



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

080601

The AOAC Research Institute hereby certifies that the method known as:

RapidChek[®] SELECTTM Salmonella Test

manufactured by

Romer Labs

130 Sandy Drive

Newark, DE 19713 USA

This method has been evaluated in the AOAC[®] *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (November 20, 2021 – December 31, 2022). Renewal may be granted at the end of one year 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

November 20, 2021

Date

METHOD AUTHORS ORIGINAL VALIDATION: Mark Muldoon, Jingkun Li, Meredith Sutzko, Ann Christine Olsson-Allen, and George Teaney MODIFICATION 2010: Meredith Sutzko MODIFICATION 2011: Meredith Sutzko MODIFICATION MARCH 2012: Meredith Sutzko MODIFICATION DECEMBER 2018: Meredith I. Sutzko, Mark T. Muldoon, and Lois C. Fleck MODIFICATION OCTOBER 2020: Meredith I. Sutzko, Mark T. Muldoon, and Lois C. Fleck	SUBMITTING COMPANY Strategic Diagnostics, Inc. 128 Sandy Drive Newark, DE 19713	CURRENT SPONSOR Romer Labs 130 Sandy Drive Newark, DE 19713
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KIT NAME(S) RapidChek® SELECT™ <i>Salmonella</i> Test	CATALOG NUMBERS Original catalog numbers: 3000032, 7000187, 7000188 – 7000198 Updated catalog numbers: 10001176, 10001375 - 10001386
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INDEPENDENT LABORATORY Silliker, Inc., Food Science Center 160 Armory Drive South Holland, IL 60473 USA	MODIFICATION 2010 Q Laboratories 1400 Harrison Ave, Cincinnati, OH 45214	AOAC EXPERTS AND PEER REVIEWERS Thomas Hammack ^{1,6} , Purnendu Vasavada ² , Edward Richter ³ , Wendy McMahon ⁴ , Wayne Ziemer ⁵ , Yi Chen ^{1,7} ¹ US FDA CFSAN, College Park, MD, USA ² University of Wisconsin, River Falls, WI, USA ³ Richter International, Columbus, OH, USA ⁴ Siliker, South Holland, IL, USA ⁵ Consultant, Georgia, USA ⁶ Modifications 2010, October 2020 ⁷ Modification December 2018
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APPLICABILITY OF METHOD Target organism – <i>Salmonella</i> spp. Matrixes – USDA/FSIS MLG Ch. 4.3 - Liquid eggs (25 g), raw ground beef (25 g), raw ground chicken (25 g), chicken carcass rinsate (30 mL), sliced cooked turkey (25 g) Modification 2010: FDA BAM Ch. 5 - painted concrete (1" x 1"), plastic (4" x 4"), rubber (4" x 4"), stainless steel (4" x 4") Modification 2012: USDA/FSIS MLG Ch. 4.4 - ground beef (375 g) Modification 2018: FDA BAM Ch. 5 - stainless steel (12" x 12") Modification 2020: MLG Ch. 4.10 - raw ground pork (25 g; 375 g), raw ground pork trim (25 g; 375 g) Performance claims - The immunochromatographic test strip-based RapidChek SELECT <i>Salmonella</i> method was evaluated and shown be comparable to the reference methods.	REFERENCE METHODS Rose, B.E. (2004) Isolation and identification of <i>Salmonella</i> from meat, poultry and egg products (chapter 4, revision #3; 10/01/04). In : USDA/FSIS Microbiology Laboratory Guidebook (2) FDA Bacteriological Analytical Manual, Chapter 5. (3) Rose, B.E. (2004) Isolation and identification of <i>Salmonella</i> from meat, poultry and egg products (chapter 4, revision #4; 2/04/08). In : USDA/FSIS Microbiology Laboratory Guidebook (4) U.S. Department of Agriculture Microbiological Laboratory Guidebook, Chapter 4.10, <i>Salmonella</i> (9)
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ORIGINAL CERTIFICATION DATE August 04, 2006	CERTIFICATION RENEWAL RECORD Renewed annually through December 2022.
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METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. 2010 Level 2	1. Matrix extension environmental surfaces
2. 2011	2. Filter pad component change
3. March 2012 Level 2	3. Matrix Extension – ground beef (375 g)
4. December 2012 Level 1	4. Name change from Strategic Diagnostics to Romer
5. October 2018 Level 1	5. Editorial changes (update copyright, confirmation sections)
6. December 2018 Level 2	6. Matrix Extension – stainless steel (12x12 in)
7. May 2019 Level 1	7. Updated catalog numbers.
8. October 2020 Level 2	8. Matrix extension to include 25 g and 375 g of raw ground pork and raw ground pork trim
9. November 2021 Level 1	9. Updated USDA/FDA information.

Under this AOAC® <i>Performance Tested</i> SM License Number, 080601 this method is distributed by: NONE	Under this AOAC® <i>Performance Tested</i> SM License Number, 080601 this method is distributed as: NONE
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PRINCIPLE OF THE METHOD (1)

The RapidChek SELECT *Salmonella* Test Kit method is an immunoassay-based test that uses anti-*Salmonella* spp. antibodies (Abs) and colloidal gold-antibody conjugates incorporated into a lateral flow test strip. The method utilizes 2 proprietary enrichment broths (primary and secondary). Following the primary enrichment (16-22 h, 42°C) of the food sample, an aliquot (0.1 mL) is transferred to a tube containing 1 mL of secondary enrichment broth. For raw ground beef, raw ground chicken, and chicken carcass rinsate samples, this is enriched for 16-22 h (42°C), whereas for sliced cooked turkey meat and liquid eggs the secondary enrichment is 6-8 h (42°C).

Following secondary enrichment, the test strip is placed into the tube containing the secondary enrichment broth. The liquid sample flows through the test strip where it re-hydrates antibody-coated colloidal gold reagents specific to *Salmonella* spp impregnated in the strip. If antigens are present in the sample, they will bind to the antibody-gold conjugate to form an antigen/antibody complex. As this complex migrates through the nitrocellulose matrix, it passes a zone of anti-*Salmonella* antibody immobilized on the nitrocellulose membrane (the test line). If antigen is present, the complex is captured in this zone and is visualized by the formation of a red line. A second zone on the membrane (the control line) is designed to capture any antibody-gold complex not bound in the first zone. As a result, when *Salmonella* antigen is present, the formation of 2 red lines is observed, whereas when *Salmonella* is not present, only 1 line forms.

This validation report was prepared for claims to detect *Salmonella* in specific foods including raw ground beef, raw ground chicken, chicken carcass rinsates, sliced cooked turkey meat, and liquid whole eggs using the SDI RapidChek SELECT *Salmonella* Test Kit method.

DISCUSSION OF THE VALIDATION STUDY (1)

The RapidChek SELECT *Salmonella* test method was shown to be highly effective in detecting *Salmonella* in raw ground beef, raw ground chicken, chicken carcass rinsates, sliced cooked turkey, and liquid eggs. Two hundred (200) samples were tested by both the test method and the FSIS/USDA reference method using both naturally-contaminated (ground chicken) and *Salmonella*-inoculated food samples (target levels of 1 to 10 CFU/ 25 g sample) in method comparison studies.

Several major *Salmonella* serogroups found in food (B, C3, D1, and E1) were used for inoculation in these studies. Overall, ninety (90) samples were found to be positive by the RapidChek SELECT *Salmonella* method and 74 were found to be positive by the reference method. There were no false positives or false negatives found with the test method in those food studies. The accuracy of the test method was 122% when compared to the cultural reference method. Overall method agreement averaged 96% for naturally- and artificially contaminated food samples. In raw meat and poultry matrixes (raw ground beef, raw ground chicken, and chicken carcass rinsates) the total aerobic plate count ranged from 4.5 x 10³ to 1.29 x 10⁶ CFU/g presenting a potential challenge to recovery of low level *Salmonella* recovery. In the naturally contaminated raw ground chicken study, 3 lots of 20 samples were tested. Nineteen (19) samples were found positive by the

test method whereas 8 were found positive by the reference method. With the reference method, competitive microflora may have limited the detectable growth of *Salmonella* in the sample. The RapidChek SELECT *Salmonella* method was tested with 113 strains of *Salmonella* representing 18 serogroups and 50 strains of non-*Salmonella* bacteria commonly found in food. The test method detected 111 of the *Salmonella* strains and none of the non-*Salmonella* bacteria, resulting in a sensitivity of 98% and specificity of 100%. The method was highly robust and stable under control (4 to 25°C) and accelerated stability conditions (37 to 45°C).

Table 1. Results from the Inclusivity Study. (1)

Serogroup	Serovar and strain	Source	Test Reactivity	Serogroup	Serovar and strain	Source	Test Reactivity
A	S. Paratyphi A ATCC ^a 9150	Not known	+	C2	S. Muenchen ATCC 8388	Clinical	+
B	S. Agona USDA ^b	Not known	+	C2	S. Newport ATCC 6962	Clinical	+
B	S. Agona ATCC 51957	Clinical	+	C2	S. Newport ATCC H1275	Food	+
B	S. Agona NFC ^c 1035: S-R2	Food	+	C3	S. Albany ATCC 51960	Clinical	+
B	S. Brandenburg ARS ^d 21	Food	+	C3	S. Amherstiana DSM 006	Food	+
B	S. Brandenburg ARS 20	Food	+	C3	S. Kentucky DSM 7-19	Litter	+
B	S. Brandenburg DSM ^e 15	Not known	+	C3	S. Kentucky ARS 25	Litter	+
B	S. Brandenburg USDA-MFS 9190	Food	+	C3	S. Kentucky ARS 26	Environmenta l	+
B	S. Bredeney DSM 04	Food	+	C3	S. Kentucky ATCC 9263	Poultry	+
B	S. Derby ATCC 6960	Food	+	C3	S. Kentucky DSM 6-290	Food	+
B	S. Heidelberg ATCC 8326	Not known	+	C3	S. Kentucky DSM 7-147	Poultry	+
B	S. Heidelberg WVU 5F114	Litter	+	C3	S. Kentucky DSM 76-P	Litter	+
B	S. Heidelberg WVU 5F140	Litter	+	C3	S. Kentucky DSM 7-82	Drag swab	+
B	S. Heidelberg WVU 5F128	Feed	+	C3	S. Virginia DSM 145	Food	+
B	S. Heidelberg WVU 5F155	Feed	+	C4	S. Jerusalem Tyson 25	Food	+
B	S. Heidelberg WVU 6F71	Food	+	D1	S. Dublin ATCC 15480	Animal	+
B	S. Paratyphi B ATCC 19940	Human	+	D1	S. Enteritidis ARS 12	Litter	+
B	S. Paratyphi B ATCC M4-00-02932	Clinical	+	D1	S. Enteritidis ATCC 13076	Clinical	+
B	S. sp #28	Food	+	D1	S. Enteritidis ATCC 8391	Clinical	+
B	S. Saintpaul ATCC 9712	Not known	+	D1	S. Enteritidis M1 BGA 164/93	Clinical	+
B	S. Saintpaul FSIS 051	Food	+	D1	S. Enteritidis T 22	Food	+
B	S. Stanley DSM 305	Not known	+	D1	S. Gallinarum ATCC 9184	Not known	+
B	S. Typhimurium ATCC 23564	Clinical	+	D1	S. Javiana ATCC 10721	Food	+
B	S. Typhimurium ATCC 19585	Not known	+	D1	S. Panama T3	Food	+
B	S. Typhimurium ARS 3	Water	+	D1	S. Typhi (Keystone)	Clinical	+

B	S. Typhimurium ATCC 14028	Clinical	+	D2	S. Maarsen ATCC 15793	Animal	+
B	S. Typhimurium ATCC 23555	Not known	+	E1	S. Anatum 5F28	Waterers	+
B	S. Typhimurium ATCC 23566	Clinical	+	E1	S. Anatum 5F29	Waterers	+
B	S. Typhimurium ATCC 23595	Clinical	+	E1	S. Anatum ATCC 9270	Food	+
B	S. Typhimurium ARS 1	Not known	+	E1	S. London DSM 80	Not known	+
B	S. Typhimurium ATCC 23853	Clinical	+	E1	S. Muenstar WVU 5F22	Swabs	+
B	S. Typhimurium ATCC 25241	Clinical	+	E1	S. Muenstar WVU 8F3	Poultry	+
B	S. Typhimurium ATCC 7823	Not known	+	E1	S. Muenstar WVU 8F9	Feces	+
B	S. Typhimurium DSM 124-193	Clinical	+	E1	S. Muenster WVU 5F24	Litter	+
B	S. Typhimurium DSM 126-193	Clinical	+	E1	S. Muenster WVU 5F30	Food	+
B	S. Typhimurium ARS 2	Food	+	E2	S. Newbrunswick DSM 92	Food	+
B	S. Typhimurium DSM 129-204	Food	+	E2	S. Newington ATCC 29628	Not known	+
B	S. Typhimurium ATCC 23594	Food	+	E4	S. Senftenburg 6F1	Turkey	+
C1	S. Paratyphi C ATCC 13428	Clinical	+	E4	S. Senftenburg 6F11	Litter	+
C1	S. Branderup DSM 16	Food	+	E4	S. Senftenburg ARS 8	Water	+
C1	S. Cholerasuis T 18	Food	+	F	S. Abaetetuba ATCC 35640	Clinical	+
C1	S. Infantis ARS 22	Environmenta l	+	F	S. Rubislaw Tyson 21	Litter	+
C1	S. Infantis ATCC 51741	Not known	+	G1	S. Clifton DSM 38	Food	+
C1	S. Livingstone M7 00-02940	Clinical	+	G1	S. Poona ARS 119	Food	+
C1	S. Mbandaka T23	Food	+	G1	S. Poona ARS 121	Food	+
C1	S. Montevideo ARS 31	Litter	+	G1	S. Poona DSM 109	Food	+
C1	S. Montevideo ARS 32	Feces	+	G1	S. Poona DSM 338	Clinical	+
C1	S. Montevideo ARS 33	Litter	+	G2	S. Cubana 12007-02	Not known	+
C1	S. Oranienburg ATCC 9239	Clinical	+	G2	S. Grumpensis DSM 333	Food	+
C1	S. Thompson ARS 13	Litter	+	G2	S. Wichita DSM 147	Food	+
C1	S. Thompson ARS 14	Feces	+	G2	S. Worthington 6F14	Not known	+
C1	S. Thompson ARS 15	Drag swab	+	G2	S. Worthington 6F51	Food	+
C1	S. Thompson ATCC 8391	Clinical	+	G2	S. Worthington ARS 146	Not known	+
C2	S. Blockley DSM14	Food	+	H	S. Boecker DSM 19	Not known	-
C2	S. Hadar ATCC 51956	Not known	+	I	S. Hvittingfoss DSM 070	Not known	+
C2	S. Hadar FSIS 044	Clinical	+	K	S. Cerro Tyson 9	Food	+
				N	S. Urbana ATCC 9261	Not known	-

Table 2. Results from the Exclusivity Study (1)

Species and strain designation	Test Reactivity	Species and strain designation	Test Reactivity
Aeromonas hydrophila #10	-	Escherichia coli ATCC 51755	-
Aeromonas hydrophila #8	-	Escherichia coli O106	-
Aeromonas veronii 9071	-	Escherichia coli O129	-
Aeromonas veronii ATCC 51106	-	Escherichia coli R7-32C4	-
Citrobacter braakii ATCC 51113	-	Hafnia alvei ATCC 25927	-
Citrobacter diversus 130R2	-	Klebsiella pneumoniae #9	-
Citrobacter farmeri ATCC 51112	-	Klebsiella pneumoniae 107G6	-
Citrobacter freundii ATCC 8090	-	Klebsiella pneumoniae ATCC 13882	-
Citrobacter freundii 35	-	Morganella morganii L9-8.2	-
Citrobacter koseri ATCC 27026	-	Morganella morganii L13-8.2	-
Citrobacter sedlaki ATCC 51115	-	Proteus mirabilis 68	-
Citrobacter werkmanii ATCC 51114	-	Proteus mirabilis 70	-
Citrobacter youngae ATCC 11102	-	Proteus mirabilis ATCC 14153	-
Enterobacter aerogenes ATCC 15038	-	Proteus mirabilis ATCC 4630	-
Enterobacter agglomerans 107b4	-	Proteus sp. CW38	-
Enterobacter agglomerans oc44	-	Proteus vulgaris #19R7	-
Enterobacter cloacae #2	-	Proteus vulgaris 6380	-
Enterobacter cloacae ATCC 13047	-	Proteus vulgaris 8427	-
Enterobacter cloacae ATCC 27508	-	Pseudomonas aeruginosa 112-1	-
Escherichia coli 111-1	-	Pseudomonas fluorescens ATCC 49838	-
Escherichia coli 96C5	-	Shigella sp. 24/11-7.3	-
Escherichia coli 99G1	-	Shigella sp. ATCC 23354	-
Escherichia coli ATCC 11775	-	Vibrio metschnikovii 62A2	-
Escherichia coli ATCC 35218	-	Vibrio sp. 62A1	-
Escherichia coli ATCC 35421	-	Vibrio sp. 62A12	-

Table 3. Results from the Internal Laboratory Method Comparison Study: Ground Beef. (1)

Matrix	Strain	Method	Number of Samples	Inoculation Level, CFU/25 g	Presumptive Positives	Confirmed Positives	Sensitivity Rate	Specificity Rate	% Method Agreement
Ground Beef	<i>Salmonella</i> Typhimurium	RapidChek	5	0	0	0	-	100	100
		SELECT	20	2.3	11	11	100	100	95
	ATCC 14028	FSIS/ USDA	5	0	0	0	-	-	-
			20	2.3	8	7	-	-	-

Table 4. Results from the Internal Laboratory Method Comparison Study: Naturally-contaminated Ground Chicken. (1)

Matrix	Method	Lot	Number of Samples	Presumptive Positives	Confirmed Positives	Sensitivity Rate	Specificity Rate	% Method Agreement
Ground Chicken	RapidChek	1	20	5	5	100	100	90
		2	20	10	10	100	100	100
		3	20	4	4	100	100	70
		All	60	19	19	100	100	87
	FSIS/ USDA	1	20	3	1	-	-	-
		2	20	3	3	-	-	-
		3	20	10	4	-	-	-
		All	60	16	8	-	-	-

Table 5. Results from the Internal Laboratory Method Comparison Study: Liquid Eggs. (1)

Matrix	Strain	Method	Number of Samples	Inoculation Level, CFU/25 g	Presumptive Positives	Confirmed Positives	Sensitivity Rate	Specificity Rate	% Method Agreement
Liquid Eggs	<i>Salmonella</i> Enteritidis	RapidChek	5	0	0	0	-	100	100
		SELECT	20	1.08	14	14	100	100	95
	ATCC 13076	FSIS/ USDA	5	0	0	0	-	-	-
			20	1.08	15	15	-	-	-

Table 6. Results from the Internal Laboratory Method Comparison Study: Sliced Cooked Turkey (1)

Matrix	Strain	Method	Number of Samples	Inoculation Level, CFU/ 25 g	Presumptive Positives	Confirmed Positives	Sensitivity Rate	Specificity Rate	% Method Agreement
Sliced Cooked Turkey	<i>Salmonella</i> Kentucky ATCC 9263	RapidChek	5	0	0	0	-	100	100
		SELECT	20	3.75	17	17	100	100	100
	FSIS/ USDA	5	0	0	0	-	-	-	
		20	3.75	17	17	-	-	-	

Table 7. Results from the Internal Laboratory Method Comparison Study- Chicken Carcass Rinsates (1)

Matrix	Strain	Method	Number of Samples	Inoculation Level, CFU/ 30 mL	Presumptive Positives	Confirmed Positives	Sensitivity Rate	Specificity Rate	% Method Agreement
Chicken Carcass Rinsate	<i>Salmonella</i> Anatum ATCC 9270	RapidChek	5	0	0	0	-	100	100
		SELECT	20	0.69	18	18	100	100	100
	FSIS/ USDA	5	0	0	0	-	-	-	
		20	0.69	17	17	-	-	-	

DISCUSSION OF MODIFICATION APPROVED 2010 (5)

The RapidChek SELECT method has demonstrated no statistically significant difference in sensitivity, specificity, and accuracy as compared to the FDA reference method in the detection of *Salmonella* species in environmental surfaces. Overall, there were no false positive or false negative results observed using the lateral flow device. In total, the RapidChek SELECT method reported 62 confirmed positive results, while the reference method reported 59 confirmed positive results. Both methods have been shown to be capable of detecting low levels of *Salmonella* spp. in environmental surfaces and the RapidChek SELECT method has performed as well or better than the reference method with the selected environmental surfaces.

Table 1. Results from the Method Comparison Studies (5)

Surface	Analyte	Method	Number of Samples	Surface Area	Inoculation Level, cfu's/surface	Presumptive Positives	Confirmed Positives	Reference Method	Chi square ^a	Sensitivity Rate ^b	False Neg Rate ^c	Specificity Rate ^d	False Positive ^e
Stainless Steel	S. Typhimurium ATCC 19585	RapidChek SELECT Salmonella	5	4" x 4"	0	0	0	0	0.098	-	-	100	0
			20		1.00E+03	10	10	11		100	0	-	-
			5	4" x 4"	0	0	0	0	0.46	-	-	100	0
			20		1.40E+01	13	13	15		100	0	-	-
Rubber	S. Kentucky ATCC		5	4" x 4"	0	0	0	0	0.429	-	-	100	0
			20		1.25E+02	14	14	12		100	0	-	-
Painted Concrete	S. Enteritidis ATCC		5	1" x 1"	0	0	0	0	0.14	-	-	100	0
			20		4.00E+02	16	16	15		100	0	-	-
Plastic	S. Anatum ATCC	5	4" x 4"	0	0	0	0	0.936	-	-	100	0	
		20		1.05E+02	9	9	6		100	0	-	-	

^a Mantel-Haenszel Chi-square analysis

$$\chi^2 = \frac{(n-1)(AF - (B+C+D)E)^2}{(A+B+C+D)(A+E)(B+C+D+F)(E+F)}$$

Where n = A+B+C+D+E+F

^b Sensitivity Rate = (No. of test method presumptive positives)/(No. of test method confirmed positives) x 100

^c False Negative Rate = 100 - Sensitivity Rate

^d Specificity Rate = (No. of test method negatives)/(No. of confirmed negatives) x100

^e False Positive Rate = 100 - Specificity Rate

DISCUSSION OF MODIFICATION APPROVED MARCH 2012 (6)

The RapidChek SELECT *Salmonella* test method was shown to be highly effective in detecting *Salmonella* spp. In 375 gram samples of ground beef as compared to the USDA/FSIS reference method. Twelve (12) samples tested positive with the RapidChek SELECT method and fifteen (15) samples tested positive with the USDA/FSIS reference method. There were no false positive or false negative results observed using the lateral flow device. The RapidChek SELECT method in 375g composite samples has performed as well as the standard reference method in detecting low levels of *Salmonella* spp. in ground beef.

Table 1. Results from the Method Comparison Study Beef 375 g (6)															
Matrix	Analyte	Method	Number of Samples	Inoculation Level, CFU/25g	Presumptive Positives	Confirmed Positives		Reference Method		Chi square ^a	Sensitivity Rate ^b	False Negative Rate ^c	Specificity Rate ^d	False Positive Rate ^e	Accuracy ^f
						SDIX Secondary	Reference Method Secondary	BAX	Confirmed						
Ground Beef	S. Typhimurium ATCC 14028	RapidChek SELECT Salmonella	5	0	0	0	0	0	0	1	-	-	100	0	-
			20	1.4	12	12	12	17	15		100	0	-	-	80

^a Mantel-Haenszel Chi-square analysis: $X^2 = \frac{(n-1)(AF - (B+C+D)E)^2}{(A+B+C+D)(A+E)(B+C+D+F)(E+F)}$ Where n = A+B+C+D+E+F

^b Sensitivity Rate = (No. of test method presumptive positives)/(No. of test method confirmed positives) x 100

^c False Negative Rate = 100 - Sensitivity Rate

^d Specificity Rate = (No. of test method negatives)/(No. of confirmed negatives) x 100

^e False Positive Rate = 100 - Specificity Rate

^f Accuracy = (No. of test method positives)/(No. of reference method positives) x 100

DISCUSSION OF MODIFICATION APPROVED DECEMBER 2018 (7)

The Romer Labs RapidChek® SELECT™ *Salmonella* test system was validated against the FDA BAM cultural reference method for the detection of *Salmonella* species on 12 x 12" stainless steel environmental surfaces in an unpaired study design. The total number of presumptive positive results obtained with the RapidChek® method was thirty-seven (37), whereas the number of positive samples identified by the FDA BAM method was twenty-five (25). Probability of Detection (POD) analysis showed that the number of positive test results obtained by the RapidChek® test method was significantly different than the number of positive test results obtained by the FDA BAM reference method (dPOD_c) at the 95% confidence interval for both dPOD_c and dPOD_R for stainless co-inoculated with *Salmonella* and *Citrobacter*. There was no significant difference realized between alternative and reference method on surfaces spiked only with *Salmonella* Derby. POD analysis showed no significant difference between the RapidChek® presumptive and confirmed results (dPOD_{CP}) at the 95% confidence interval.

Table 2. Comparison of presumptive and confirmed results (7)

Matrix	Inoculation Strain	Inoculation Level	N ^b	Presumptive			Confirmed			dPOD _{CP} ^f	95% CI ^g
				x ^c	POD _{CP} ^d	95% CI	x	POD _{CC} ^e	95% CI		
Stainless Steel	<i>Salmonella</i> Derby with 10x <i>C. freundii</i>	Control	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		Low	20	19	0.95	0.76, 1.00	19	0.95	0.76, 1.00	0.00	-0.19, 0.19
		High	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Derby	Control	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		Low	20	18	0.90	0.70, 0.97	18	0.90	0.70, 0.97	0.00	-0.21, 0.21
		High	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_{CP} = Candidate presumptive positive outcomes divided by the total number of trials.

^ePOD_{CC} = Candidate confirmatory positive outcomes divided by the total number of trials.

^fdPOD_{CP} = Difference in POD values between the candidate presumptive and candidate confirmatory results.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 3. Comparison of candidate and reference method results (7)

Matrix	Inoculation Strain	Inoculation Level	N ^b	Candidate			Reference			dPOD _{CR} ^f	95% CI ^g
				x ^c	POD _C ^d	95% CI	x	POD _R ^e	95% CI		
Stainless Steel	<i>Salmonella</i> Derby with 10x <i>C. freundii</i>	Control	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		Low	20	19	0.95	0.76, 1.00	10	0.50	0.30, 0.70	0.45	0.18, 0.66
		High	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
	<i>Salmonella</i> Derby	Control	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		Low	20	18	0.90	0.70, 0.97	15	0.75	0.53, 0.89	0.15	-0.09, 0.38
		High	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_C = Candidate presumptive positive outcomes divided by the total number of trials.

^ePOD_{CC} = Candidate confirmatory positive outcomes divided by the total number of trials.

^fdPOD_{CR} = Difference in POD values between the candidate presumptive and candidate confirmatory results.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

DISCUSSION OF MODIFICATION APPROVED OCTOBER 2020 (8)

The Romer Labs RapidChek® SELECT™ *Salmonella* test system was validated against the FSIS MLG cultural reference method for the detection of *Salmonella* species in raw ground pork and pork trim at 25 g and 375 g sample sizes in an unpaired study design. The total number of low-level inoculated presumptive positive results obtained with the RapidChek® method was forty-nine (49), whereas the number of positive samples identified by the USDA MLG method was thirty-four (34). Probability of Detection (POD) analysis showed that the number of positive test results obtained by the RapidChek® test method was significantly different than the number of positive test results obtained by the USDA MLG reference method (dPOD_c) at the 95% confidence interval for ground pork at both sample sizes (25 g and 375 g). There was no significant difference realized between alternative and reference methods in the pork trim method comparison studies. POD analysis showed no significant difference between the RapidChek® presumptive and confirmed results (dPOD_{cp}) at the 95% confidence interval in raw ground pork and pork trim.

Table 2. Comparison of presumptive and confirmed results (8)

Matrix	Inoculation Strain	MPN ^a per Test Portion	N ^b	Presumptive			Confirmed			dPOD _{cp} ^f	95% CI ^g
				x ^c	POD _{cp} ^d	95% CI	x	POD _{cc} ^e	95% CI		
25 g Ground Pork	<i>Salmonella</i> Derby	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.19 (95% CI)	20	13	0.65	0.43, 0.82	13	0.65	0.43, 0.82	0.00	-0.13, 0.13
		4.25 (95% CI)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
375 g Ground Pork	<i>Salmonella</i> Derby	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.19 (95% CI)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	-0.13, 0.13
		4.25 (95% CI)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
25 g Pork Trim	<i>Salmonella</i> Typhimurium	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		2 (95% CI)	20	14	0.70	0.48, 0.86	14	0.70	0.48, 0.86	0.00	-0.13, 0.13
		>1.84 (95%CI)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
375 g Pork Trim	<i>Salmonella</i> Typhimurium	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.9 (95% CI)	20	12	0.60	0.39, 0.78	12	0.60	0.39, 0.78	0.00	-0.13, 0.13
		4.25 (95% CI)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_{cp} = Candidate presumptive positive outcomes divided by the total number of trials.

^ePOD_{cc} = Candidate confirmatory positive outcomes divided by the total number of trials.

^fdPOD_{cp} = Difference in POD values between the candidate presumptive and candidate confirmatory results.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Table 3. Comparison of candidate and reference method results (8)

Matrix	Inoculation Strain	MPN ^a per Test Portion	N ^b	Candidate			Reference			dPOD _c ^f	95% CI ^g
				x ^c	POD _c ^d	95% CI	x	POD _R ^e	95% CI		
25 g Ground Pork	<i>Salmonella</i> Derby	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.19 (95% CI)	20	13	0.65	0.43, 0.82	4	0.20	0.08, 0.42	0.45	0.14, 0.66
		4.25 (95% CI)	5	5	1.00	0.57, 1.00	1	0.20	0.00, 0.62	0.80	0.19, 1.00
375 g Ground Pork	<i>Salmonella</i> Derby	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.19 (95% CI)	20	10	0.50	0.30, 0.70	4	0.20	0.08, 0.42	0.30	0.01, 0.53
		4.25 (95% CI)	5	5	1.00	0.57, 1.00	1	0.20	0.00, 0.62	0.80	0.19, 1.00
25 g Pork Trim	<i>Salmonella</i> Typhimurium	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		2 (95% CI)	20	14	0.70	0.48, 0.85	17	0.85	0.64, 0.95	-0.15	-0.39, 0.11
		>1.84 (95%CI)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43
375 g Pork Trim	<i>Salmonella</i> Typhimurium	-	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
		0.9 (95% CI)	20	12	0.60	0.39, 0.78	9	0.45	0.26, 0.66	0.15	-0.15, 0.41
		4.25 (95% CI)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

^aMPN = Most Probable Number is calculated using the LCF MPN calculator provided by AOAC RI, with 95% confidence interval.

^bN = Number of test portions.

^cx = Number of positive test portions.

^dPOD_c = Candidate method confirmed positive outcomes divided by the total number of trials.

^ePOD_R = Reference method confirmed positive outcomes divided by the total number of trials.

^fdPOD_c = Difference in POD values between the candidate method confirmed and reference method confirmed results.

^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

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