aim of achieving a high level (10–50 cells/25 g) or a low level (1–5 cells/25 g) of inoculation. A most probable number (MPN) determination was performed prior to sample dispatch, and levels of salmonellae were adjusted by addition of more seed or more product if necessary.

For milk chocolate, the seed was prepared by inoculating freeze-dried *Salmonella* culture into milk chocolate tempered at 55°C. It was then mixed with an electric mixer and held at room temperature for 2–3 weeks to stabilize. The stabilized seed was mixed with tempered chocolate to obtain target levels.

Naturally contaminated raw ground turkey was used for inoculation at high or low levels. Different batches of turkey were tested and, on the basis of contamination levels, used as uninoculated control or inoculated sample at high or low level. Samples were frozen prior to shipment.

Sample Dispatch

Dried foods and chocolate were shipped at ambient temperatures. Raw turkey was shipped frozen on dry ice.

Each collaborator received 15 samples of the food to be analyzed: 5 inoculated at high level, 5 inoculated at low level, and 5 uninoculated.

The appropriate samples were dispatched to participating laboratories the week prior to analysis. On arrival at the laboratory, samples were stored at room temperature with the exception of raw turkey, which was stored frozen.

Sample Analysis

A different food product was scheduled for testing each week. On the day when sample analysis was initiated by collaborators, an MPN determination also was performed by the organizing laboratory.

Each sample was analyzed by the BAM method (7th Ed.) and the TSVIA method using the enrichment protocol described in section F of the method, *Preparation of Sample*. Equal portions (0.5–1 mL) from each of the 2M broths arising from the 2 selective-enrichment broths were combined in a small screw-capped tube and heat treated in boiling water for 15 min prior to performing the TSVIA.

Confirmation for the immunoassay was by streaking from the M broth culture onto selective agar, as specified in the BAM reference culture method.

Analysis of Data

Data from each food type were collated, and numbers of false-negative results were determined for the TECRA method and for the reference culture method. The false-negative rate (the number of false-negative results divided by the total number of confirmed positive samples) and the false-positive rate (the number of false-positive results divided by the number of method negative results) were determined. A false-positive result was determined at the first assay or decision point for the procedure, that is, at the immunoassay result for the TECRA method and at the plate reading stage for the cultural method. The percentage agreement was determined as 100 times the number of samples giving identical results for both methods divided by the total number of samples. A pairwise comparison of the proportion of positive samples for the TECRA method with the reference method was made. McNemar's test ($p > 0.05$) was used to determine whether the 2 methods were significantly different.

998.09 *Salmonella* in Foods, Colorimetric Polyclonal Enzyme Immunoassay Screening Method with Rappaport-Vassiliadis (R10) Broth and Tetrathionate Broth (TECRA *Salmonella* Visual Immunoassay)

First Action 1998

The method is a screening procedure for the presence of *Salmonella* in all 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. Enrichment broths and M broths from samples positive by an enzyme immunoassay (EIA) method must be streaked on selective media as in **967.26B**, and typical or suspicious colonies must be identified as in **967.26C**, **967.27**, and **967.28**.

Determination of positive results may be performed (1) visually by aid of a color comparator card, where a result is valid when negative and positive controls match those described on card, or (2) instrumentally, with a filter photometer, where a result is valid only when negative and positive controls possess acceptable optical density readings.

A. Principle

Detection of *Salmonella* antigens is based on an EIA using highly purified antibodies prepared from antigens unique to *Salmonella* spp. Polyclonal antibodies to *Salmonella* antigen are adsorbed onto the internal surface of a 96-well microtiter

tray. A sample to be assayed is placed into a well of the tray. If *Salmonella* antigens are present, they will attach to the antibody adsorbed on the well. All other material in sample is washed away.

A conjugate is added that will bind to *Salmonella* antigens if they are attached to the antibody adsorbed on the surface of the well. Wells are washed to remove unbound conjugate and enzyme substrate is added. A dark blue-green color indicates the presence of *Salmonella* antigen in sample.

B. Method Performance

See Table 998.09 for method performance data.

C. Reagents

Items (a)–(m) are available as TECRA *Salmonella* Visual Immunoassay from BioEnterprises Pty. Ltd. (28 Barcoo St, Roseville, NSW 2069, Australia) and International BioProducts, Inc. (Redmond, WA). Substitutions must be pretested for equivalency.

Caution: Never pipette by mouth; reagents may contain low levels of toxic substances. Use appropriate chemical and microbiological safety procedures.

(a) *Antibody adsorbed strips*.—Removawell (Dynatech Laboratories, Inc.) strips with polyclonal antibodies to *Salmonella*. Store at 2°–8°C when not in use.

(b) *Tray*.—Sufficient to secure individual wells or strips.

(c) *Control antigen*.—Positive control (lyophilized). Purified *Salmonella* antigen, which reacts with antibodies to *Salmonella*, one vial. Reconstituted control antigens are stable 2 months when stored at 2°–8°C.

(d) *Control diluent*.—One vial (6 mL/vial). Contains Tris–saline–Tween with 0.01% thimerosal. Also used as negative control.

(e) *Conjugate*.—Two vials (lyophilized). Contains anti-*Salmonella* antibodies (from sheep) conjugated to horseradish peroxidase in borate buffer (0.001M) with 0.01% thimerosal. Reconstituted conjugate is stable 28 days when stored at 2°–8°C.

(f) *Conjugate diluent*.—Two vials (13.5 mL/vial). Contains borate buffer (0.005M) with 0.01% thimerosal.

(g) *Substrate*.—One vial (lyophilized). Contains 0.011 g 2,2'-azinodi(3-ethylbenzthiazoline sulfonate) and 0.123 g NaH₂PO₄. Reconstituted substrate is stable 2 months when stored at 2°–8°C.

(h) *Substrate diluent*.—One vial (22 mL/vial). Contains 0.116 g citric acid, 0.0011 g H₂O₂, and 0.0185 g NaOH in H₂O.

(i) *Stop solution*.—One vial (6 mL/vial). Contains 0.15 g NaF in H₂O. *Caution*: Avoid contact with skin. If contact occurs, wash area with H₂O. If spill occurs, sweep or pipette contents into beaker and dilute with water. Wash area with copious amounts of water.

(j) *Wash solution concentrate*.—One vial (25 mL/vial). Contains Tris–saline–Tween (58 g Tris/L, 234 g NaCl/L, 40 mL Tween 20/L) in 0.01% thimerosal. Reconstituted wash solution is stable 2 months when stored at 2°–8°C.

(k) *Color comparator card*.—For visual interpretation of positive and negative tests.

(l) *M broth*.—5.0 g yeast extract, 12.5 g tryptone, 2.0 g D-mannose, 5.0 g sodium citrate, 5.0 g NaCl, 5.0 g K₂HPO₄,

0.14 g MnCl₂, 0.8 g MgSO₄, 0.04 g FeSO₄, and 0.75 g Tween 80. Suspend ingredients in 1 L H₂O and heat to boiling for 1–2 min. Dispense 10 mL portions into 16 × 125 mm screw-cap test tubes. Cap tubes loosely and autoclave 15 min at 121°C. Tighten caps securely for storage. Final pH should be 7.0 ± 0.2.

(m) *Diagnostic reagents*.—Necessary for culture confirmation of presumptive EIA tests. Enrichment broths and M broths from samples positive by EIA method must be streaked on selective media as in 967.26B, and typical or suspicious colonies must be identified as in 967.26C, 967.27, and 967.28.

D. Apparatus

(a) *Incubators*.—35°–37°C, 41°–43°C.

(b) *Pipets*.—Capable of delivering 2 and 200 µL volumes for immunoassay, and 1 and 0.1 mL volumes for sample enrichment.

(c) *Water bath*.—Capable of maintaining 100°C. Autoclave set at 100°C is an acceptable alternative, as are generators of flowing steam.

(d) *Plastic squeeze bottle*.—500 mL, for dispensing wash solution. Automatic washer may be used.

(e) *Plastic film wrap*.—To cover wells during incubation.

(f) *EIA reader*.—Optional. Photometer with 414 ± 10 nm screening filter that will read through microtiter plates, or dual-wavelength reader with filters at 414 ± 10 nm and 490 ± 10 nm.

E. General Instructions

(1) Components of kit must be refrigerated when not in use. Kit is intended for one-time use only; do not reuse wells containing sample, reagents, or wash solution.

(2) Include single positive and negative control antigens with each group of test samples. All controls must function properly for test to be valid.

(3) Use data record sheet to identify location of each test sample.

(4) Use separate pipets for each sample and kit reagent to avoid cross-contamination. If plastic troughs are used to dispense conjugate and substrate, ensure that they are always kept separate.

(5) Components in kit are intended for use as integral unit.

F. Preparation of Sample

(a) *Pre-enrichment*.—Pre-enrich product in noninhibitory broth to initiate growth of *Salmonella* spp. Methods used may vary with product and should be performed as indicated in 967.26A or BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, chapter 5, section C. Pre-enrichment broths should be incubated at 35°–37°C.

(b) *Selective enrichment*.—Transfer 0.1 mL incubated pre-enrichment mixtures to Rappaport-Vassiliadis R10 broth (9.9 mL) and 1 mL to TT broth (9 mL). For all foods other than raw foods or foods having a high microbial load, incubate 6–8 h at 41°–43°C. Selective enrichments of raw foods or foods having a high microbial load must be incubated 16–20 h at 41°–43°C. An air incubator is suitable for this purpose.

(c) *Post-enrichment*.—Remove selective broths from incubation and mix by hand or by Vortex mixer. Remove 1 mL from TT tube and transfer to separate tube of sterile M broth (10 mL)

Table 998.09. Statistical analysis of collaborative study results for comparison of TECRA assay with method in BAM, 7th Ed.

Food (No. labs)	Method MPN ^a , agreement, cfu/g % ^b samples	Samples positive						Incidence of false negatives among total positive samples, % ^d				Sensitivity rate, % ^e				Incidence of false positives among total negative samples, % ^f				Specificity rate, % ^g	
		ELISA			Culture			χ ^{2c}	ELISA		Culture		ELISA	Culture		ELISA	Culture		ELISA	Culture	
		Total	Pres.	Conf.	Pres.	Conf.	Pres.		Conf.	Pres.	Conf.	Pres.		Conf.	Pres.		Conf.				
Milk chocolate (14)	<0.003 0.009 0.093	70 70 70	0 12 45	0 12 45	6 15 47	0 12 45	— ^h	— 0.0 0.0	— 0.0 0.0	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	
Dried whole egg (13)	<0.003 0.009 0.430	65 65 65	0 36 60	2 37 (35r) ⁱ 60	1 36 60	0 35 60	0.0 0.0 —	— 0.0 (2.8r) 0.0	— 2.8 0.0	— 100 (97r) 100	— 97 100	— 97 100	— 100 (97r) 100	— 99 (100r) 100	— 99 (100r) 100	— 99 (100r) 100	— 99 (100r) 100	— 99 (100r) 100	— 99 (100r) 100	— 99 (100r) 100	
Nonfat dry milk (18)	<0.003 0.24 2.4	80 80 80	0 75 75	0 74 75	0 75 75	0 75 75	— 0.0 0.0	— 1.3 0.0	— 0.0 0.0	— 99 100	— 100 100	— 100 100	— 99 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	
Black pepper (15)	<0.003 0.014 0.088	75 75 75	0 37 66	0 35 66 (64r)	3 38 67	0 37 66	— 0.5 —	— 5.4 0.0	— 0.0 0.0	— 95 100 (95r)	— 100 100	— 100 100	— 95 100 (95r)	— 100 100	— 100 100 (99r)	— 100 100 (99r)	— 100 100 (99r)	— 100 100 (99r)	— 100 100 (99r)	— 100 100 (99r)	
Soy flour (15)	<0.003 0.24 2.4	75 75 75	0 69 68	0 69 68	1 69 68	0 69 68	— — —	— 0.0 0.0	— 0.0 0.0	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	— 100 100	
Raw turkey (12)	<0.003 0.043 0.46	60 60 60	0 57 59	3 (4v) ^j 51 54	0 51 54	33 59 56	— 0.4 0.16	— 10.5 6.9	— 5.3 3.4	— 89.4 93.1	— 94.7 96.5	— 94.7 96.5	— 89.4 93.1	— 94.7 96.5	— 95 (93.3v) 98.4	— 95 (93.3v) 98.4	— 95 (93.3v) 98.4	— 95 (93.3v) 98.4	— 95 (93.3v) 98.4	— 95 (93.3v) 98.4	

^a Most probable number of colony-forming units per gram of food.^b Rate reflects the number of confirmed determinations that were equivalent between the TECRA and the culture methods.^c χ^2 is defined by McNemar as $(a - b)^2 / (a + b)$ where a = samples positive by TECRA and negative by culture method, and b = samples negative by TECRA and positive by culture method. An χ^2 value greater than 3.84 indicates significance at $p < 0.05$.^d Incidence of false negatives is 100 - sensitivity rate.^e Sensitivity rate is defined as 100 times the total number of analyzed positive test portions among "known" positive test portions divided by the total number of "known" negative test portions, where "known" positive is defined as samples confirmed positive by the reference method.^f Incidence of false positives is 100 - specificity rate.^g Specificity rate is defined as 100 times the total number of analyzed negative test portions among "known" negative test portions divided by the total number of "known" negative test portions, where "known" negative is defined as samples confirmed negative by the reference method and negative controls.^h —, statistical analysis not applicable.ⁱ r, reader only.^j v, visual only.

that has been warmed to 35°–37°C. Also remove 1 mL from Rappaport-Vassiliadis R10 tube and transfer to a separate tube of prewarmed M broth (10 mL). For all foods other than raw foods or foods having a high microbial load, incubate M broth tubes 16–20 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C incubator and incubate for additional 16–18 h. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35°–37°C and return selective enrichment broth tubes to 41°–43°C and incubate for an additional 6 h.

(d) *Preparation of sample for EIA analysis.*—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Combine equal volumes (0.5–1 mL) from each M broth tube in a clean screw-cap tube and heat in boiling H₂O bath or in flowing steam 15 min. Refrigerate (2°–8°C) remaining M broth and TT and Rappaport-Vassiliadis R10 tubes from (c) for culture confirmation of any EIA-positive samples (see 967.26B, 967.26C, 967.27 and 967.28). Cool heated M broths to 25°–37°C prior to analysis.

G. EIA

(a) The following reagents must be prepared prior to commencing assay: (1) Prepare working-strength wash solution by diluting contents of one vial of wash solution concentrate to 2 L with distilled or deionized H₂O into plastic reagent bottle. Plastic squeeze bottle is ideal for washing trays manually. (2) Prepare reconstituted positive control by transferring 3 mL control diluent to vial of lyophilized positive control antigen; mix thoroughly. The remaining control diluent acts as negative control. (3) Prepare reconstituted conjugate by adding vial of conjugate diluent to vial of lyophilized conjugate. Let conjugate rehydrate

at room temperature. Gently mix reconstituted conjugate. (4) Prepare reconstituted substrate by adding vial of substrate diluent to lyophilized substrate. Be sure substrate has dissolved and mixture is at room temperature prior to use. Reconstituted substrate will appear pale green. (5) Use stop solution as received. No reconstitution is required.

(b) Secure desired number of test (Removawell) strips in tray, allowing one well per food sample plus 2 wells for controls. *Press wells firmly into place.* Remove sealing film from top of wells to be used. Transfer 0.2 mL of each heated M broth sample to single well. Transfer 0.2 mL of reconstituted positive control and negative control into individual wells. Record sample position on sample record sheet provided.

(c) Cover tray with plastic film and incubate 30 min at 35°–37°C in standard laboratory incubator. Tray must be covered to prevent evaporation.

(d) After incubation, wash plate by hand with plastic squeeze bottle containing working-strength wash solution or use automatic washer charged with working-strength wash solution as follows: (1) Quickly invert tray, emptying its contents into container. (2) Remove any residual liquid by *firmly* tapping tray facedown on paper towel. (3) Completely fill each well with working-strength wash solution. (4) Repeat (1)–(3) another 2 times.

(e) Empty tray according to (d)(1) and (d)(2); then add 0.2 mL reconstituted conjugate to each well. Cover tray and incubate 30 min at 35°–37°C.

(f) Empty contents of tray and wash tray thoroughly 4 times according to (d)(1) and (d)(3); then empty tray according to (d)(1) and (d)(2).

Table 1. Test products, organisms, and levels

Product	<i>Salmonella</i> serovar	Inoculation level	MPN/g
Milk chocolate		Control	<0.003
	<i>Senftenberg</i> (H ₂ S neg)	Low	0.009
	<i>Senftenberg</i> (H ₂ S neg)	High	0.093
Dried egg		Control	<0.003
	B:r:1 complex	Low	0.009
	B:r:1 complex	High	0.430
Nonfat dry milk		Control	<0.003
	<i>Bovis morbificans</i>	Low	0.240
	<i>Bovis morbificans</i>	High	2.40
Black pepper		Control	<0.003
	<i>Anatum</i>	Low	0.014
	<i>Anatum</i>	High	0.088
Soy flour		Control	<0.003
	<i>Heidelberg</i>	Low	0.240
	<i>Heidelberg</i>	High	2.40
Raw ground turkey		Control	<0.003
	B:G, B:E h1 complex	Low	0.043
	C ₁ :G complex	High	0.46

(g) Add 0.2 mL reconstituted substrate to each well. Incubate at room temperature (20°–25°C) for a minimum of 10 min until positive control has reached color equivalent to positive control on color comparator card or to $A \geq 1.0$. Because color development tends to concentrate around edges of wells, it is important to tap sides of plate gently to mix contents prior to reading result. In this way, accurate readings will be obtained.

(h) Add 0.02 mL stop solution to each well. Incubation time should be ca 10–20 min. If >30 min has elapsed and A of 1.0 has not been attained, test is invalid.

H. Reading

Results of tests can be determined (1) visually or (2) with a microtiter tray reader. (1) Place tray on white background, and then compare individual test wells with color comparator. Positive control should give strong blue-green color indicating that all reagents are functional. If positive control is lighter than "Positive" on color comparator card, test is invalid; refer to "Troubleshooting Guide" in package insert, C(k). If negative control is darker than "Negative" on color comparator card, it is probable that the tray was inadequately washed and assay must be repeated.

(2) A maximum absorbance of the blue-green end product occurs at 414 nm; therefore, tray can be read at 414 ± 10 nm. For dual-wavelength readers, set reader to zero (blank) on air and set second reference wavelength at 490 ± 10 nm. For single-wavelength readers, set reader to zero (blank) with a well containing 200 μ L water or substrate. $A \geq 0.3$ indicates positive

results. Positive control should give $A \geq 1.0$, negative control should give $A < 0.2$.

Ref.: *J. AOAC Int.* 82, 635–639(1999)

Results and Discussion

Table 1 gives the product types, serotypes, and levels of salmonellae present at the time of analysis. Table 2 shows product analysis by collaborator number for both Australia and the United States. In Australia, 18 collaborators participated in the study, which included nonfat dry milk, ground black pepper, and soy flour. In the United States, 20 laboratories participated in the study, which included milk chocolate, dry egg, and raw ground turkey. However, not all collaborators analyzed all foods, and data from some laboratories were excluded because of method deviations or because uninoculated control samples gave positive results. Some collaborators also failed to set up samples or to return data.

Results for sample analysis of each food type are given in Tables 3 to 8.

Nonfat Dry Milk

Table 3 gives results of analysis of nonfat dry milk by 16 collaborators. Of the 240 samples analyzed, 239 gave identical results for the TECRA method and the reference method. The remaining sample gave a false-negative result with the TECRA method, as well as an unconfirmed positive result for the TECRA method with the plate reader only. No significant dif-

Table 2. Collaborator participation in study by product type

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

^a Y, participated in trial.

^b N, did not participate.

^c Results not used in analysis because one or more control samples tested positive for *Salmonella*.

^d Results not used in analysis because of method error.

^e Received samples but did not set up or did not return data.

Table 3. Collaborative study results for detection of *Salmonella* spp. in milk powder

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

^a r+, positive with reader only.

Table 4. Collaborative study results for detection of *Salmonella* spp. in black pepper

Collaborator	Uninoculated samples					Low inoculum samples					High inoculum samples				
	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	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
12	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
14	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+
16	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+
17	-	-	-	-	-	+	+	-	-	+	+	-	+(r-) ^b	+	+
TECRA assay (confirmed result)															
1	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+
3	-	-	-	-	-	+	+	-	+	+	+	+	-	-	+
4	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+
5	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+
6	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+
8	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+
9	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
12	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
14	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+
16	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+
17	-	-	-	-	-	+	+	-	-	+	+	-	+	+	+
BAM															
1	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+
3	-	-	-	-	-	+	+	-	+	+	+	+	-	-	+
4	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+
5	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+
6	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+
8	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+
9	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
12	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
14	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+
16	- ^c	-	-	-	-	-	-	+	-	-	+	+	+	-	+
17	-	- ^c	-	- ^c	-	+	+	- ^c	-	+	+	- ^c	+	+	+

^a No reader result.^b r-, negative with reader only.^c Suspect colonies on plates.

Table 5. Collaborative study results for detection of *Salmonella* spp. in soy flour

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

^a No reader result.^b Suspect colonies on plates.

Table 6. Collaborative study results for detection of *Salmonella* spp. in milk chocolate

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

^a Borderline reading classified as negative because positive control was greater than 1.5.^b Suspect colonies on plates.

Table 7. Collaborative study results for detection of *Salmonella* spp. in dried egg

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

^a Borderline reader result (positive control was 2.24).

Table 8. Collaborative study results for detection of *Salmonella* spp. in raw turkey

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

^a r+, positive with plate reader only.^b Visual reading positive.^c Suspect colonies on plates.

ferences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Ground Black Pepper

Table 4 gives the results for analysis of ground black pepper by 15 collaborators. This was a raw product with high levels of competitive flora and very low inoculation levels. For the 225 samples analyzed, there was exact agreement between the confirmed results with the TECRA and the cultural method. There were 5 false positives for the cultural method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Soy Flour

Table 5 shows the results of analysis for soy flour by 15 collaborators. For the 225 samples analyzed, there was exact agreement between the confirmed data for the TECRA method and the cultural method. One false positive occurred for the cultural method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Milk Chocolate

Table 6 shows the results of analysis for milk chocolate by 14 collaborators. For the 210 samples analyzed, there was exact agreement between confirmed results for the TECRA method and the cultural method. There were 11 false positives for the cultural method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples with that for the reference method.

Dried Whole Egg

Table 7 shows results from 13 collaborators for dry egg. For the 195 samples analyzed, using confirmed results, there were no false negatives for the TECRA method and one false negative for the cultural method. There was one false positive for the TECRA method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Raw Turkey

Table 8 shows results from 12 collaborators for naturally contaminated raw turkey. For the 180 samples tested, using confirmed data, there was one false negative at the low level for the TECRA method. There were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. The incidence of false-positive results was very high for the cultural method, with a total of 41 false positives, compared with 5 for the TECRA method. It should, however, be taken into account that the comparison was with the method in BAM 7th Ed., not the current 8th Ed. The method for raw flesh food in the BAM 8th Ed. uses elevated temperature with RV and TT broth, which would be more selective. No significant

differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the TECRA method with that for the reference method.

Recommendation

No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the new enrichment procedure for the TECRA method with that for the reference method.

Therefore, it is recommended that the new enrichment procedure for the TECRA method be adopted official First Action. This method is not intended to replace AOAC Official Method 989.14 but to be available as an alternative procedure for enrichment.

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ⓧ Notes

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