

MICROBIOLOGICAL METHODS

***Salmonella* in Foods: New Enrichment Procedure for TECRA Salmonella Visual Immunoassay Using a Single RV(R10) Only, TT Only, or Dual RV(R10) and TT Selective Enrichment Broths (AOAC Official Method 998.09): Collaborative Study¹**DENISE HUGHES,² ANGELA E. DAILIANIS, and LOUISE HILL

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A collaborative study was conducted to compare a new enrichment procedure for the TECRA® *Salmonella* Visual Immunoassay (TSVIA) with the reference method given in the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (7th Ed.). Three food types (milk powder, 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. The TECRA method was evaluated using both Rappaport-Vassiliadis R10 (RV(R10)) and tetrathionate (TT) broths for selective enrichment. M broth cultures arising from each of the 2 selective enrichment broths were tested in the TSVIA using 2 individual wells, one for each selective broth, and a single well to test the pooled selective enrichment broths. The results for the pooled enrichment broths were reported elsewhere. This study presents the results for the use of single enrichment broths, i.e., RV(R10) only or TT only, with the TSVIA. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for either RV(R10)

or TT used as a single enrichment broth for the TSVIA with that for the reference method.

The TECRA® *Salmonella* Visual Immunoassay (TSVIA) is an enzyme-linked immunosorbent assay (ELISA) performed in the sandwich configuration. High affinity capture antibodies specific to *Salmonella* have been adsorbed onto the surface of the Removawells. 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*. The presence of *Salmonella* is indicated when the bound conjugate converts the substrate to a green color.

AOAC Method 989.14 *Salmonella* in Foods (using the TSVIA) was adopted First Action in 1989 and Final Action in 1990. In 1996, a modified version of the TSVIA, which was supplemented with antibodies to *S. Pullorum* and *S. Gallinarum*, was given First Action approval (1).

In previous AOAC studies on the 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 replace SC with 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 RV(R10) and TT broths at 42°C for selective enrichment. It was considered that the use of a single selective enrichment broth, particularly one which could be prepared in advance, would provide a simple, convenient cultural procedure. For this reason, data for both individual and pooled se-

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² The collaborative study reporting the modified dual enrichment procedure [*J. AOAC Int.* 82, 634–647(1999)] erroneously includes Table 998.09B rather than Table 998.09A.

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lective enrichment broths were generated in the study. However, only the results for the use of single enrichment broths are reported here; results for the pooled enrichments have been reported separately (3).

The reference method for the study was U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (BAM; 7th Ed.; 4) because this method was current at commencement of the precollaborative study. Since this time, a new 8th Ed. of BAM (5) has been published in which the method for raw flesh foods and highly contaminated 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 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 (6). All foods were artificially contaminated except raw turkey, which was naturally contaminated.

In order 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 resuspended in sterile non-fat milk and lyophilized at room temperature for 24 h. Freeze-dried inocula were ground to a fine powder before use.

Inoculation of Samples

For dried products (milk powder, pepper, soy flour, and dried egg) a concentrated seed was made by addition of freeze-dried inoculum to approximately 500 g test product. This seeded product was mixed well and stored at room temperature for at least 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 the concentrated seed was added to the test product to achieve high (10–50 cells/25 g) and low levels (1–5 cells/25 g). A most probable number (MPN) determination was performed before sample shipment, and levels of *Salmonella* 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 the high and low levels. Different batches of turkey were

tested and based on contamination levels were used as uninoculated control, low, and high levels. Samples were frozen before shipment.

Sample Shipment

Dried foods and chocolate were shipped at ambient temperature, and raw turkey was shipped frozen on dry ice. Each collaborator received 15 samples of the food to be analyzed: 5 high, 5 low, and 5 uninoculated. The appropriate samples were shipped to participating laboratories on the week before 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 sample analysis was initiated by collaborators, an MPN determination was also performed by the organizing laboratory.

Each sample was analyzed by the BAM method (7th Ed.) and the the TSVIA using the enrichment protocol described in E, *Preparation of Sample*. M broths from RV(R10) and TT were heat treated and assayed individually, i.e., 2 separate wells were used for each sample, one well to analyze TT only and one well to analyze RV(R10) only. The immunoassay was confirmed 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 determined for the TECRA method using combined and individual selective enrichments 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) was determined. A false-positive result was determined at the first assay or decision point for the procedure, i.e., 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 was made of the proportion of positive samples using each of the selective enrichment broths with the TSVIA and the proportion of positive samples for the reference method. McNemar's test ($p > 0.05$) was used to determine whether the methods were significantly different.

AOAC Official Method 998.09
***Salmonella* in Foods**
 Colorimetric Polyclonal Enzyme
 Immunoassay Screening Method
 (TECRA *Salmonella* Visual Immunoassay)
 First Action 1998
 Revised First Action 1999
 Final Action 2001

[Method is a screening procedure for presence of *Salmonella* in all foods; it is not a confirmatory test because

polyclonal antibodies used in test may cross-react with small percentage of non-*Salmonella*. Enrichment and M broths from samples positive by enzyme immunoassay (EIA) method must be streaked on selective media as in **967.26B** (17.9.02) and typical or suspicious colonies must be identified as in **967.26C** (17.9.02), **967.27** (17.9.03), and **967.28** (17.9.07).]

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

See Tables **998.09A–C** for the results of the interlaboratory study supporting acceptance of the method.

Caution: Reagents may contain low levels of toxic substances; never mouth-pipette. Use appropriate chemical and microbiological safety procedures.

A. Principle

Detection of *Salmonella* antigens is based on EIA using highly purified antibodies prepared from antigens unique to *Salmonella*. Polyclonal antibodies to *Salmonella* antigen are absorbed onto the internal surface of a 96-well microtiter tray. Suspension from product to be assayed is placed into a well of the tray. If *Salmonella* antigens are present, they will attach to specific antibody adsorbed on the well. All other material is washed away.

Conjugate is added and will bind to *Salmonella* antigens if they are attached to adsorbed antibody 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 the suspension.

B. Reagents

Items (a)–(m) are available as TECRA Salmonella Visual Immunoassay (TECRA International Pty Ltd, 13 Frenchs Forest, NSW 2086, Australia, and International BioProducts, Inc., Redmond, WA). Substitutions must be pretested for equivalency.

(a) *Antibody adsorbed strips*.—Removawell® (Dynatech Laboratories, Inc., Chantilly, VA) strips. Polyclonal antibodies to *Salmonella* 96 wells. Store wells at 2–8°C when not in use.

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

(c) *Control antigen*.—Positive control (lyophilized). One vial purified *Salmonella* antigen, which reacts with antibodies to *Salmonella*. 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 horse-radish 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'-azino-di(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 water. If spill occurs sweep/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/L Tris, 234 g/L NaCl, 40 mL/L Tween 20) in 0.01% thimerosal. Reconstituted wash solution is stable for 2 months when stored at 2–8°C.

(k) *Data record sheet*

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

(m) *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, 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.

(n) *Diagnostic reagents*.—Necessary for culture confirmation of presumptive EIA tests. Enrichment and M broths from suspensions positive by EIA method must be streaked on selective media as in **967.26B** (17.9.02) and typical or suspicious colonies must be identified as in **967.26C** (17.9.02), **967.27** (17.9.03), and **967.28** (17.9.07).

C. Apparatus

(a) *Incubators*.—35–37 and 41–43°C.

(b) *Pipets*.—Delivering 20 and 200 µL volumes for immunoassay; 1 and 0.1 mL for enrichment.

(c) *Water bath*.—Maintaining 100°C. Autoclave set at 100°C is 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) *Enzyme immunoassay reader*.—Optional. Photometer with 414 ± 10 nm screening filter which will read through microtiter plates, or dual wavelength reader with filters at 414 ± 10 and 490 ± 10 nm.

D. General Instructions

Refrigerate components of kit when not in use. Kit is intended for one-time use only. Do not reuse wells containing suspension, reagents, or wash solution.

Include single positive and negative control antigens with each group of test samples. All controls must function prop-

Table 998.09A. Statistical analyses of interlaboratory study results for TECRA assay using 2 selective enrichment broths (RV(R10) and TT)

Food (No. labs)	MPN, ^a CFU/g	Method agreement, % ^c samples	Samples positive				χ^2	Incidence of false negatives among total positive samples, % ^d				Sensitivity rate, % ^e				Incidence of false positives among total negative samples, % ^f				Specificity rate, % ^g	
			Total	ELISA	Presumptive Confirmed	Culture		ELISA	ELISA	Culture	ELISA	ELISA	Culture	ELISA	Culture	ELISA	Culture	ELISA	Culture	ELISA	Culture
Milk chocolate (14)	<0.003	100	70	0	1	0	6	0								1.4	8.6	99	91		
	0.009	100	70	12	12	12	15	12				100	100	100	100	0.0	2.3	100	98		
	0.093	100	70	45	44	45	47	45	0.0	0.0	0.0	100	100	100	100	0.0	2.1	100	98		
Dried whole egg (13)	<0.003	100	65	0	4	1	0	0								4.6	0.0	95	100		
	0.009	98.4	65	36	37	36	35	35	0.0	2.8	0.0	100	97	100	100	1.1	0.0	99	100		
	0.430	100	65	60	56	60	60	60	0.0	0.0	0.0	100	100	100	100	0.0	0.0	100	100		
Nonfat dry milk (18)	<0.003	100	80	0	0	0	0	0								0.0	0.0	100	100		
	0.24	98.8	80	75	74(75) ^h	74	75	75	0.0	0.0	0.0	99	100	100	100	0.0(1.2) ^h	0.0	100(99) ^h	100		
	2.4	100	80	75	75	75	75	75	0.0	0.0	0.0	100	100	100	100	0.0	0.0	100	100		
Black pepper (15)	<0.003	100	75	0	0	0	3	0								0.0	4.0	100	96		
	0.014	100	75	37	37	37	38	37	0.0	0.0	0.0	100	100	100	100	0.0	0.9	100	99		
	0.088	100	75	66	66(64) ^h	66	67	66	0.0	0.0	0.0	100(97) ^h	100	100	100	0.0	1.2	100	99		
Soy flour (15)	<0.003	100	75	0	0	0	1	0								0.0	1.3	100	99		
	0.24	98.7	75	69	70	69	70	69	0.0	0.0	0.0	100	100	100	100	1.2	1.2	99	99		
	2.4	100	75	68	68	68	68	68	0.0	0.0	0.0	100	100	100	100	0.0	0.0	100	100		
Raw turkey (12) (7th Ed. BAM)	<0.003	100	60	0	3	0	33	0								5.0	55	95	45		
	0.043	93.3	60	57	58	56	59	54	0.0	1.8	5.3	98.2	94.7	98.2	94.7	3.2	7.9	96.8	92.1		
	0.46	95.0	60	59	53	59	59	56	1.3	0.0	5.1	100	94.9	100	94.9	0.0	4.9	100	95.1		

^a MPN = Most probable number of colony forming units per gram of food.^b Rate reflects number of confirmed determinations equivalent between TECRA and 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. χ^2 value > 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 total number of known test portions. 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. Known negative is defined as samples confirmed negative by the reference method and negative controls.^h Statistical analysis not applicable; r = reader only.

Table 998.09B. Statistical analyses of interlaboratory study results for TECRA assay with selective enrichment in Rappaport-Vassiliadis (R10) broth only, compared to BAM, 7th Ed.

Food (No. labs)	MPN, ^a CFU/g	Method agreement, % ^b	Total samples	ELISA				Presumptive	Confirmed	χ^2 ^c	Incidence of false negatives among total positive samples, % ^d		Sensitivity rate, % ^e		Incidence of false positives among total negative samples, % ^f		Specificity rate, % ^g	
				Total	Presumptive	Confirmed	Confirmed				ELISA	Culture	ELISA	Culture	ELISA	Culture	ELISA	Culture
Milk chocolate (14)	<0.003	100	70	0	0	0	6	0					0	8.6	100	91		
	0.009	100	70	12	13	12	15	12			0.0	0.0	100	100	1.72	2.3	98	98
	0.093	100	70	45	44	45	47	45		0.0	0.0	0.0	100	100	0.0	2.1	100	98
Dried whole egg (13)	<0.003	98.5	65	0	2	1	0	0	0.0				1.5	0.0	99	100		
	0.009	98.5	65	36	37(35r) ^h	36	35	35	0.0	0.0(2.8r)	2.8	100(97r)	97	1.1(0.0r)	0.0	99(100r)	100	100
	0.430	100	65	60	56	60	60	60		0.0	0.0	100	100	0.0	0.0	100	100	
Nonfat dry milk (18)	<0.003	100	80	0	0	0	0	0					0.0	0.0	100	100		
	0.24	98.8	80	75	74	74	75	75	0.0	1.3	0.0	99	100	0.0	0.0	100	100	
	2.4	100	80	75	75	75	75	75	0.0	0.0	0.0	100	100	0.0	0.0	100	100	
Black pepper (15)	<0.003	100	75	0	0	0	3	0					0.0	4.0	100	96		
	0.014	97.3	75	37	35	35	38	37	0.0	5.4	0.0	95	100	0.0	0.9	100	99	
	0.088	100	75	66	66(64r)	66	67	66		0.0	0.0	100(95r)	100	0.0(1.2r)	1.2	100(99r)	99	
Soy flour (15)	<0.003	100	75	0	0	0	1	0					0.0	1.3	100	99		
	0.24	100	75	69	69	69	69	69		0.0	0.0	0.0	0.0	0.0	0.0	100	100	
	2.4	100	75	68	69	68	68	68		0.0	0.0	0.0	0.0	0.0	0.0	100	100	
Raw turkey (12) (7th Ed. BAM)	<0.003	100	60	0	3(4v) ^h	0	33	0					5.0(6.6v)	55	95(93.3v)	45		
	0.043	85	60	57	52	51	59	54	0.4	10.5	5.3	89.4	94.7	1.6	7.9	98.4	92.1	
	0.46	90	60	58	53	54	59	56	0.16	6.9	3.4	93.1	96.5	0.0	4.8	100	95.2	

^a MPN = Most probable number of colony forming units per gram of food.^b Rate reflects number of confirmed determinations equivalent between TECRA and culture methods.^c χ^2 is defined by McNemar as $((a - b) - 1)^2 / (a + b)$, where a = samples positive by TECRA and negative by culture method and b = samples negative by TECRA and positive by culture method. χ^2 value > 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 total number of known test portions. 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. Known negative is defined as samples confirmed negative by the reference method and negative controls.^h Statistical analysis not applicable; r = reader only; v = visual only.

Table 998.09C. Statistical analyses of interlaboratory study results for TECRA assay with selective enrichment in tetrathionate broth only, compared to BAM, 7th Ed.

Food (No. labs)	MPN, ^a CFU/g	Method agreement, % ^b	Total samples	Samples positive				χ^2	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			ELISA	Culture	ELISA	Culture	ELISA	Culture		
				Presumptive	Confirmed	Presumptive	Confirmed									
Milk chocolate (14)	<0.003	100	70	0	1	6	0	0.0					0	8.6	100	91
	0.009	100	70	12	12	15	12		0.0	0.0	100	100	0.0	2.3	100	98
	0.093	98.6	70	45	44	47	45	0.5	2.2	0.0	98	100	0.0	2.1	100	98
Dried whole egg (13)	<0.003	98.4	65	0	3(4v) ^h	0	0	0.0					3.0(4.6v)	0.0	97(95v)	100
	0.009	100	65	36	35	35	35		0.0	0.0	100	100	1.1	0.0	99	100
	0.430	100	65	60	60	60	60		0.0	0.0	100	100	0.0	0.0	100	100
Nonfat dry milk (18)	<0.003	100	80	0	0	0	0						0.0	0.0	100	100
	0.24	98.8	80	75	74	75	75	0.0	1.3	0.0	99	100	0.0	0.0	100	100
	2.4	100	80	75	75	75	75	0.0	1.3	0.0	99	100	0.0	0.0	100	100
Black pepper (15)	<0.003	100	75	0	0	3	0						0.0	4.0	100	96
	0.014	98.7	75	37	36	37	37	0.0	2.7	0.0	97	100	0.0	0.9	100	99
	0.088	97.3	75	66	64	66	66	0.5	3.0	0.0	97	100	0.0	1.2	100	99
Soy flour (15)	<0.003	100	75	0	0	1	0		0.0	0.0	100	100	0.0	1.3	100	99
	0.24	100	75	69	69	69	69		0.0	0.0	100	100	1.2	0.0	99	100
	2.4	100	75	68	68	68	68		0.0	0.0	100	100	0.0	0.0	100	100
Raw turkey (12) (7th Ed. BAM)	<0.003	100	60	0	2(3r) ^h	33	0						3.3(5.0r)	55	96.7(95.0r)	45
	0.043	91.6	60	56	55	59	54	0.0	5.6	3.6	94.6	96.4	3.1	7.8	96.9	92.2
	0.46	91.6	60	56	56	59	56		0.0	5.1	100	100	0.0	4.7	100	95.3

^a MPN = Most probable number of colony forming units per gram of food.^b Rate reflects number of confirmed determinations equivalent between TECRA and 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. χ^2 value > 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 total number of known test portions. 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. Known negative is defined as samples confirmed negative by the reference method and negative controls.^h Statistical analysis not applicable; r = reader only; v = visual only.

erly for test to be valid. Use data record sheet to identify location of each test sample.

Use separate pipets for each test 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.

Components in kit are intended for use as integral unit.

E. 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 (17.9.02), or in BAM, 7th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, Ch. 5, sec. C. Pre-enrichment broths should be incubated at 35–37°C.

(b) *Selective enrichment*.—Transfer 0.1 mL incubated pre-enrichment mixtures to RV(R10) broth (9.9 mL) or 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) which has been warmed to 35–37°C. Alternatively, remove 1 mL from RV(R10) tube and transfer to separate tube of 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. For raw foods or foods having a high microbial load, incubate M broth tubes 6 h at 35–37°C.

(d) *Preparation for EIA analysis*.—Remove M broth tubes from incubation and mix tubes by hand or Vortex mixer. Transfer 1 mL from each M broth tube into clean screw-cap tube and heat in boiling water bath or in flowing steam 15 min. Refrigerate (2–8°C) remaining M broth and TT or RV(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 before analysis.

F. Enzyme Immunoassay

Prepare the following reagents before commencing assay:

(a) *Working strength wash solution*.—Prepare by diluting contents of one vial wash solution concentrate to 2 L with distilled or deionized H₂O into reagent bottle. Plastic squeeze bottle is ideal for washing trays manually.

(b) *Reconstituted positive control*.—Prepare by transferring 3 mL control diluent to vial of lyophilized positive control antigen; mix thoroughly. The control diluent remaining acts as negative control.

(c) *Reconstituted conjugate*.—Prepare by adding vial of conjugate diluent to vial of lyophilized conjugate. Let conjugate rehydrate at room temperature. Gently mix reconstituted conjugate.

(d) *Reconstituted substrate*.—Prepare by adding vial of substrate diluent to lyophilized substrate. Be sure substrate has

dissolved and mixture is at room temperature before use. Reconstituted substrate will appear pale green.

(e) *Stop solution*.—Use as received. No reconstitution is required.

Secure desired number of test (Removawell) strips in tray, allowing one well per food test 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 suspension to single well. Transfer 0.2 mL reconstituted positive control and negative control into individual wells. Record test sample position on sample record sheet provided. Cover tray with plastic film and incubate 30 min at 35–37°C in standard laboratory incubator. Tray must be covered to prevent evaporation.

After incubation, wash plate by hand, using 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 face-down on paper towel several times. (3) Completely fill each well with working strength wash solution. (4) Repeat (1)–(3) twice more.

Empty tray according to (1) and (2); then add 0.2 mL reconstituted conjugate to each well. Cover tray and incubate 30 min at 35–37°C. Empty contents of tray and wash thoroughly 4 times according to (1)–(3); then empty tray according to (1) and (2). Add 0.2 mL reconstituted substrate to each well. Incubate at room temperature (20–25°C) for minimum of 10 min, until positive control has reached color equivalent to positive control on color comparator card or to A ≥ 1.0. Because color development tends to concentrate around edges of wells, to obtain accurate readings it is important to tap sides of plate gently to mix contents before reading result. 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.

G. Reading

Results of tests can be determined (1) visually or (2) with 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 Control” on color comparator card, test is invalid; refer to “Troubleshooting Guide” in package insert. If negative control is darker than “Negative” on color comparator card, the tray was probably inadequately washed, and assay must be repeated.

(2) A maximum of blue-green end product occurs at 414 nm; therefore, read tray 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 readers to zero (blank) using a well containing 200 µL water or substrate. A ≥ 0.3 indicates positive results. Positive control should give A ≥ 1.0, negative control A < 0.2.

Refs.: *J. AOAC Int.* **82**, 634(1999); **86**, 776–781(2003)

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, 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 3–8. Different results for RV(R10) and TT are indicated by footnotes. Performance parameters for TECRA method using RV(R10) are shown in Table 998.09B; the TECRA method using TT is shown in Table 998.09C. Table 998.09A, which details the results of the dual RV(R10) and TT enrichment, is included for completeness.

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 VIA methods and the reference method. The remaining sample was negative for the 2 VIA methods and positive with the cultural method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of

the proportion of positive samples for each of the TECRA methods with that for the reference method.

Ground Black Pepper

Table 4 gives the results of analysis of ground black pepper by 15 collaborators. This was a raw product with high levels of competitive flora and very low inoculation levels. In all, 225 samples were analyzed. For the VIA using RV(R10) only, there were 2 false negatives at the low level and exact agreement of the confirmed results at the high level. For the VIA using TT only, there was one false negative at the low level and 2 false negatives at the high level. There were 5 false positives for the reference method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for each of the 2 VIA methods with that for the reference method.

Soy Flour

Table 5 shows the results of analysis of soy flour by 15 collaborators. For the 225 samples analyzed, there was exact agreement between the confirmed data for the 2 VIA methods and the cultural method. One false positive occurred for the VIA with RV(R10) only and one false positive for the cultural method. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for each of the 2 VIA methods with that for the reference method.

Table 1. Test products, organisms, and levels

Product	<i>Salmonella</i> serovar	Inoculation level	MPN/g
Milk chocolate		Control	<0.003
	<i>S. Senftenberg</i> (H ₂ S neg)	Low	0.009
	<i>S. 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>S. Bovis moribificans</i>	Low	0.240
	<i>S. Bovis moribificans</i>	High	2.40
Black pepper		Control	<0.003
	<i>S. Anatum</i>	Low	0.014
	<i>S. Anatum</i>	High	0.088
Soy flour		Control	<0.003
	<i>S. Heidelberg</i>	Low	0.240
	<i>S. 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

Table 2. Collaborator participation in study by product type^a

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

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

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 VIA using RV(R10) only and the cultural method. There was one false negative for the VIA with TT only. There were 11 false positives for the cultural method. Statistical analysis was performed for the VIA with TT only. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the 2 VIA methods 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 2 VIA methods and one false negative for the cultural method. There was one false positive for each of the VIA methods. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the

proportion of positive samples for each of the 2 VIA methods 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 were 3 false negatives at the low level and 3 false negatives at the high level for the cultural method. When only RV(R10) was used with the VIA, there were 6 false negatives at the low level and 4 false negatives at the high level. When only TT was used with the VIA, there were 3 false negatives at the low level but none at the high level. The incidence of false-positive results was very high for the cultural method with a total of 41 false positives, compared to 4 for RV(R10) with the reader, 5 for RV(R10) read visually, 4 for TT read visually, and 5 for TT with the reader. It should, however, be taken into account that the comparison was with the BAM, 7th Ed., not the current 8th Ed.

Table 3. Interlaboratory study results for detection of *Salmonella* in milk powder using single selective enrichment broths^a

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	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
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	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
FDA BAM															
1	—	—	—	—	—	+	+	+	+	+	+	+	+	—	+
2	—	—	—	—	—	+	—	+	+	+	+	+	+	+	+
3	—	—	—	—	—	+	+	+	+	+	+	+	+	+	—
4	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
5	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
7	—	—	—	—	—	—	+	+	+	+	—	+	+	+	+
8	—	—	—	—	—	+	+	—	+	+	+	+	+	+	+
9	—	—	—	—	—	+	—	+	+	+	+	+	+	+	+
10	—	—	—	—	—	+	+	+	+	+	+	—	+	+	+
11	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
12	—	—	—	—	—	+	+	+	+	+	+	+	—	+	+
13	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
14	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
15	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
16	—	—	—	—	—	+	+	+	—	+	+	+	+	+	+
17	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+

^a There were no differences in results for the VIA method using RV(R10) and the VIA method using TT.

Table 4. Interlaboratory study results for detection of *Salmonella* in pepper^a

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	-	-	-	-	-	+	+	-	+	+(R-)	+	+	-	-	+(Rr-)
4	-	-	-	-	-	-	-	+(T-)	-	-	+	+	+	+	+
5	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+
6	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+
8	-	-	-	-	-	+	-	-	+	-	+	+	+(T-)	+	+
9	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
12	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
14	-	-	-	-	-	-	+	+	+(R-)	-	+	+	-	+	+
16	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+
17	-	-	-	-	-	+	+	-	-	+	+	-	-(Rv+)	+	+
TECRA assay (confirmed result)															
1	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+(R-)
2	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+
3	-	-	-	-	-	+	+	-	+	+(R-)	+	+	-	-	+
4	-	-	-	-	-	-	-	+(T-)	-	-	+	+	+	+	+
5	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+
6	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+
8	-	-	-	-	-	+	-	-	+	-	+	+	+(T-)	+	+
9	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
12	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
14	-	-	-	-	-	-	+	+	+(R-)	-	+	+	-	+	+
16	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+
17	-	-	-	-	-	+	+	-	-	+	+	-	+(T-)	+	+
FDA BAM															
1	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+
3	-	-	-	-	-	+	+	-	+	+	+	+	-	-	+
4	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+
5	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+
6	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	-	-	+	+	+	+	+
8	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+
9	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+
11	-	-	-	-	-	-	+	-	+	+	+	+	+	+	-
12	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	-	+	-	+	+	+	+	+
14	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+
16	- ^c	-	-	-	-	-	-	+	-	-	+	+	+	-	+
17	-	- ^c	-	- ^c	-	+	+	- ^c	-	+	+	- ^c	+	+	+

^a R = RV only; T = TT only; Rr = RV with plate reader only; Rv = RV visual reading only.^b Visually positive, no reader result for RV only.^c Suspect colonies on plates.

Table 5. Interlaboratory study results for detection of *Salmonella* 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	-	-	-	-	-	+	+	-(T+) ^a	+	+	+	+	+	+	+
3	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-
4	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+
5	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+
8	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+
9	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+
10	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+
11	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
13	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+
14	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+
15	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
16	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
17	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+
TECRA assay (confirmed result)															
1	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
2	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+
3	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-
4	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+
5	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+
8	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+
9	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+
10	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+
11	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
13	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+
14	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+
15	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
16	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
17	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+
FDA BAM															
1	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
2	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+
3	-	-	- ^b	-	-	+	+	+	+	+	+	+	+	+	-
4	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+
5	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+
7	-	-	-	-	-	+	+	+	+	-	+	+	+	+	+
8	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+
9	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+
10	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+
11	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
12	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+
13	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+
14	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+
15	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
16	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
17	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+

^a T = TT only.^b Suspect colonies on plates.

Table 6. Interlaboratory study results for detection of *Salmonella* 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	—	—	—	—	—	—	—	—	—	—	—	+(T-) ^a	+	+	—
3	—	—	—	—	—	+	—	—	—	—	+	+	+	+	+
4	—	—	—	—	—	—	—	—	—	+	+	—	+	+	+
5	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
6	—	—	—	—	—	—	—	—	—	—	+	+	+	—	+
7	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
8	—	—	—	—	—	—	—	—	-(R+) ^b	—	—	—	—	—	—
9	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
10	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—
14	—	—	—	—	—	+	+	—	+	—	—	+	+	—	—
16	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—
17	—	—	—	—	—	— ^c	—	+	—	—	+	+	—	+	+
18	—	—	—	—	—	—	—	—	—	—	+	+	—	+	—
20	—	—	—	—	—	—	—	—	—	+	—	+	+	+	—
TECRA assay (confirmed result)															
1	—	—	—	—	—	—	—	—	—	—	—	+(T-) ^a	+	+	—
3	—	—	—	—	—	+	—	—	—	—	+	+	+	+	+
4	—	—	—	—	—	—	—	—	—	+	+	—	+	+	+
5	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
6	—	—	—	—	-(T+) ^b	—	—	—	—	—	+	+	+	—	+
7	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
8	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—
9	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
10	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—
14	—	—	—	—	—	+	+	—	+	—	—	+	+	—	—
16	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	+	—	—	+	+	—	+	+
18	—	—	—	—	—	—	—	—	—	—	+	+	—	+	—
20	—	—	—	—	—	—	—	—	—	+	—	+	+	+	—
FDA BAM															
1	—	—	—	—	—	—	—	—	—	—	—	+	+	+	—
3	—	—	—	—	—	+	—	—	—	—	+	+	+	+	+
4	—	—	—	—	—	—	—	—	—	+	+	—	+	+	+
5	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
6	—	—	—	—	—	—	—	—	—	—	+	+	+	—	+
7	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
8	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—
9	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
10	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—
14	— ^d	—	—	— ^d	— ^d	+	+	—	+	—	— ^d	+	+	—	—
16	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—
17	—	—	— ^d	— ^d	— ^d	— ^d	— ^d	+	—	— ^d	+	+	— ^d	+	+
18	—	—	—	—	—	—	—	—	—	—	+	+	—	+	—
20	—	—	—	—	—	—	—	—	—	+	—	+	+	+	—

^a T = TT only.^b R = RV only.^c Borderline reading for TT classified as negative because positive control > 1.5.^d Suspect colonies on plates.

Table 7. Interlaboratory study results for detection of *Salmonella* in dried egg^a

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	—	—	+(R—)	—	—	—	—	—	—	+	+	+	+	+	+
3	—	—	—	—	—	+	—	—	+	+	+	+	+	+	+
4	—	—	—	—(Tv+)	—	+	+	+	+	+	+	+	+	+	+
5	—	—	—	—	—	+	—	+	+	+	+	+	+	+	+
6	—	—	—	—	—	+	—	+	—	+	—	—	—	—	—
7	—	—	—	—	—	—	+	—	+	—	+	+	+	+	+
8	—(R+)	+(R—)	— ^b	+	—	—	+	+	+	+	—	—	+(T—)	—	—
9	—	—	—	—	—	+	—	—	+	—	+	+	+	+	+
14	—	—	—	—	—	+	—	+	—	—	+	+	+	+	+
15	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
16	—	—	—	—	—	+	+	—	+	+	+	+	+	+	+
18	—	—	—	—	—	—	—	+(T—)	+	+	+	+	+	+	+
20	—	—	—	—	—	—	+(Rr—)	+	+(Rr—)	+	+	+	+	+	+
TECRA assay (confirmed result)															
1	—	—	+(R—)	—	—	—	—	—	—	+	+	+	+	+	+
3	—	—	—	—	—	+	—	—	+	+	+	+	+	+	+
4	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
5	—	—	—	—	—	+	—	+	+	+	+	+	+	+	+
6	—	—(R+)	—	—	—	+	—	+	—	+	—	—	—	—	—
7	—	—	—	—	—	—	+	—	+	—	+	+	+	+	+
8	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
9	—	—	—	—	—	+	—	—	+	—	+	+	+	+	+
14	—	—	—	—	—	+	—	+	—	—	+	+	+	+	+
15	—	—	—	—	—	—	—	+	—	—	+	+	+	+	+
16	—	—	—	—	—	+	+	—	+	+	+	+	+	+	+
18	—	—	—	—	—	—	—	+(T—)	+	+	+	+	+	+	+
20	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+
FDA BAM															
1	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
3	—	—	—	—	—	+	—	—	+	+	+	+	+	+	+
4	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
5	—	—	—	—	—	+	—	+	+	+	+	+	+	+	+
6	—	—	—	—	—	+	—	+	—	+	—	—	—	—	—
7	—	—	—	—	—	—	+	—	+	—	+	+	+	+	+
8	—	—	—	—	—	—	—	+	—	+	+	+	+	+	+
9	—	—	—	—	—	+	—	—	+	—	+	+	+	+	+
14	—	—	—	—	—	+	—	+	—	—	+	+	+	+	+
15	—	—	—	—	—	—	—	+	—	—	+	+	+	+	+
16	—	—	—	—	—	+	+	—	+	+	+	+	+	+	+
18	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+
20	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+

^a R = RV only; Rr = RV with plate reader only; T = TT only; Tv = TT with visual reading only; v = with visual reading only; r = with plate reader only.^b Borderline reader result (positive control 2.24).

Table 8. Interlaboratory study results for detection of *Salmonella* in raw turkey^a

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-,V-)	-(Tr+)	—	—	—	+(R-)	+(Rr-)	+(R-)	+(R-)	+	+(Rr-)	+(Rv-)	+(Rv-)	+	+
9	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
12	—	—	—	—	—	—	+(Rr-)	+	+	+	—	+	+	+	+
15	—	—	—	—	—	+	+	+	+(R-)	+	—	-(T+)	-(T+)	—	—
16	—	-(R+)	—	-(R+)	-(Rv+)	+(R-)	+	+	+	+	+	+	+	+	+
17	—	—	+	—	—	+	+	—	+	+	+	+	+	+	+
18	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
19	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
20	—	—	—	—	—	+(Tv-)	+(T-)	+(T-)	+(T-)	+	+	+	+	+	+
TECRA assay (confirmed result)															
3	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
4	—	—	—	—	—	+	+	+	—	+	+	+	+	+	+
5	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
8	—	—	—	—	—	+	+	+(R-)	+(R-)	—	+(R-)	+	+	+	+
9	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
12	—	—	—	—	—	—	+(R-)	+(R-)	+	+(T-)	—	+(R-)	+(T-)	+(R-)	+(T-)
15	—	—	—	—	—	+	+	+	+(R-)	+	+	+(R-)	+(R-)	+	+
16	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
17	—	—	—	—	—	+	+	—	+	+	+	+	+	+	+
18	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
19	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
20	—	—	—	—	—	+	+(T-)	+	+	+(T-)	+	+	+(T-)	+	+
FDA BAM															
3	— ^b	— ^b	— ^b	— ^b	— ^b	+	+	+	+	+	+	+	+	+	+
4	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
5	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
8	—	—	— ^b	—	—	+	+	+	+	—	+	+	+	+	+
9	— ^b	— ^b	— ^b	— ^b	— ^b	+	+	+	+	+	+	+	+	+	+
12	— ^b	— ^b	— ^b	— ^b	— ^b	— ^b	+	+	— ^b	+	— ^b	+	+	— ^b	— ^b
15	—	—	—	—	—	+	+	+	+	+	+	+	+	—	+
16	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+
17	—	— ^b	—	— ^b	—	+	+	—	+	+	+	+	+	+	+
18	— ^b	— ^b	— ^b	— ^b	— ^b	+	+	+	+	+	+	+	+	+	+
19	— ^b	— ^b	— ^b	— ^b	— ^b	+	—	+	+	+	+	+	+	+	+
20	— ^b	— ^b	— ^b	— ^b	— ^b	+	— ^b	+	+	+	+	+	+	+	+

^a R = RV only; Rr = RV with reader only; Rv = RV with visual reading only; T = TT only; Tr = TT with reader only; Tv = TT with visual reading only; r = with reader only; v = with visual reading only.

^b Suspect colonies on plates.

The method for raw flesh food in the BAM, 8th Ed., uses elevated temperature with RV and TT broths, which would be expected to be more selective. No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for each of the 2 VIA methods with that for the reference method.

Recommendations

No significant differences ($p > 0.05$) were observed for the pairwise comparison of the proportion of positive samples for the new enrichment procedures using single selective enrichment broths, with that for the reference method. We, therefore, recommend that the new enrichment procedures for TECRA method, with the optional use of RV(R10) or TT, be adopted by AOAC as Official First Action. This method is not intended to replace 989.14 but is meant as an alternative procedure for enrichment.

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