ANSR™ for *Salmonella*: a simple, rapid amplified nucleic acid assay for detection of *Salmonella* spp. in foods and environmental samples

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Summary

ANSR for *Salmonella* is a new isothermal nucleic acid amplification assay based on the nicking enzyme amplification reaction (NEAR™) technology [1]. The amplification mechanism involves the binding of an oligonucleotide "template" to a specific sequence of target DNA. The template contains a recognition site for a specific endonuclease. The nicked strand is recognized as damaged and repaired by the action of a thermostable DNA polymerase, displacing the original strand with the newly-synthesized repaired portion. This displaced DNA "product" then binds to a second template and the same reactions lead to formation of a second product. The second product is homologous to the target sequence and is detected using a specific molecular beacon probe. A fluorescent signal is generated in real time, with amplification and detection complete within 10 minutes.

The entire assay is conducted at a constant temperature of 56°C using a temperature-controlled fluorescence detection instrument. Assay software analyzes the fluorescent signal through time and a data interpretation algorithm interprets results as negative, positive, or invalid based on baseline, rate-of-change, and other criteria. Each tube of ANSR reagents also contains an internal positive control, signaling in a second fluorescence channel irrespective of the presence of target DNA, and indicating proper functioning of the amplification reagents.

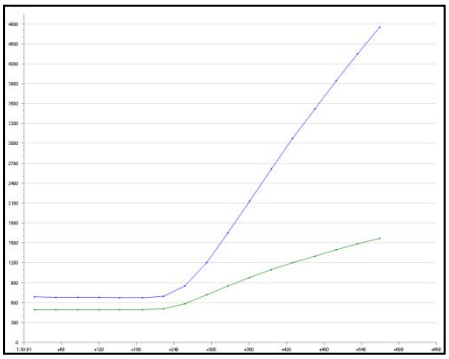
Workflow

The ANSR for *Salmonella* assay is completed in 30 minutes following a series of simple steps:

1. Add 50 µL enrichment culture to a 1.2 mL cluster tube.
2. Add 450 µL lysis buffer to the cluster tube.
3. Transfer the cluster tubes to a 80°C heater block and incubate for 20 minutes.
4. Approximately 3 minutes before the end of the lysis step, preheat the ANSR reaction tubes to 56°C by placing the ANSR reaction tubes in the incubator/reader.
5. At the end of the 20 minute lysis incubation, remove and discard the caps from the reaction tubes.
6. Using an 8-channel micropipettor and 50 µL filtered tips, transfer 50 µL of the lysed samples to the reaction tubes. Mix by rapidly pipetting up and down at least 10 times.

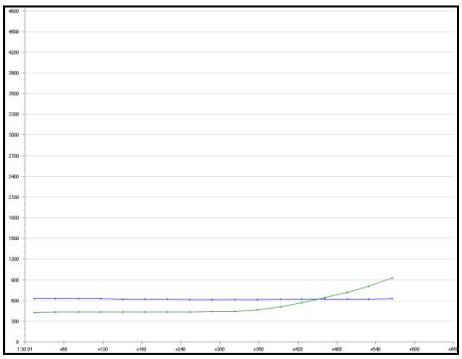
7. Place the permanent caps on the reaction tubes and close the lid of the incubator/reader.
8. Click **Start** in the ANSR software to begin the 10 minute assay.

Fig. A



Curve generated from the ANSR system of a positive sample. The blue line (top) represents the test sample and the green line (bottom)represents the internal positive control. The axes are millivolts over time.

Fig. B



Curve generated from the ANSR system of a negative sample. The blue line (top) represents the test sample and the green line (bottom) represents the internal positive control. The axes are millivolts over time.

ANSR reader

The ANSR reader is an incubator/fluorescence reader operating at a constant temperature of 56^{o}C. Two fluorescence channels are used, one for the test reaction and the other for the internal positive control reaction. The reader is connected to a laptop computer equipped with the ANSR assay software. The reader has a capacity of 16 tests per 10 minute assay run. The assay lysis step at 80^{o}C can be extended up to a maximum of 60 minutes for purposes of staging sets of samples into the reader. In this way, up to 48 samples can be processed in 1 hour. At the completion of the assay, results will be displayed as positive, negative, or invalid by the ANSR software. If the result is invalid, the test must be repeated.

Sample enrichment

The ANSR for *Salmonella* test has been validated for use with a variety of foods and environmental samples. One of three enrichment procedures is used depending on the sample type.

ANSR for Salmonella *Enrichment Broth 1* – For processed foods with a moderate to heavy load of competing background flora, including processed meats, nut products, and spices. Incubate for 16-24 hours at 36 ± 1°C.

ANSR for Salmonella *Enrichment Broth 2* – For raw meats and chicken carcass rinse. Incubate for 12-24 hours at 42 ± 1°C (10-24 hours for raw ground beef).

ANSR for Salmonella *Enrichment Broth 3* – For processed foods with a low microbial load and for environmental sponge or swab samples. Incubate for 16-24 hours at 36 ± 1°C.

For 25 g samples, 225 mL of enrichment broth is used. For larger sample sizes (e.g., 325 g or 375 g), a 4:1 ratio of broth to sample is used. Chicken carcass rinse samples (30 mL) should be collected in accordance with USDA-FSIS procedures [2] and enriched in 225 mL ANSR for *Salmonella* Enrichment Broth 2.

Test performance

Inclusivity

Inclusivity testing was conducted using 113 strains of *Salmonella enterica* and *Salmonella bongori*. This test panel represented 109 serovars and all *Salmonella* genetic subgroups. Testing was conducted after growth in all three ANSR media and dilution to approximately 1 x 10^5 cfu/mL, about 10-fold above the limit of detection of the ANSR method. Results are shown in Appendix 1. All strains were detected in all media with the exception of the rare serovar *S.* Weslaco (group T) which was uniformly negative.

Exclusivity

Exclusivity was tested using a panel of 38 non-salmonellae, most closely related members of Enterobacteriaceae. Testing was conducted from overnight Tryptic Soy Broth cultures of the test organisms, without dilution. Results are shown in Appendix 2. All test strains produced negative results.

Food testing

The ANSR method was assessed for the ability to detect *Salmonella* spp. in a variety of foods in comparison with the USDA-FSIS [2] or FDA/BAM [3] reference culture procedures.

Foods were inoculated with *Salmonella* spp. at a target level of approximately 1 cfu/test portion, a level intended to produce fractional positive results (5-15 positives out of 20 replicate test portions). A different *Salmonella* serovar was used with each food. Chicken carcass rinse samples were naturally contaminated and were tested without inoculation. High moisture products were inoculated with a culture dilution and stored for 48-72 hours at 2-8°C prior to analysis. Low moisture products were inoculated with lyophilized cells and stored for a minimum of 14 days at room temperature before

testing. Actual contamination levels were established by most probable number (MPN) analysis on the day of testing.

For each food type, a minimum of 40 inoculated test portions were prepared, half for testing by ANSR and half for testing by the reference culture method. In addition, five negative control portions were prepared for testing by each method. Samples were enriched according to the procedures outlined above. Enriched samples were tested with the ANSR method at 10 and/or 12 and 24 hours for raw meats, or at 16 and 24 hours for other foods. For confirmation of ANSR assay results, enrichments were streaked to selective/differential agars, and presumptive isolates identified using standard biochemical and serological procedures.

Results are shown in Appendix 3 and Appendix 4. There were no significant differences in the number of positive results obtained with the ANSR and reference methods in any trial as determined by chi-square analysis [4] at $P < 0.05$. There were no unconfirmed positive results on negative control samples, for specificity of 100%.

Environmental sample testing

The ANSR method was assessed for the ability to detect *Salmonella* spp. in sponge or swab samples taken from various inoculated environmental surfaces in comparison with the FDA/BAM reference culture procedure [2]. Environmental surface areas of 4 in. x 4 in. (stainless steel, sealed concrete, and rubber) or 1 in. x 1 in. (plastic and ceramic tile) were inoculated with an appropriate culture dilution and allowed to air dry for 16-24 hours. Again, inoculation levels were chosen to produce fractional positive data sets. For each method, 20 or more replicate areas were inoculated, along with five negative control areas. Stainless steel, concrete, and rubber surfaces were sampled with sponges containing Dey-Engley (DE) neutralizing broth. Plastic and ceramic tile surfaces were sampled with swabs containing DE broth. After sampling, swabs or sponges were placed in Whirl-Pak bags and held for a minimum of 2 hours at room temperature. Samples for ANSR analysis were enriched according to the procedures described above and tested after 16 and 24 hour incubation periods.

Results are shown in Appendix 5. In all trials except the one with stainless steel, there were no significant differences in the number of positive results by the ANSR and reference culture methods as determined by chi-square analysis. In the stainless steel surface trial, there were 20 positives by the ANSR method vs. 8 by the USDA-FSIS method; this difference was significant at $P < 0.05$ ($\chi^2 = 16.7$). There was a single unconfirmed positive result in the environmental surface trials.

Summary

Results of validation studies show that ANSR for *Salmonella* is an effective procedure for detection of *Salmonella* spp. in raw and processed meat products, selected processed foods, and environmental sponge or swab samples from a variety of environmental surfaces. Inclusivity was greater than 99% in testing of 113 strains belonging to *S. enterica* and *S. bongori*. Exclusivity was 100% in testing of 38 strains

of non-salmonellae. Method sensitivity was comparable to that of the FDA/BAM and USDA-FSIS methods as determined by chi-square analysis. There were no statistically significant differences in the number of positive results obtained with the ANSR and reference culture methods in any trial, with the exception of the trial with stainless steel surface in which there were significantly more positives by the ANSR method. Sensitivity of the ANSR assay relative to confirmation from ANSR-associated enrichment cultures was 98.7%. There was only one unconfirmed positive result from uninoculated control test portions, for overall specificity of 98.3%.

In addition to high sensitivity and specificity, the ASNR for *Salmonella* method offers the advantages of single-step enrichment, minimal labor and assay hardware requirements, and post-enrichment assay results within 30 minutes.

References

[1] Van Ness, J., Van Ness, L.K., & Galas, D. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 4504-4509

[2] USDA-FSIS (2011) *Microbiology Laboratory Guidebook*, chapter 4.05
http://www.fsis.usda.gov/PDF/MLG_4_05.pdf

[3] U.S. FDA (2011) *Bacteriological Analytical Manual*, chapter 5
http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm

[4] Mantel, N. & Haenszel, W. (1959) *J. Nat. Cancer Inst.* **22**,719-748

Appendix 1

Results of inclusivity testing for the ANSR for *Salmonella* test

					ANSR result at ~ 1 x 10^5 cfu/mL		
Strain no.	***Salmonella* serovar**[a]	**O group**	**Source**	**Origin (if known)**	**ANSR Broth 1**	**ANSR Broth 2**	**ANSR Broth 3**
GT2652	Arizonae (III)	51	CDC		+	+	+
GT799	Arizonae (III)	51	ATCC 13314		+	+	+
GT3136	Treforest	51	CDC		+	+	+
GT3089	Humber (II)	53	CDC		+	+	+
GT3090	Tranoroa (II)	55	CDC		+	+	+
GT3091	Artis (II)	56	CDC		+	+	+
GT3092	Tokai (II)	57	CDC		+	+	+
GT3093	Betioky (II)	59	CDC		+	+	+
GT3094	Luton (II)	60	CDC		+	+	+
GT2704	*S. bongori ser*. Brookfield	66	CDC		+	+	+
GT2705	*S. bongori ser*. Malawi	66	CDC		+	+	+
GT1614	*S. bongori ser*. Maregrosso	66	CDC		+	+	+
GT3199	Crossness	67	CDC		+	+	+
GT657	Kiel	A	Deibel Labs		+	+	+
GT2403	Paratyphi A	A	CDC		+	+	+
GT2284	Agona	B	CDC		+	+	+
GT2304	Heidelberg	B	CDC		+	+	+
GT2306	Java	B	CDC		+	+	+
GT2309	Paratyphi B	B	CDC		+	+	+
GT2360	Saint-Paul	B	CDC		+	+	+
GT546	Schwarzengrund	B	Mass. State Lab		+	+	+
GT2373	Typhimurium	B	ATCC 13311	Mutton	+	+	+
Neogen 190	Typhimurium	B	CDC		+	+	+
GT2365	Typhimurium var. Copenhagen	B	CDC		+	+	+
GT2378	Braenderup	C1	CDC		+	+	+
GT2886	Cholerasuis	C1	CDC		+	+	+
A144	Infantis	C1	ATCC 51741		+	+	+

Continued

GT2479	Mbandaka	C1	CDC		+	+	+
GT2483	Montevideo	C1	CDC		+	+	+
A149	Oranienberg	C1	ATCC 9239	Ill. State Hospital	+	+	+
GT2528	Paratyphi C	C1	CDC		+	+	+
GT2516	Tennessee	C1	CDC		+	+	+
GT2518	Thompson	C1	CDC		+	+	+
GT2524	Virchow	C1	CDC		+	+	+
GT2545	Bovismorbificans	C2	CDC		+	+	+
GT2547	Duesseldorf	C2	CDC		+	+	+
GT623	Hadar	C2	Mass. State Lab		+	+	+
GT2553	Muenchen	C2	CDC		+	+	+
GT2557	Newport	C2	CDC		+	+	+
GT2558	Newport var. Puerto Rico	C2	CDC		+	+	+
GT2579	Tulear (II)	C2	CDC		+	+	+
GT662	Albany	C3	U. Mass.		+	+	+
GT2549	Haardt	C3	CDC		+	+	+
GT2581	Kentucky	C3	CDC		+	+	+
GT2882	Virginia	C3	CDC		+	+	+
GT2674	Bornum	C4	CDC		+	+	+
GT2103	Eimsbuettel	C4	CDC		+	+	+
GT2884	Berta	D1	CDC		+	+	+
GT2583	Daressalaam	D1	CDC		+	+	+
GT2584	Dublin	D1	CDC		+	+	+
GT2881	Eastbourne	D1	CDC		+	+	+
Neogen 195	Enteritidis	D1	CDC		+	+	+
Neogen 207	Enteritidis	D1	CDC		+	+	+
GT2124	Enteritidis	D1	ATCC 13076		+	+	+
GT896	Gallinarum	D1	GENE-TRAK Systems		+	+	+
GT2589	Javiana	D1	CDC		+	+	+
GT2885	Pullorum	D1	CDC		+	+	+
GT2125	Typhi	D1	ATCC 6539		+	+	+
GT2620	Fresno	D2	CDC		+	+	+
GT2621	Gateshead	D2	CDC		+	+	+
GT2622	Strasbourg	D2	CDC		+	+	+
GT2626	Anatum	E1	CDC		+	+	+
GT2637	Butantan	E1	CDC		+	+	+

Continued

GT2638	Give	E1	CDC		+	+	+
GT2158	Lexington	E1	CDC		+	+	+
GT2641	Meleagridis	E1	CDC		+	+	+
GT2510	Muenster	E1	USDA, Athens, GA		+	+	+
Neogen 469	Uganda	E1	Ampcor	Pork Sausage	+	+	+
GT911	Weltervreden	E1	CDC		+	+	+
GT619	Binza	E2	U. Mass.		+	+	+
GT908	Kinshasa	E2	CDC		+	+	+
GT2101	Newbrunswick	E2	CDC		+	+	+
GT2643	Arkansas	E3	CDC		+	+	+
GT2645	Illinois	E3	CDC		+	+	+
GT2646	Minneapolis	E3	CDC		+	+	+
GT3233	Chittagong	E4	CDC		+	+	+
GT2650	Krefeld	E4	CDC		+	+	+
GT2883	Senftenberg	E4	CDC		+	+	+
GT3178	Simsbury	E4	CDC		+	+	+
GT2676	Westerstede	E4	CDC		+	+	+
GT1857	Pretoria	F	CDC		+	+	+
GT2703	Rubislaw	F	CDC		+	+	+
GT2680	Poona	G1	CDC		+	+	+
GT1858	Havana	G2	CDC		+	+	+
GT4701	Worthington	G2	GENE-TRAK Systems		+	+	+
GT5149	Ferlac	H	ATCC 43976		+	+	+
GT2711	Florida	H	CDC		+	+	+
GT913	Hvittingfoss	I	CDC		+	+	+
GT2716	Kirkee	J	CDC		+	+	+
GT2691	Cerro	K	CDC		+	+	+
GT2721	Minnesota	L	CDC		+	+	+
GT2723	Dakar	M	CDC		+	+	+
GT3025	Urbana	N	CDC		+	+	+
GT3028	Adelaide	O	CDC		+	+	+
GT3034	Inverness	P	CDC		+	+	+
GT3037	Champaign	Q	CDC		+	+	+
GT3039	Bern (IV)	R	CDC		+	+	+
GT3058	Springs (II)	R	CDC		+	+	+
GT3059	Waycross	S	CDC		+	+	+
GT3060	Weslaco	T	CDC		-	-	-
GT4467	Houten (IV)	U	Silliker		+	+	+
GT3066	Guinea	V	CDC		+	+	+

Continued

GT2707	*S. bongori ser*. Camdeni	V	CDC		+	+	+
GT3069	Dugbe	W	CDC		+	+	+
GT3073	Quimbamba	X	CDC		+	+	+
GT3083	Djakarta	Y	CDC		+	+	+
GT2706	*S. bongori ser*. Balboa	Y	CDC		+	+	+
GT2708	*S. bongori ser*. Bongor	Y	CDC		+	+	+
GT3084	Flint (IV)	Z	CDC		+	+	+
GT3085	Greenside (II)	Z	CDC		+	+	+
GT3086	Hooggraven (II)	Z	CDC		+	+	+
GT3087	Wassenaar (IV)	Z	CDC		+	+	+
GT1615	*S. enterica* subsp. *indica* (VI)		CDC		+	+	+

[a] All strains are serovars of *Salmonella enterica* unless otherwise indicated. Serovars of subspecies other than subsp. I are indicated in parentheses.

Appendix 2

Results of exclusivity testing for the ANSR for *Salmonella* test

Strain no.	Organism	Source	Origin (if known)	ANSR result at 1 x 10^9 cfu/mL in TSB
GT1485	*Citrobacter amalonaticus*	ATCC 25405	Feces	-
GT1475	*Citrobacter diversus*	ATCC 27156		-
GT1477	*Citrobacter freundii*	ATCC33128		-
GT1476	*Citrobacter youngae*	ATCC 29935	Meat	-
GT1483	*Cronobacter sakazakii*	ATCC 29544	Human	-
GT1710	*Edwardsiella hoshinae*	ATCC 33379	Bird	-
GT569	*Edwardsiella tarda*	ATCC 15947	Feces	-
GT1487	*Enterobacter aerogenes*	ATCC 29940	Human	-
GT1482	*Enterobacter amnigenus*	ATCC 33072	Soil	-
GT1497	*Enterobacter cancerogenus*	ATCC 35317		-
GT1481	*Enterobacter cloacae*	ATCC 29941		-
GT2990	*Enterobacter cloacae*	GENE-TRAK	Dairy plant	-
GT1486	*Enterobacter gergoviae*	ATCC 33028		-
GT1480	*Enterobacter intermedia*	ATCC 33110		-
GT1460	*Escherichia blattae*	CDC		-
GT1214	*Escherichia coli*	ATCC 12038		-
GT1459	*Escherichia fergusonii*	ATCC 35473	Feces	-
GT1216	*Escherichia hermannii*	ATCC 33650	Human	-
GT1217	*Escherichia vulneris*	ATCC 33821	Human	-
GT241	*Hafnia alvei*	ATCC 29927	Human	-
GT1503	*Klebsiella oxytoca*	ATCC 13182	Human	-
GT1478	*Klebsiella planticola*	ATCC 33531	Radish	-
GT1499	*Klebsiella pneumoniae* subsp. *ozaenae*	ATCC 11296		-
GT3600	*Kluyvera ascorbata*	ATCC 33433	Human	-
GT303	*Morganella morganii*	ATCC 25830	Human	-
GT1467	*Pantoea agglomerans*	ATCC 29917		-
GT358	*Pasteurella multocida*	ATCC 19427		-
GT1493	*Proteus mirabilis*	ATCC 25933	Human	-
GT366	*Proteus myxofaciens*	ATCC 19692		-
GT367	*Proteus penneri*	ATCC 33519		-
GT368	*Proteus vulgaris*	ATCC 13315		-
GT371	*Providencia alcalifaciens*	ATCC 9886	Feces	-
GT373	*Providencia rettgeri*	ATCC 29944		-
GT374	*Providencia rustigiani*	ATCC 33673		-
GT375	*Providencia stuartii*	ATCC 29914		-
GT1909	*Pseudomonas aeruginosa*	ATCC 27853	Blood	-
GT392	*Serratia marcescens*	ATCC 29937	Human	-
GT1713	*Serratia rubidae*	ATCC 15338		-

Appendix 3

Results of comparative testing of raw meats with the ANSR for *Salmonella* and USDA-FSIS reference methods

Food type	Inoculum strain	Inoculation level		No. Samples	Number of positive samples				USDA Ref. Method	Sensitivity (%)[e] (12 h)	Specificity (%)[f] (12 h)	χ^{2g} (12 h)
					ANSR Method							
		cfu/g[b]	cfu/ portion[b]		Assay[c] 10 h	Conf.[d] 10 h	Assay[c] 12 h	Conf.[d] 12 h				
Chicken carcass rinse	Nat. contam.[a]	-	-	20	Not done	Not done	7	6	8	100%	-	0.43
Raw ground turkey	*S.* Heidelberg	0.036	0.90	20	Not done	Not done	15	15	19	100%	-	3.06
	-	< 0.030	< 0.75	5			0	0	0	-	100%	-
Raw ground turkey[h]	*S.* Heidelberg	0.027	0.67	20	Not done	Not done	7[i]	7[i]	10	100%	-	0.90
	-	0	0	5			0	0	0	-	100%	-
Raw ground beef	*S.* Newport	0.036	0.90	20	12	13	13	13 (14)[j]	14	93%	-	0.11
	-	< 0.030	< 0.75	5	0	0	0	0	0	-	100%	-

[a] *Salmonella* spp. of serogroups C_2, D_1, and E_4 were isolated from positive samples.

[b] Determined by most probable number analysis.

[c] Number of test portions positive by ANSR assay not considering subsequent culture confirmation.

[d] Number test portions positive by the ANSR assay and confirmed by culture from ANSR-associated enrichments.

[e] Sensitivity = ANSR confirmed positives divided by maximum number of culture positives from ANSR enrichments.

[f] Specificity = ANSR negatives divided by total number of negative test portions. Calculated only for uninoculated control samples.

[g] χ^2 by Mantel-Haenszel formula [4]; $\chi^2 > 3.84$ indicates a statistically significant difference at $p < 0.05$.

[h] Trial performed by independent laboratory.

[i] Also tested after 24 h enrichment with identical results.

[j] There was one ANSR assay-negative, culture-positive result.

Appendix 4

Results of comparative testing of processed foods with the ANSR for *Salmonella* and USDA-FSIS and FDA/BAM reference methods.

Food type	Inoculum strain	Inoculation level		No. samples	Number of positive samples								
					ANSR Method				USDA Ref. Method	FDA Ref. Method	Sensitivity (%)[d] (24 h)	Specificity (%)[e] (24 h)	χ^{2f} (24 h)
		cfu/g[a]	cfu/ portion[a]		Assay 16 h[b]	Conf. 16 h[c]	Assay 24 h[b]	Conf. 24 h[c]					
Hot dogs, 25 g	*S.* Oranienburg	0.036	0.90	20	8	8	9	9	13	-	100%	-	1.58
	-	< 0.030	< 0.75	5	0	0	0	0	0	-	-	100%	-
Hot dogs, 325 g	*S.* Oranienburg	0.036	0.90	20	11	11	11	11	13	-	100%	-	0.41
	-	< 0.030	< 0.75	5	0	0	0	0	0	-	-	100%	-
Oat cereal	*S.* Agona	0.092	2.3	20	13	13	15	15	-	13	100%	-	0.46
	-	< 0.030	< 0.75	5	0	0	0	0	-	0	-	100%	-

[a] Determined by most probable number analysis.

[b] Number of test portions positive by ANSR assay not considering subsequent culture confirmation.

[c] Number test portions positive by the ANSR assay and confirmed by culture from ANSR-associated enrichments.

[d] Sensitivity = ANSR confirmed positives divided by maximum number of culture positives from ANSR enrichments.

[e] Specificity = ANSR negatives divided by total number of negative test portions. Calculated only for uninoculated control samples.

[f] χ^2 by Mantel-Haenszel formula [4]; $\chi^2 > 3.84$ indicates a statistically significant difference at $p < 0.05$.

Appendix 5

Results of comparative testing of environmental samples with the ANSR *Salmonella* and FDA/BAM reference methods.

Food type	Inoculum strain	Inoculation level (cfu/surface)[a]	No. samples	Number of positive samples					Sensitivity (%)[d] (24 h)	Specificity (%)[e] (24 h)	χ^{2f} (24 h)
				ANSR method				FDA ref. method			
				Assay 16 h[b]	Conf. 16 h[c]	Assay 24 h[b]	Conf. 24 h[c]				
Stainless steel	*S.* Heidelberg + competitor cocktail	140/1,500	20	20	20	20	20	8	100%	-	16.7
	-	-	5	0	0	0	0	0	-	100%	-
Plastic	*S.* Javiana	210	30	12	12	13	13	13	100%	-	0.00
	-	-	5	0	0	1	0	0	-	80%	-
Sealed concrete	*S.* Infantis	21,700	20	11	11	11	11	16	100%	-	2.78
	-	-	5	0	0	0	0	0	-	100%	-
Ceramic tile	*S.* Meleagridis	110	30	19	19	19	19	24	100%	-	2.02
	-	-	5	0	0	0	0	0	-	100%	-
Ceramic tile[g]	*S.* Meleagridis	53	20	10	10	10	10	7	100%	-	0.90
	-	-	5	0	0	0	0	0	-	100%	-
Rubber	*S.* Arizonae	210	20	7	7	8	8	7	100%	-	0.10
	-	-	5	0	0	0	0	0	-	100%	-

[a] Determined by dilution plating of the inoculum cultures.

[b] Number of test portions positive by ANSR assay not considering subsequent culture confirmation.

[c] Number test portions positive by the ANSR assay and confirmed by culture from ANSR-associated enrichments.

[d] Sensitivity = ANSR confirmed positives divided by maximum number of culture positives from ANSR enrichments.

[e] Specificity = ANSR negatives divided by total number of negative test portions. Calculated only for uninoculated control samples.

[f] χ^2 by Mantel-Haenszel formula [4]; $\chi^2 > 3.84$ indicates a statistically significant difference at $p < 0.05$.

[g] Trial performed by independent laboratory.