CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

111002

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

RapidChek® SELECT™ *Salmonella* Enteritidis Test System & RapidChek® CONFIRM™ *Salmonella* Enteritidis Immunomagnetic Separation (IMS) Kit

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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

November 20, 2021
Date

2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

METHOD AUTHORS	SUBMITTING COMPANY	CURRENT SPONSOR
Mark Muldoon	**Strategic Diagnostics, Inc.** **128 Sandy Drive** **Newark, DE 19713**	**Romer Labs** **130 Sandy Drive** **Newark, DE 19713**

KIT NAME(S)
RapidChek® SELECT™ *Salmonella* Enteritidis Test System & RapidChek® CONFIRM™ *Salmonella* Enteritidis Immunomagnetic Separation (IMS) Kit

CATALOG NUMBERS
Original catalog numbers: 3000027, 7000220, 7000220P, 7000220S, 7000221, 7000222, 7000225, 7000228
Updated catalog numbers: 10001175, 10001396, 10001714, 10001715, 10001397, 10001398, 10001403

INDEPENDENT LABORATORY
Q Laboratories, Inc.
1400 Harrison Avenue
Cincinnati, OH 45214
USA

AOAC EXPERTS AND PEER REVIEWERS
Thomas Hammack[1], Michael Broadsky[2], Edward Richter[3]
[1] USDA FDA CFSAN, College Park, MD, USA
[2] Brodsky Consultants, Thornhill, Ontario, Canada
[3] Richter International, Columbus, OH, USA

APPLICABILITY OF METHOD
Target organism – *Salmonella* Enteritidis and other *Salmonella* Group D1 bacteria

Matrixes – Poultry house environmental drag swabs, egg pools, chicken carcass rinsates

Performance claims - RapidChek SELECT™ *Salmonella* Enteritidis Test System was validated for the low-level detection of *Salmonella* Enteritidis (SE) (1-5 CFU/sample) in poultry house drag swabs, shell egg pools, and chicken carcass rinsates. Method sensitivity was 100% and method specificity was 100%. Accuracy of the test method was 137%.

REFERENCE METHODS
FDA CFSAN BAM. Chapter 5: *Salmonella.* (9)

USDA FSIS. MLG. Chapter 4: Isolation and identification of *Salmonella* from meat, poultry and egg products. (10)

ORIGINAL CERTIFICATION DATE	CERTIFICATION RENEWAL RECORD
November 11, 2010	**Renewed annually through December 2022.**

METHOD MODIFICATION RECORD	SUMMARY OF MODIFICATION
1. December 2012 Level 1	**1. Name change from Strategic Diagnostics to Romer.**
2. May 2019 Level 1	**2. Updated catalog numbers.**
3. November 2021 Level 1	**3. Updated USDA/FDA information.**

Under this AOAC® *Performance Tested*℠ License Number, 111002 this method is distributed by:	Under this AOAC® *Performance Tested*℠ License Number, 111002 this m is distributed as:
NONE	**NONE**

PRINCIPLE OF THE METHOD (1)
The RapidChek SELECT™ *Salmonella* Enteritidis Test Kit is designed to detect *Salmonella* Enteritidis (including other Group D1 serovars) in poultry house drag swabs, shell egg pool samples and chicken carcass rinsate samples. The test kit permits the presumptive detection and identification of the target pathogen in 40 or 48 hours, dependent on sample type, when present at levels as low as 1-5 organisms per sample.
This immunoassay test uses a double antibody sandwich format in a lateral flow test strip. It utilizes a murine monoclonal antibody specific for *Salmonella* Group D1 including *Salmonella* Enteritidis (SE). The antibody is sprayed and immobilized on the surface of a nitrocellulose membrane comprising a "test line". The same monoclonal antibody is also labeled with colloidal gold and is contained within a reagent pad upstream from the test line on the membrane. As the sample moves by capillary action from the filter pad into the antibody–gold pad, the antibody–gold reagent specifically binds to the target organism and moves with the liquid sample onto the test membrane. The sample passes through the test line where the immobilized antibody captures the antigen–antibody–gold complex, causing the formation of an antibody–antigen "sandwich" and development of red color at the test line. Antibody–antigen sandwiches are not formed in the absence of the *Salmonella* Group D1 including SE, resulting in no red color development at the test line. Anti-mouse antibody immobilized at the control line captures excess monoclonal antibody-gold reagent passing through the test line. The presence of red color at the control line indicates that the strip has flowed correctly. Therefore, the presence of only one line (control line) on the membrane indicates a negative sample and the presence of two lines indicates a positive sample.
The immunomagnetic confirmation kit utilizes the same monoclonal antibody described above attached to magnetic particles for the purification of SE and other Group D1 serovars from a complex enriched liquid media sample. The antibody- coated magnetic particles are used to concentrate *Salmonella* Group D1bacteria present within an enriched sample making confirmation of the presumptive positive result much more robust and easier to interpret. Essentially, the coated magnetic particles are added to a presumptive positive enrichment. If SE is present, it will bind to the magnetic particles via the coated antibody. A magnet is then used to concentrate the bound, coated magnetic particles and the remaining enrichment is discarded leaving only magnetic particles bound to the *Salmonella* Group D1serovars present in the enrichment. Confirmation procedures are then continued with the concentrated sample.

DISCUSSION OF THE VALIDATION STUDY (1)

The RapidChek SELECT™ *Salmonella* Enteritidis Test Method was validated for the detection of *Salmonella* Enteritidis (SE) in poultry house drag swab samples, shell egg pools, and carcass rinsate samples. For the detection of SE in poultry house drag swab samples, a immunomagnetic separation (IMS) method was used to aid in the isolation and confirmation of SE from those samples.

The test method showed equivalency to both reference methods used for the detection of SE in poultry house drag swabs and shell egg pools (FDA-BAM) as well as carcass rinsates (USDA-FSIS). The test method gave a sensitivity of 100% and a specificity of 100% across all sample types. There were no false positives or false negatives found in the study. The overall accuracy was 137%, indicating that, in general, the test method gave more positives (52) than the reference methods (38). The overall Chi square was 4.95, indicating that the test method was overall more sensitive than the reference method in this study.

The test method was highly selective for *Salmonella* Enteritidis and other *Salmonella* Group D1 serotypes and did not cross-react with other commonly occurring bacteria spanning 10 bacterial genera including several non-Group D1*Salmonella*. Both the lateral flow test strip and the IMS reagent demonstrated very good accelerated stability at elevated temperatures.

Table 4. Results from the Test Strip Inclusivity Study. (1)

Sample Number	Serovar	Strain Number	RapidChek Select SE Test Strip Result	Sample Number	Serovar	Strain Number	RapidChek Select SE Test Strip Result
1	Salmonella Enteritidis	ARS 11	+	43	Salmonella Enteritidis	ISU-18-4h	+
2	Salmonella Enteritidis	ARS 12	+	44	Salmonella Enteritidis	ISU-18-5d	+
3	Salmonella Enteritidis	M1 BGA 164-93	+	45	Salmonella Enteritidis	ISU-18-6n	+
4	Salmonella Enteritidis	Tyson 22	+	46	Salmonella Enteritidis	ISU-18-9f	+
5	Salmonella Enteritidis	ATCC 13076	+	47	Salmonella Enteritidis	ISU-18-10g	+
6	Salmonella Enteritidis	ATCC 8391	+	48	Salmonella Enteritidis	ISU-19-11g	+
7	Salmonella Enteritidis var. Jena	ATCC 49221	+	49	Salmonella Enteritidis	ISU-20-19i	+
8	Salmonella Enteritidis var. Jena	ATCC 49222	+	50	Salmonella Enteritidis	ISU-20-32m	+
9	Salmonella Enteritidis var. Jena	ATCC 49223	+	51	Salmonella Enteritidis	ISU-20-33n	+
10	Salmonella Enteritidis var. Essen	ATCC 49218	+	52	Salmonella Enteritidis	ISU-20-36p	+
11	Salmonella Enteritidis var. Essen	ATCC 49219	+	53	Salmonella Enteritidis	ISU-20-35q	+
12	Salmonella Enteritidis var. Essen	ATCC 49220	+	54	Salmonella Enteritidis	ISU-20-36r	+
13	Salmonella Enteritidis var. Danysz	ATCC 49217	+	55	Salmonella Enteritidis	ISU-21-5f	+
14	Salmonella Enteritidis var. Chaco	ATCC 49214	+	56	Salmonella Enteritidis	ISU-22-5a	+
15	Salmonella Enteritidis var. Chaco	ATCC 49215	+	57	Salmonella Enteritidis	ISU-22-6b	+
16	Salmonella Enteritidis	ISU-1-2P	+	58	Salmonella Enteritidis	ISU-23-5e	+
17	Salmonella Enteritidis	ISU-1-4K	+	59	Salmonella Enteritidis	ISU-23-5h	+
18	Salmonella Enteritidis	ISU-1-6J	+	60	Salmonella Enteritidis	ISU-24-3a	+
19	Salmonella Enteritidis	ISU-1-38s	+	61	Salmonella Enteritidis	ISU-24-4b	+
20	Salmonella Enteritidis	ISU-1-78t	+	62	Salmonella Enteritidis	ISU-24-5c	+
21	Salmonella Enteritidis	ISU-5-4j	+	63	Salmonella Enteritidis	ISU-25-1f	+
22	Salmonella Enteritidis	ISU-6-19i	+	64	Salmonella Dublin	ISU-2-1a	+
23	Salmonella Enteritidis	ISU-7-2i	+	65	Salmonella Dublin	ISU-3-1a	+
24	Salmonella Enteritidis	ISU-7-6f	+	66	Salmonella Dublin	ISU-4-1a	+
25	Salmonella Enteritidis	ISU-8-27e	+	67	Salmonella Berta	ISU-16-2b	+
26	Salmonella Enteritidis	ISU-8-13a	+	68	Salmonella Berta	ISU-16-3i	+
27	Salmonella Enteritidis	ISU-9-13e	+	69	Salmonella Berta	ISU-16-7j	+
28	Salmonella Enteritidis	ISU-10-3e	+	70	Salmonella Berta	ISU-16-10f	+
29	Salmonella Enteritidis	ISU-10-9g	+	71	Salmonella Berta	ISU-16-12k	+
30	Salmonella Enteritidis	ISU-10-13d	+	72	Salmonella Javiana	ATCC 10721	+
31	Salmonella Enteritidis	ISU-10-13p	+	72	Salmonella Panama	Tyson 3	+
32	Salmonella Enteritidis	ISU-11-2a	+	73	Salmonella Pullorum	ATCC 9120	+
33	Salmonella Enteritidis	ISU-11-2f	+	74	Salmonella Pullorum	ATCC 19945	+
34	Salmonella Enteritidis	ISU-12-39s	+	75	Salmonella 9,12:nonmotile	ISU-10-3a	+
35	Salmonella Enteritidis	ISU-12-42e	+	76	Salmonella 9,12:nonmotile	ISU-10-5b	+
36	Salmonella Enteritidis	ISU-12-53p	+	77	Salmonella 9,12:nonmotile	ISU-10-9c	+
37	Salmonella Enteritidis	ISU-13-10f	+	78	Salmonella 9,12:nonmotile	ISU-10-19i	+
38	Salmonella Enteritidis	ISU-13-11e	+	79	Salmonella 9,12: poorly motile	ISU-10-5"o"	+
39	Salmonella Enteritidis	ISU-14-8g	+	80	Salmonella 9,12: poorly motile	ISU-10-9n	+
40	Salmonella Enteritidis	ISU-15-2h	+	81	Salmonella 9,12: poorly motile	ISU-10-13h	+
41	Salmonella Enteritidis	ISU-17-43h	+	82	Salmonella 9,12: poorly motile	ISU-10-15m	+
42	Salmonella Enteritidis	ISU-18-3b	+				

Table 5. Results from the Test Strip Exclusivity Study. (1)

Bacteria	Strain Number	RapidChek Select SE Test Strip Result
Salmonella Typhimurium (B)	ATCC 14028	-
Salmonella Heidelberg (B)	WVU 5F114	-
Salmonella Montevideo (C1)	ARS 32	-
Salmonella Thompson (C1)	ARS 15	-
Salmonella Hadar (C2)	ATCC 51956	-
Salmonella Kentucky (C3)	ATCC 9263	-
Salmonella Albany (C3)	ATCC 51960	-
Salmonella Maarsen (D2)	ATCC 15793	-
Salmonella Muenster (E1)	WVU 5F22	-
Salmonella Illinois (E3)	ATCC 11646	-
Salmonella Senftenberg (E4)	WVU 6F11	-
Salmonella Abaetetuba (F)	ATCC 35640	-
Salmonella Poona (G1)	DSM 109	-
Salmonella Cubana (G2)	ATCC 12007	-
Salmonella Pomona (M)	ATCC 10729	-
Bacillus subtilis	ATCC 6633	-
Aeromonas veronii	ATCC 51106	-
Citrobacter koseri	ATCC 27026	-
Citrobacter freundii	ATCC 8090	-
Enterobacter cloacae	ATCC 27508	-
Enterobacter aerogenes	ATCC 15038	-
Escherichia coli	ATCC 35218	-
Escherichia coli	ATCC 51755	-
Escherichia hermannii	ATCC 55236	-
Escherichia hermannii	ATCC 33650	-
Klebsiella pneumoniae	ATCC 29018	-
Klebsiella pneumoniae	ATCC 35596	-
Proteus vulgaris	ATCC 8427	-
Proteus mirabilis	ATCC 4630	-
Serratia liquefaciens	ATCC 27592	-
Vibrio parahaemolyticus	ATCC 17802	-
Vibrio parahaemolyticus	ATCC 27519	-

Table 1. Results from the Poultry House Drag Swab Method Comparison Study-Internal Validation

Matrix	Analyte	Method	Number of Samples	Inoculation Level, CFU/sample	Presumptive Positives	Confirmed Positives	Reference Method Positives	Chi square[a]	Sensitivity Rate[b]	False Negative Rate[c]	Specificity Rate[d]	False Positive Rate[e]	Accuracy[f]
Poultry House Drag Swabs	S. Enteritidis ARS 11	RapidChek SELECT	5	0	0	0	0	8.070	100.0%	0.0%	100.0%	0.0%	325.0%
			20	3	13	13	4						

[a]Mantel-Haenszel Chi-square analysis. [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 test method negatives) x 100. [e]False Positive Rate = 100 - Specificity Rate. [f]Accuracy = (No. of test method positives)/(No. of reference method positives) x 100.

Table 2. Results from the Egg Pool Method Comparison Study-Internal Validation.

Matrix	Analyte	Method	Number of Samples	MPN, CFU/sample	Presumptive Positives	Confirmed Positives	Reference Method Positives	Chi square[a]	Sensitivity Rate[b]	False Negative Rate[c]	Specificity Rate[d]	False Positive Rate[e]	Accuracy[f]
Egg Pools	S. Enteritidis ATCC 13076	RapidChek SELECT	5	0	0	0	0	0.609	100.0%	0.0%	100.0%	0.0%	113.3%
			20	<3	17	17	15						

[a]Mantel-Haenszel Chi-square analysis. [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 test method negatives) x 100. [e]False Positive Rate = 100 - Specificity Rate. [f]Accuracy = (No. of test method positives)/(No. of reference method positives) x 100.

Table 3. Results from the Chicken Carcass Rinsate Method Comparison Study-Internal Validation.

Matrix	Analyte	Method	Number of Samples	Inoculation Level, CFU/sample	Presumptive Positives	Confirmed Positives	Reference Method Positives	Chi square[a]	Sensitivity Rate[b]	False Negative Rate[c]	Specificity Rate[d]	False Positive Rate[e]	Accuracy[f]
Chicken Carcass Rinsates	S. Enteritidis ARS 11	RapidChek SELECT	5	0	0	0	0	0.406	100.0%	0.0%	100.0%	0.0%	84.6%
			20	1	11	11	13						

[a]Mantel-Haenszel Chi-square analysis. [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 test method negatives) x 100. [e]False Positive Rate = 100 - Specificity Rate. [f]Accuracy = (No. of test method positives)/(No. of reference method positives) x 100.

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