



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
960801

The AOAC Research Institute hereby certifies the method known as:

Reveal 2.0 for *Salmonella* Test System

manufactured by

Neogen Corporation
620 Leshar Place
Lansing, Michigan 48912
USA

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

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SUBMITTING COMPANY

Neogen Corporation
 620 Lesher Place
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METHOD NAME

Reveal 2.0 for *Salmonella* Test System

CATALOG NUMBER

9706

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⁴ Modification: 2010
 Modification April 2020 reviewed internally by AOAC Research Institute.

APPLICABILITY OF METHOD

Target organism – ORIGINAL VALIDATION: *Salmonella* spp.
MODIFICATION December 2010 *Salmonella enterica* serogroups A-E.

Matrixes – raw ground beef, raw ground chicken, raw ground sausage, raw ground pork, cooked chicken, beef skin, pork skin, chicken rinse water, picked crab meat, shrimp, fishmeal, liquid eggs, poultry feed, powdered milk, soybean meal, frozen whole eggs

MODIFICATION DECEMBER 2010 – chicken carcass rinse, raw ground turkey, raw ground beef, hot dogs, raw shrimp, ready-to-eat meal product, dry pet food, ice cream, spinach, cantaloupe, peanut butter, sprout irrigation water, stainless steel

Performance claims – The method was found to be not statistically different when compared to the reference methods.

REFERENCE METHODS

Bacteriological Analytical Manual (1992) 8th Ed. (2)
 USDA (2008) *Microbiology Laboratory Guidebook* (7)
 US FDA (2007) *Bacteriological Analytical Manual* (8)

ORIGINAL CERTIFICATION DATE

August 1996

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2024.

METHOD MODIFICATION RECORD

1. December 2010, Level 3
2. November 2019 Level 1
3. April 2020 Level 2
4. December 2023 Level 1

SUMMARY OF MODIFICATION

1. Additional polyclonal antibody to improve detection of certain *Salmonella* serovars. Architectural change to a “naked strip” format without plastic housing allowing the test to run in a vertical position instead of horizontally.
2. Editorial/clerical changes.
3. Evaluation of a change to the device nitrocellulose membrane blocking procedure.
4. Editorial/clerical changes.

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 NONE

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 NONE

PRINCIPLE OF THE METHOD (1)

The Reveal for *Salmonella* (RSS) Test System uses the medium called Revive. This medium provides *Salmonella* spp. with readily available nutrients and other factors required for survival, recovery, and repair from a stressed or injured condition. After treatment with Revive, selective enrichment then favors growth of *Salmonella* spp. to levels detectable by the RSS test device.

A portion (100µL) of the enrichment culture is placed into the round sample port of the RSS test device, initiating a lateral flow through a reagent zone containing specific anti-*Salmonella* antibodies conjugated to colloidal gold particles. If antigens are present, they will bind to the conjugated antibodies. This complex continues to flow through the device until it reaches a zone containing a stationary anti-*Salmonella* antibody. The immune complex with the conjugated antibody is captured and aggregates, forming a visible line. A procedural control line all will develop above the positive line, indicating proper test completion. Absence of a control line indicates an invalid test. RSS test devices should not be interpreted after 20 minutes from the addition of the sample culture. The viewing window can become blurred and faded, making interpretation difficult.

DISCUSSION OF THE VALIDATION STUDY (1)

An in-house comparative study was performed to compare the RSS to the traditional method described in the *Bacteriological Analytical Manual* (BAM; 2). In this study, 48 samples were artificially inoculated with *Salmonella* spp., and 12 samples were left uninoculated as controls. All 60 samples were tested by the RSS test system and the BAM method. Both methods showed 48 positive and 12 negative samples, for 100% agreement. In the mini-collaborative study, 3 laboratories participated and compared the RSS test system with the method recommended by the U.S. Department of Agriculture's Food Safety Inspection Service (FSIS, 3), using 14 artificially inoculated samples and 7 negative controls, for a total of 63 samples. All 63 samples were tested with the RSS test system (with the RSS sample enrichments culture confirmed by FSIS techniques) and the FSIS method, of which 31 were positive and 8 were negative by both methods, for a 61.9% agreement. Five samples were positive by the RSS test with culture confirmation (FSIS methods were used for culture confirmation) and negative by FSIS. Nine samples were positive by the RSS test but negative by culture confirmation and negative by FSIS. Two samples were negative by the RSS test but positive by culture confirmation and by FSIS.

Table 3. In-house data for inclusivity and exclusivity of RSS (1)

<i>Salmonella</i> serovar		Inclusivity		Result ^a
		Concentration, cfu/mL		
<i>S. agona</i>		1.4 x 10 ⁵		Positive
<i>S. typhimurium</i>		2.3 x 10 ⁵		Positive
<i>S. montevideo</i>		8.0 x 10 ⁵		Positive
<i>S. branderup</i>		1.3 x 10 ⁵		Positive
<i>S. enteritidis</i>		1.7 x 10 ⁵		Positive
Species		ATCC No.	Exclusivity	
			Concentration, cfu/mL	
<i>Escherichia coli</i>	11775	1.2 x 10 ⁸		Negative
		1.2 x 10 ⁷		Negative
		1.2 x 10 ⁶		Negative
<i>Escherichia coli</i>	11839	1.1 x 10 ⁸		Negative
		1.1 x 10 ⁷		Negative
		1.1 x 10 ⁶		Negative
<i>Proteus vulgaris</i>	13315	1.3 x 10 ⁸		Negative
		1.3 x 10 ⁷		Negative
		1.3 x 10 ⁶		Negative
<i>Proteus mirabilis</i>	29906	1.1 x 10 ⁸		Negative
		1.1 x 10 ⁷		Negative
		1.1 x 10 ⁶		Negative
<i>Aeromonas caviae</i>	15468	2.7 x 10 ⁸		Negative
		2.7 x 10 ⁷		Negative
		2.7 x 10 ⁶		Negative
<i>Aeromonas hydrophila</i>	15467	2.0 x 10 ⁸		Negative
		2.0 x 10 ⁷		Negative
		2.0 x 10 ⁶		Negative
<i>Citrobacter freundii</i>	29220	2.6 x 10 ⁸		Negative
		2.6 x 10 ⁷		Negative
		2.6 x 10 ⁶		Negative
<i>Citrobacter freundii</i>	12012	1.5 x 10 ⁸		Negative
		1.5 x 10 ⁷		Negative
		1.5 x 10 ⁶		Negative

^a Two RSS devices were tested on each culture. Pure, unstressed cultures were used.

Table 4. Independent laboratory data for exclusivity of RSS (1)

Species	ATCC No.	Enrichment	Result
<i>Escherichia coli</i>	8677	Revive/SC ^a	Negative
<i>Escherichia coli</i>	12435	Revive/SC	Negative
<i>Escherichia vulneris</i>	33833	Revive/SC	Negative
<i>Escherichia fergusonii</i>	35469	Revive/SC	Negative
<i>Klebsiella pneumoniae</i>	9997	Revive/SC	Negative
<i>Klebsiella oxytoca</i>	43165	Revive/SC	Negative
<i>Klebsiella trevisanii</i>	33558	Revive/SC	Negative
<i>Aeromonas hydrophila</i>	7965	Revive/SC	Negative
<i>Aeromonas jandaei</i>	49568	Revive/SC	Negative
<i>Enterobacter aerogenes</i>	35028	Revive/SC	Negative
<i>Enterobacter cloacae</i>	13047	Revive/RV ^b	Negative
<i>Edwardsiella tarda</i>	15947	Revive/RV	Negative
<i>Hafnia alvei</i>	29926	Revive/RV	Negative
<i>Citrobacter freundii</i>	8090	Revive/RV	Negative
<i>Citrobacter diversus</i>	25408	Revive/RV	Negative
<i>Yersinia enterocolitica</i>	23715	Revive/RV	Negative
<i>Yersinia mollaretii</i>	43969	Revive/RV	Negative
<i>Proteus mirabilis</i>	7002	Revive/RV	Negative
<i>Proteus vulgaris</i>	13315	Revive/RV	Negative
<i>Proteus penneri</i>	33519	Revive/RV	Negative

^a SC = Selenite Cystine broth

^b Rappaport-Vassiliadis broth

Table 5. AOAC-RI in-house data for analysis by Reveal for *Salmonella* (RSS) test system (1)

Sample	Level, cfu/25 g	Total	Positive Samples		Reference Method ^c	X ^{2d}	Sensitivity, %	False negatives, %	Specificity, %	False positives, %
			Presumed ^a	Confirmed ^b						
Raw ground beef	6	1	1	1	1	--	100	0	100	0
	127	1	1	1	1	--	100	0	100	0
	16	1	1	1	1	--	100	0	100	0
	133	1	1	1	1	--	100	0	100	0
Raw ground chicken	Control	1	0	0	0	--	-	-	100	0
	9	1	1	1	1	--	100	0	100	0
	170	1	1	1	1	--	100	0	100	0
	5	1	1	1	1	--	100	0	100	0
Picked crab meat	105	1	1	1	1	--	100	0	100	0
	Control	1	0	0	0	--	-	-	100	0
	5	1	1	1	1	--	100	0	100	0
	101	1	1	1	1	--	100	0	100	0
Poultry feed	4	1	1	1	1	--	100	0	100	0
	83	1	1	1	1	--	100	0	100	0
	Control	1	0	0	0	--	-	-	100	0
	7	1	1	1	1	--	100	0	100	0
Chicken rinse water	137	1	1	1	1	--	100	0	100	0
	9	1	1	1	1	--	100	0	100	0
	170	1	1	1	1	--	100	0	100	0
	Control	1	0	0	0	--	-	-	100	0
Powdered milk	11	1	1	1	1	--	100	0	100	0
	222	1	1	1	1	--	100	0	100	0
	6	1	1	1	1	--	100	0	100	0
	111	1	1	1	1	--	100	0	100	0
Shrimp	Control	1	0	0	0	--	-	-	100	0
	7	1	1	1	1	--	100	0	100	0
	142	1	1	1	1	--	100	0	100	0
	9	1	1	1	1	--	100	0	100	0
Shrimp	169	1	1	1	1	--	100	0	100	0
	Control	1	0	0	0	--	-	-	100	0

Table 5. AOAC-RI in-house data for analysis by Reveal for *Salmonella* (RSS) test system – Continued (1)

Sample	Level, cfu/25 g	Total	Positive Samples		Reference Method ^c	X ^{2d}	Sensitivity, %	False negatives, %	Specificity, %	False positives, %
			Presumed ^a	Confirmed ^b						
Pork skin	6	1	1	1	1	--	100	0	100	0
	119	1	1	1	1	--	100	0	100	0
	7	1	1	1	1	--	100	0	100	0
	152	1	1	1	1	--	100	0	100	0
	Control	1	0	0	0	0	--	-	-	100
Liquid eggs	7	1	1	1	1	--	100	0	100	0
	151	1	1	1	1	--	100	0	100	0
	7	1	1	1	1	--	100	0	100	0
	152	1	1	1	1	--	100	0	100	0
	Control	1	0	0	0	0	--	-	-	100
Fishmeal	4	1	1	1	1	--	100	0	100	0
	72	1	1	1	1	--	100	0	100	0
	12	1	1	1	1	--	100	0	100	0
	230	1	1	1	1	--	100	0	100	0
	Control	1	0	0	0	0	--	-	-	100
Soybean meal	3	1	1	1	1	--	100	0	100	0
	63	1	1	1	1	--	100	0	100	0
	6	1	1	1	1	--	100	0	100	0
	112	1	1	1	1	--	100	0	100	0
	Control	1	0	0	0	0	--	-	-	100
Beef skin (hide)	3	1	1	1	1	--	100	0	100	0
	58	1	1	1	1	--	100	0	100	0
	8	1	1	1	1	--	100	0	100	0
	155	1	1	1	1	--	100	0	100	0
	Control	1	0	0	0	0	--	-	-	100

^a RSS positive result

^b RSS culture confirmed by BAM and FSIS cultural confirmation

^c BAM and FSIS cultural methods

^d χ^2 value for RSS device compared with culture confirmed by BAM and FSIS methods.

Table 6. AOAC-RI Independent laboratories data for analysis by Reveal for *Salmonella* (RSS) test system (1)

Sample	Level, cfu/25 g	Total	Positive Samples RSS		Reference Method ^c	X ^{2d}	Sensitivity, %	False negatives, %	Specificity, %	False positives, %
			Presumed ^a	Confirmed ^b						
Raw ground beef	0.06	3	3	3	3	-	100	0	100	0
Control	-	3	3	3	3	-	100	0	100	0
Raw ground chicken	0.43	3	3	3	3	-	100	0	100	0
Control	1.49	3	3	3	3	-	100	0	100	0
Raw ground sausage	0.09	3	3	3	3	-	100	0	100	0
Control	0.23	3	2	3	3	-	66	33	100	0
Cooked chicken	1.49	3	3	3	3	-	100	0	100	0
Control	0.09	3	2	3	3	-	66	33	100	0
Raw ground pork	0.09	3	3	3	2	-	100	0	100	0
Control	0.04	3	2	2	3	-	100	0	100	0
Liquid whole eggs	-	3	3	2	3	-	100	0	66	33
Control	2.4	3	3	3	2	-	100	0	100	0
Frozen whole eggs	4.62	3	1	0	1	-	100	0	0	100
Control	0.01	3	1	0	2	-	100	0	0	100
Control	-	3	1	0	0	-	-	-	0	100

^a RSS positive result

^b RSS culture confirmed by FSIS cultural confirmation

^c FSIS cultural methods

^d χ^2 value for RSS device compared with culture confirmed by BAM and FSIS methods.

DISCUSSION OF MODIFICATION STUDY APPROVED DECEMBER 2010 (6)

Results of the internal and independent laboratory studies show that the Reveal *Salmonella* 2.0 method is an effective procedure for detection of *Salmonella* spp. in a variety of foods and environmental samples. For food samples, considering data from both internal and independent laboratory trials utilizing Revive or direct RV enrichment, relative sensitivity of the Reveal method compared to the USDA-FSIS and FDA/BAM reference procedures was 88% and 117%, respectively (99% overall). There were no statistically significant differences in the number of positives obtained by the Reveal and reference methods in any food trials.

The choice of enrichment procedure for use with the Reveal test depends on the sample type. For raw products that may contain significant numbers of competing bacteria, e.g., raw meats, chicken carcass rinse, and fresh spinach, direct enrichment in RV broth has proven effective. This procedure has also been found to be applicable to detection of *Salmonella* spp. in hot dogs.

For foods in which there is a likelihood that any salmonellae present will be in a sublethally injured state, a two-stage enrichment utilizing nonselective Revive broth followed by RV broth is used.

In some cases, an additional enrichment step in M broth following either the Revive/RV or direct RV procedures is required to eliminate matrix effects caused by residual antigen or pigment in the test sample. Examples include pet food (possible presence of *Salmonella* antigen from dead cells) and fresh spinach (pigment). The Reveal test may also be used in conjunction with traditional reference method enrichment procedures. Three foods (hot dogs, fresh spinach, and peanut butter) were tested by Reveal following reference method primary enrichment and secondary enrichment in RV broth. In these trials, relative sensitivity of the Reveal method compared to the USDA-FSIS and FDA/BAM reference procedures was 88 and 100%, respectively (92% overall). Results are available in 44–48 h when these procedures are used.

Table 1. Inclusivity testing results for Reveal Salmonella 2.0 test (6)

Serovar ^a	O serogroup	Strain No.	Source	Origin	Reveal 2.0 result (approx. 10 ⁷ CFU/mL) ^b		
					RV Direct ^c	Revive-RV ^c	Revive-RV-M Broth ^c
S. Kiel	A	GT654	Deibel Labs		Pos	Pos	Pos
S. Paratyphi-A	A	GT2403	CDC		Pos	Pos	Pos
S. Bredeney	B	GT2288	CDC		Pos	Pos	Pos
S. Abortus-bovis	B	GT2322	CDC		Pos	Pos	Pos
S. Abortus-equi	B	GT2408	CDC		Pos	Pos	Pos
S. Agona	B	GT2284	CDC		Pos	Pos	Pos
S. Brandenburg	B	GT2286	CDC		Pos	Pos	Pos
S. California	B	GT625	Mass. State Lab		Pos	Pos	Pos
S. Derby	B	GT2990	CDC		Pos	Pos	Pos
S. Essen	B	GT3254	CDC		Pos	Pos	Pos
S. Heidelberg	B	GT2304	CDC		Pos	Pos	Pos
S. Indiana	B	GT328	Mass. State Lab		Pos	Pos	Pos
S. Java	B	GT2306	CDC		Pos	Pos	Pos
S. Paratyphi-B	B	GT2309	CDC		Pos	Pos	Pos
S. Saint-Paul	B	GT2360	CDC		Pos	Pos	Pos
S. San Diego	B	GT2363	CDC		Pos	Pos	Pos
S. Schleissheim	B	GT2396	CDC		Pos	Pos	Pos
S. Schwarzengrund	B	GT546	Mass. State Lab		Pos	Pos	Pos
S. Stanley	B	GT2364	CDC		Pos	Pos	Pos
S. Stanleyville	B	GT909	CDC		Pos	Pos	Pos
S. Typhimurium	B	GT2373	ATCC 13311	Mutton	Pos	Pos	Pos
S. Typhimurium var. copenhagen	B	GT2365	CDC		Pos	Pos	Pos
S. Amersfoort	C ₁	GT2375	CDC		Pos	Pos	Pos
S. Bareilly	C ₁	GT2376	CDC		Pos	Pos	Pos
S. Braenderup	C ₁	GT2378	CDC		Pos	Pos	Pos
S. Choleraesuis	C ₁	GT2886	CDC		Pos	Pos	Pos
S. Choleraesuis var. kuzendorf	C ₁	GT3197	CDC		Pos	Pos	Pos
S. Djugu	C ₁	GT663	Deibel Labs		Pos	Pos	Pos
S. Hartford	C ₁	GT2439	CDC		Pos	Pos	Pos
S. Infantis	C ₁	GT2477	CDC		Pos	Pos	Pos
S. Livingstone	C ₁	GT2478	CDC		Pos	Pos	Pos
S. Lomita	C ₁	GT617	U. Mass.		Pos	Pos	Pos
S. Mbandaka	C ₁	GT2479	CDC		Pos	Pos	Pos
S. Montevideo	C ₁	GT2483	CDC		Pos	Pos	Pos
S. Oranienberg	C ₁	A149	ATCC 9239	Ill. State hosp.	Pos	Pos	Pos
S. Paratyphi-C	C ₁	GT2528	CDC		Neg	Pos	Neg
S. Tennessee	C ₁	GT2516	CDC		Pos	Pos	Pos
S. Thompson	C ₁	GT2518	CDC		Pos	Pos	Pos
S. Virchow	C ₁	GT2524	CDC		Pos	Pos	Pos
S. Aesch	C ₂	GT3180	CDC		Pos	Pos	Pos
S. Blockley	C ₂	GT2544	CDC		Pos	Pos	Pos
S. Bovismorbificans	C ₂	GT2545	CDC		Pos	Pos	Pos
S. Duesseldorf	C ₂	GT2547	CDC		Neg	Pos	Pos

Table 1. (continued) (6)

Serovar ^a	O serogroup	Strain No.	Source	Origin	Reveal 2.0 result (approx. 10 ⁷ CFU/mL) ^b		
					RV Direct ^c	Revive-RV ^c	Revive-RV-M Broth ^c
S. Glostrup	C ₂	GT2548	CDC		Pos	Pos	Pos
S. Hadar	C ₂	GT623	Mass. State Lab		Pos	Pos	Pos
S. Kottbus	C ₂	GT2550	CDC		Pos	Pos	Pos
S. Lindenburg	C ₂	GT910	CDC		Pos	Pos	Pos
S. Litchfield	C ₂	GT2551	CDC		Pos	Pos	Pos
S. Manhattan	C ₂	GT630	Mass. State Lab		Pos	Pos	Pos
S. Muenchen	C ₂	GT2553	CDC		Pos	Pos	Pos
S. Newport	C ₂	GT2557	CDC		Pos	Pos	Pos
S. Newport var. Puerto-Rico	C ₂	GT2558	CDC		Pos	Pos	Pos
S. Albany	C ₃	GT662	U. Massachusetts		Pos	Pos	Pos
S. Haardt	C ₃	GT2549	CDC		Pos	Pos	Pos
S. Kentucky	C ₃	GT2581	CDC		Pos	Pos	Pos
S. Kentucky	C ₃	GT548	Mass. State Lab		Pos	Pos	Pos
S. Virginia	C ₃	GT2882	CDC		Pos	Neg ^d	Pos
S. Bornum	C ₄	GT2674	CDC		Neg	Neg	Pos/Neg ^e
S. Eimsbuettel	C ₄	GT2103	CDC		Pos	Pos	Pos
S. Antarctica	D ₁	GT3195	CDC		Pos	Pos	Pos
S. Berta	D ₁	GT2884	CDC		Pos	Pos	Pos
S. Blegdam	D ₁	GT2582	CDC		Pos	Pos	Pos
S. Daressalaam (II)	D ₁	GT2583	CDC		Pos	Pos	Pos
S. Dublin	D ₁	GT2584	CDC		Pos	Pos	Pos
S. Eastbourne	D ₁	GT2881	CDC		Pos	Pos	Pos
S. Enteritidis	D ₁	GT2124	ATCC 13076		Pos	Pos	Pos
S. Gallinarum	D ₁	GT896	GENE-TRAK Systems		Pos	Pos	Pos
S. Javiana	D ₁	GT2589	CDC		Pos	Pos	Pos
S. Moscow	D ₁	GT2591	CDC		Pos	Pos	Pos
S. Panama	D ₁	GT2593	CDC		Pos	Pos	Pos
S. Pensacola	D ₁	GT2615	CDC		Pos	Pos	Pos
S. Pullorum	D ₁	GT2885	CDC		Pos	Pos	Pos
S. Rostock	D ₁	GT2616	CDC		Pos	Pos	Pos
S. Sendai	D ₁	GT2911	CDC		Pos	Pos	Pos
S. Typhi	D ₁	GT2125	ATCC 6539		Pos	Pos	Pos
S. Fresno	D ₂	GT2620	CDC		Pos	Pos	Pos
S. Gateshead	D ₂	GT2621	CDC		Pos	Pos	Pos
S. Strasbourg	D ₂	GT2622	CDC		Pos	Pos	Pos
S. Anatum	E ₁	GT2626	CDC		Pos	Pos	Pos
S. Butantan	E ₁	GT2637	CDC		Pos	Pos	Pos
S. Give	E ₁	GT2638	CDC		Pos	Pos	Pos
S. Lexington	E ₁	GT2158	FDA		Pos	Pos	Pos
S. London	E ₁	GT2640	CDC		Pos	Pos	Pos
S. Meleagridis	E ₁	GT2641	CDC		Pos	Pos	Pos
S. Muenster	E ₁	GT2510	Georgia USDA		Pos	Pos	Pos

Table 1. (continued) (6)

Reveal 2.0 result (approx. 10⁷ CFU/mL)^b

Serovar ^a	O serogroup	Strain No.	Source	Origin	RV Direct ^c	Revive-RV ^c	Revive-RV-M Broth ^c
<i>S. Nyborg</i>	E ₁	GT2642	CDC		Pos	Pos	Pos
<i>S. Orion</i>	E ₁	GT2880	CDC		Pos	Pos	Pos
<i>S. Rutgers</i>	E ₁	GT3179	CDC		Pos	Pos	Pos
<i>S. Uganda</i>	E ₁	Neogen 469	AMPCOR	Pork sausage	Pos	Pos	Pos
<i>S. Weltevreden</i>	E ₁	GT911	CDC		Pos	Pos	Pos
<i>S. Binza</i>	E ₂	GT619	U. Massachusetts		Pos	Pos	Pos
<i>S. Kinshasa</i>	E ₂	GT908	CDC		Pos	Pos	Pos
<i>S. Newbrunswick</i>	E ₂	GT2101	CDC		Pos	Pos	Pos
<i>S. Newington</i>	E ₂	A147	ATCC 29628	Duckling	Pos	Pos	Pos
<i>S. Arkansas</i>	E ₃	GT2643	CDC		Pos	Pos	Pos
<i>S. Illinois</i>	E ₃	GT2645	CDC		Pos	Pos	Pos
<i>S. Minneapolis</i>	E ₃	GT2646	CDC		Pos	Pos	Pos
<i>S. Chittagong</i>	E ₄	GT3233	CDC		Pos	Pos	Pos
<i>S. Krefeld</i>	E ₄	GT2650	CDC		Pos	Pos	Pos
<i>S. Senftenberg</i>	E ₄	GT2883	CDC		Pos	Pos	Pos
<i>S. Simsbury</i>	E ₄	GT3178	CDC		Pos	Pos	Pos
<i>S. Westerstede</i>	E ₄	GT2676	CDC		Pos	Pos	Pos

^a All are subspecies I unless otherwise indicated in parentheses.^b All tests at 1:10 dilution unless otherwise indicated.^c See text for details of culture procedures.^d Positive at approx. 10⁸ CFU/mL (undiluted).^e Variable results on multiple tests.

Table 2. Exclusivity testing results for Reveal *Salmonella* 2.0 test (6)

Species	Strain No.	Source	Origin	Reveal 2.0 result	
				TSB (approx. 10 ⁹ CFU/mL)	RV
<i>Citrobacter amalonaticus</i>	690	ATCC 25405	Feces	Neg	
<i>Citrobacter diversus</i>	1475	ATCC 27156		Neg	
<i>Citrobacter freundii</i>	1491	ATCC 8090		Neg	
<i>Citrobacter freundii</i>	679	ATCC 33128	Urine	Neg	
<i>Citrobacter youngae</i>	1476	ATCC 29935	Meat	Neg	
<i>Cronobacter sakazakii</i>	1483	ATCC 29544	Human	Neg	
<i>Edwardsiella hoshinae</i>	1710	ATCC 33379	Bird	Neg	
<i>Edwardsiella tarda</i>	569	ATCC 15947	Feces	Neg	
<i>Enterobacter aerogenes</i>	1487	ATCC 29940	Human	Neg	
<i>Enterobacter amnigenus</i>	1482	ATCC 33072	Soil	Neg	
<i>Enterobacter cloacae</i>	1481	ATCC 29941		Neg	
<i>Escherichia blattae</i>	1460	CDC		Neg	
<i>Escherichia coli</i>	1215	ATCC 19413		Neg	
<i>Escherichia coli</i>	1720	ATCC 25922	Human	Neg	
<i>Escherichia fergusonii</i>	1459	ATCC 35473	Feces	Neg	
<i>Escherichia hermannii</i>	1216	ATCC 33650	Human	Neg	
<i>Escherichia vulneris</i>	1217	ATCC 33821	Human	Neg	
<i>Hafnia alvei</i>	241	ATCC 29927	Human	Neg	
<i>Klebsiella oxytoca</i>	1503	ATCC 13182	Human	Neg	
<i>Klebsiella planticola</i>	1478	ATCC 33531	Radish	Neg	
<i>Klebsiella pneumoniae</i>	1500	ATCC 13883		Neg	
<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	1499	ATCC 11296		Neg	
<i>Kluyvera ascorbata</i>	3600	ATCC 33433	Human	Neg	
<i>Kluyvera intermedia</i>	1480	ATCC 33110	Water	Pos ^a	Neg/Pos ^b
<i>Morganella morganii</i>	303	ATCC 25830	Human	Neg	
<i>Pantoea agglomerans</i>	4361	ATCC 27155	Chicken liver	Neg	
<i>Pasteurella multocida</i>	358	ATCC 19427		Neg	
<i>Proteus mirabilis</i>	1493	ATCC 25933	Human	Neg	
<i>Proteus vulgaris</i>	368	ATCC 13315		Neg	
<i>Providencia alcalifaciens</i>	371	ATCC 9886	Feces	Neg	
<i>Providencia rettgeri</i>	373	ATCC 29944		Neg	
<i>Pseudomonas aeruginosa</i>	1909	ATCC 27853	Blood	Neg	
<i>Serratia marcescens</i>	392	ATCC 29937	Human	Neg	

^a Also positive at 10⁸/mL.^b Negative undiluted from RV and Revive/RV/M broth; weak positive from Revive/RV

Table 3. Results of food sample testing using the Reveal *Salmonella* 2.0 test with direct RV or Revive/RV enrichment (6)

Food type	Inoculum strain	Enrichment protocol ^a	Inoculation level ^b		No. of samples	No. of positive samples				Sensitivity, % ^f	Specificity, % ^g	χ^2 ^h
			CFU/g	CFU/portion ^c		Reveal Method		Reference Method				
						Assay ^d	Confirmed ^e	FDA	USDA			
Chicken carcass rinse	Naturally contaminated	Direct RV	NA ⁱ	NA ⁱ	20	6	6	—	5	120	—	0.12
Raw ground beef ^j	S. Newport	Direct RV	0.043	1.1	20	11	11	—	14	79	—	0.94
			0.015	0.38	20	5	5	—	5	100	—	0.00
			0.00	0.00	5	0	0	—	0	—	100	—
Raw ground beef ^j	S. Newport	Direct RV	0.018	0.45	20	7	7	—	7	100	—	0.00
			0.00	0.00	5	0	0	—	0	—	100	—
Raw ground turkey	Naturally contaminated	Direct RV	0.43	11	20	20	20	—	20	100	—	—
			0.040	1.0	20	11	11	—	12	92	—	0.10
Hot dogs	S. Oranienburg	Direct RV	0.0039	1.3	20	8	8	—	13	62	—	2.44
			0.00	0.00	5	0	0	—	0	—	100	—
Hot dogs ^j	S. Oranienburg	Direct RV	0.0040	1.3	20	11	11	—	14	79	—	0.94
			0.00	0.00	5	0	0	—	0	—	100	—
Raw shrimp	S. Weltevreden	Direct RV	0.015	0.37	20	8	8	4	—	200	—	1.86
			0.00	0.00	5	0	0	0	—	—	100	—
Ready-to-eat meal product	S. Typhimurium	Revive/RV	0.019	0.47	20	10	10	8	—	125	—	0.39
			0.00	0.00	5	0	0	0	—	—	100	—
Dry pet food	S. Schwarzengrund	Revive/RV/M broth	0.031	0.78	20	12	12	10	—	120	—	0.39
			0.00	0.00	5	0	0	0	—	—	100	—
Ice cream	S. Enteritidis	Revive/RV	0.11	2.7	20	15	15 ^k	19	—	79	—	3.06
			0.00	0.00	5	0	0	0	—	—	100	—
Spinach	S. Montevideo	RV/M broth	0.019	0.47	20	11	11 ^l	8	—	137	—	0.88
			0.00	0.00	5	0	0	0	—	—	100	—
Cantaloupe	S. Muenchen	Revive/RV	0.023	0.58	20	8	8	7	—	114	—	0.10
			0.00	0.00	5	0	0	0	—	—	100	—
Cantaloupe ^j	S. Muenchen	Revive/RV	0.0090	0.22	20	6	6	4	—	150	—	0.52
			0.00	0.00	5	0	0	0	—	—	100	—

^a See Text

^b MPN calculated using program found at <http://www.lcfltd.com/customer/LCFMPNCalculator.exe>

^c CFU/325 g (hot dogs) or 25 g (all other foods)

^d Number of samples positive by Reveal assay not considering subsequent confirmation

^e Number of samples positive by Reveal assay and confirmed positive from associated cultures

^f Sensitivity of Reveal method relative to that of reference method ((Reveal + /reference +) x 100)

^g Specificity of Reveal assay. Calculated only for uninoculated control samples.

^h χ^2 value by Mantel-Haenszel formula (4). $\chi^2 > 3.84$ indicates a statistically significant difference at $P < 0.05$

ⁱ Not applicable. MPNs not performed on individual carcass rinses

^j Trial performed at independent laboratory

^k There were two additional Reveal-negative, culture-positive samples

^l There was one additional Reveal-negative, culture-positive sample

Table 4. Results of food sample testing using the Reveal *Salmonella* 2.0 test with reference method enrichment cultures (6)

Food type	Inoculum strain	Enrichment protocol ^a	Inoculation level ^b		No. of samples	No. of positive samples				Sensitivity, % ^f	Specificity, % ^g	X ^{2h}
			CFU/g	CFU/portion ^c		Assay ^d	Confirmed ^e	FDA	USDA			
Hot dogs	S. Oranienburg	BPW/RV	0.0039	1.3	20	13	13		13	100	—	—
			0.00	0.00	5	0	0		0	—	100	—
Hot dogs	S. Oranienburg	BPW/RV	0.0018	0.58	20	7	6		6	100	—	—
			0.00	0.00	5	0	0		0	—	100	—
Hot dogs ⁱ	S. Oranienburg	BPW/RV	0.0040	1.3	20	10	10		14	71	—	2.25
			0.00	0.00	5	0	0		0	—	100	—
Peanut butter	S. Tennessee	Lactose/RV	0.028	0.71	20	11	10	10		100	—	—
			0.00	0.00	5	0	0	0		—	100	—
Spinach	S. Montevideo	Lactose/RV	0.019	0.47	20	9	8	8		100	—	—
			0.00	0.00	5	0	0	0		—	100	—

^a See text.

^b MPN calculated using program found at <http://www.lcfltd.com/customer/LCFMPNCalculator.exe>.

^c CFU/325 g (hot dogs) or 25 g (all other foods).

^d Number of samples positive by Reveal assay not considering subsequent confirmation.

^e Number of samples positive by Reveal assay and confirmed positive from associated cultures.

^f Sensitivity of Reveal method relative to that of reference method ([Reveal +/reference +] × 100).

^g Specificity of Reveal assay. Calculated only for uninoculated control samples.

^h χ^2 value by McNemar's test (5). $\chi^2 > 3.84$ indicates a statistically significant difference at $P < 0.05$.

ⁱ Trial performed at independent laboratory.

DISCUSSION OF MODIFICATION STUDY APPROVED APRIL 2020 (9)

Results demonstrate that the three modified Reveal *Salmonella* 2.0 device lots perform similarly to each other and compare favorably in performance to that of the control lot.

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