CERTIFICATION

AOAC® *Performance Tested*SM

Certificate No.

041602

The AOAC Research Institute hereby certifies the performance of the test kit known as:

Reveal 2.0 for Group D1 *Salmonella* (including *Salmonella* Enteritidis)

manufactured by

Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912
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 23, 2019 – December 31, 2020). 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 23, 2019
Date

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SUBMITTING COMPANY
Neogen Corporation
620 Lesher Place
Lansing, Michigan 48912

KIT NAME(S)
Reveal 2.0 for Group D1 *Salmonella* (including *Salmonella* Enteritidis)

CATALOG NUMBERS
9809

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

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APPLICABILITY OF METHOD
Target organism – *Salmonella enterica* subsp. *Enterica* ser. Enteritidis. Also detects majority of other *Salmonella* somatic group D1 serovars.

Matrices – (USDA BAM Ch 5) – raw shell eggs (20 shells), poultry feed (25 g)
(USDA FSIS MLG 4.08) – chicken carcass rinse (30 mL)

Performance claims - As determined by probability of detection analysis, Reveal 2.0 group D1 *Salmonella* method performance is equivalent to that of the reference methods.

REFERENCE METHODS
US FDA (2014) *Bacteriological Analytical Manual*, chapter 5
http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm (2)
USDA-FSIS (2014) *Microbiology Laboratory Guidebook*, chapter 4.08
http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES (3)

ORIGINAL CERTIFICATION DATE
April 11, 2016

CERTIFICATION RENEWAL RECORD
Renewed annually through 2020

METHOD MODIFICATION RECORD
1. **November 2019 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial/clerical changes to inserts.**

Under this AOAC® *Performance Tested*SM License Number, 041602 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)
Reveal 2.0 for Group D1 *Salmonella* is a lateral flow immunoassay utilizing monoclonal and polyclonal antibodies specific to *Salmonella* somatic group D1 bacteria. The test device is inserted into the secondary enrichment culture in a vertical position after incubation. Bacteria present in the culture flow up the device membrane. Target organisms are captured in a zone containing group D1 *Salmonella*-specific antibodies (capture antibodies). Simultaneously, specific antibodies labeled with colloidal gold (detector antibodies) travel up the membrane and bind to target bacteria immobilized in the capture zone. The concentrated, immobilized detector antibodies form a distinct red line visible to the naked eye. Liquid and unbound bacteria and reagents continue to travel up the membrane where they are deposited into a waste pad. Ten minutes are allowed for test development before interpretation as positive or negative based on the presence or absence of a visible line in the capture zone. A second antibody-antigen reaction produces a second line in a different region of the membrane, with this functioning as a positive control for proper test development. Positive results may be confirmed by plating of enrichment cultures to *Salmonella* selective/differential agar media, followed by biochemical and serological identification of presumptive isolates performed according to standard procedures (2,3).

DISCUSSION OF THE VALIDATION STUDY (1)
Results of this study establish that the Reveal 2.0 for Group D1 *Salmonella* test is an effective procedure for detection of *Salmonella* Enteritidis in raw shell eggs, poultry feed, and chicken carcass rinse. There were no significant differences in method performance for any of these matrices comparing the Reveal 2.0 method and the appropriate reference culture procedure. There were no false positive results on uninoculated control test portions.
Further, results of an extensive NPIP validation study with a variety of naturally contaminated poultry house environmental samples showed that performance of the Reveal 2.0 method compares favorably with that of the NPIP reference culture procedure (unpublished results).
While the target organism for the Reveal 2.0 test is *Salmonella* Enteritidis, the test also detects a majority of other group D1 serovars. Therefore, confirmation of positive assay results by serology is required for definitive identification of SE in the sample. However, compared to SE, the presence of other group D1 serovars is relatively rare in poultry production and processing environments, and most positive Reveal 2.0 results would be expected to confirm as SE. For example, in one laboratory that conducts a high volume of SE testing from
poultry associated samples, 94% of group D1 isolates over a three-year period ultimately confirmed as SE after full serotyping (D. Waltman, personal communication). Taken together, results of the present study, and those of the NPIP validation study, establish that the Reveal 2.0 test can provide a comprehensive solution for SE testing in poultry and egg production and processing, while reducing analysis time by a minimum of 1 day compared with standard culture methods.

Table 1. Results of inclusivity testing for the Reveal 2.0 Group D1 *Salmonella* test using TSB and Reveal 2.0 for SE Media primary enrichment (1)

Organism	*Strain #*	*Source*	*Origin (If Known)*	*Result from TSB/MSRV*	*Result from Reveal 2.0 Media/MSRV*
S. Enteritidis	Neogen 201	USDA[a] Athens		Positive	Positive
S. Enteritidis	Neogen 202	USDA Athens		Positive	Positive
S. Enteritidis	Neogen 203	USDA Athens		Positive	Positive
S. Enteritidis	Neogen 204	USDA Athens		Positive	Positive
S. Enteritidis	Neogen 205	USDA Athens		Positive	Positive
S. Enteritidis	Neogen 209	USDA Athens		Positive	Positive
S. Enteritidis	Neogen 195	FDA[b]	Chicken	Positive	Positive
S. Enteritidis	Neogen 199	FDA	Human	Positive	Positive
S. Enteritidis	Neogen 206	USDA Athens	Spleen	Positive	Positive
S. Enteritidis	Neogen 200	FDA	Human	Positive	Positive
S. Enteritidis	Neogen 210	USDA Athens		Positive	Positive
S. Enteritidis	Neogen 196	FDA	Chicken	Positive	Positive
S. Enteritidis	Neogen 208	USDA Athens	Heart	Positive	Positive
S. Enteritidis	Neogen 197	FDA	Chicken	Positive	Positive
S. Enteritidis	Neogen 207	USDA Athens	Egg	Positive	Positive
S. Enteritidis	Neogen 198	FDA	Human	Positive	Positive
S. Enteritidis	GT 2585	CDC[c]		Positive	Positive
S. Enteritidis	GT 2586	CDC		Positive	Positive
S. Enteritidis	GT 2587	CDC		Positive	Positive
S. Enteritidis	GT 2588	CDC		Positive	Positive
S. Enteritidis	GT 5160	Iowa State Univ.[d]		Positive	Positive
S. Enteritidis	GT 5161	Iowa State Univ.		Positive	Positive
S. Enteritidis	GT 5162	Iowa State Univ.		Positive	Positive
S. Enteritidis	GT 5163	Iowa State Univ.		Positive	Positive
S. Enteritidis	GT 5164	Iowa State Univ.		Positive	Positive
S. Enteritidis	GT 5165	Iowa State Univ.		Positive	Positive
S. Enteritidis	GT5175	Georgia Poultry Lab[e]	Drag swab	Positive	Positive
S. Enteritidis	GT5176	Georgia Poultry Lab	Drag swab	Positive	Positive
S. Enteritidis	GT5180	Georgia Poultry Lab	Carcass rinse	Positive	Positive
S. Enteritidis	GT5183	Georgia Poultry Lab	Chicken	Positive	Positive
S. Enteritidis	GT5184	Georgia Poultry Lab	Drag swab	Positive	Positive
S. Enteritidis	GT5185	Michigan State Univ.[f]		Positive	Positive
S. Enteritidis	GT5186	Michigan State Univ.		Positive	Positive
S. Enteritidis	GT5187	Michigan State Univ.		Positive	Positive
S. Enteritidis	GT5188	Michigan State Univ.		Positive	Positive
S. Enteritidis	GT5189	Michigan State Univ.		Positive	Positive
S. Enteritidis	GT5190	Michigan State Univ.		Positive	Positive
Salmonella Berta	GT2884	CDC		Positive	Positive
Salmonella Dublin	GT2584	CDC		Positive	Positive
Salmonella Pullorum	GT2885	CDC		Positive	Positive
Salmonella Israel	Neogen 497	Neogen		Positive	Positive

Salmonella Gallinarum	Neogen 429	ATCC[g]		Negative	Negative
Salmonella Panama	Neogen 447	Neogen		Positive	Positive
Salmonella Lomalinda	Neogen 539	Neogen		Positive	Positive
Salmonella Javiana	GT2589	CDC		Positive	Positive
Salmonella Typhi	GT2125	ATCC 6539		Positive	Positive
Salmonella Eastbourne	GT2881	CDC		Positive	Positive
Salmonella Miami	GT620	Univ. Massachusetts[h]		Positive	Positive
Salmonella Blegdam	GT2582	CDC		Positive	Positive
Salmonella Moscow	GT2591	CDC		Positive	Positive
Salmonella Sendai	GT2911	CDC		Positive	Positive
Salmonella Rostock	GT2616	CDC		Positive	Positive
Salmonella Antarctica	GT3195	CDC		Positive	Positive
Salmonella Pensacola	GT2615	CDC		Positive	Positive
Salmonella Napoli	GT2592	CDC		Positive	Positive

[a] U. S. Department of Agriculture, Athens, GA.
[b] U. S. Food and Drug Administration, College Park, MD.
[c] Centers for Disease Control and Prevention, Atlanta, GA.
[d] Iowa State University, Ames, IA.
[e] Georgia Poultry Lab, Tifton, GA.
[f] Michigan State University, East Lansing, MI.
[g] American Type Culture Collection, Manassas, VA.
[h] University of Massachesetts, Amherst, MA.

Table 2. Results of exclusivity testing for the Reveal 2.0 Group D1 *Salmonella* test (1)

Organism	*Strain #*	*O Group*	*Source*	*Origin (If Known)*	*Result*
Salmonella Heidelberg	GT2304	B	CDC[a]		Negative
Salmonella Typhimurium	GT2373	B	ATCC[b] 13311	Mutton	Negative
Salmonella Montevideo	GT2483	C1	CDC		Negative
Salmonella Infantis	A144	C1	ATCC 51741	Pasta	Negative
Salmonella Mbandaka	GT2479	C1	CDC		Negative
Salmonella Kentucky	GT2581	C2-C3	CDC		Negative
Salmonella Fresno	GT2620	D2	CDC		Negative
Salmonella Gateshead	GT2621	D2	CDC		Negative
Salmonella Strasbourg	GT2622	D2	CDC		Negative
Citrobacter amalonaticus	GT1485		ATCC 25405	Feces	Negative
Citrobacter diversus	GT1475		ATCC 27156		Negative
Citrobacter freundii	GT1477		ATCC33128		Negative
Citrobacter youngae	GT1476		ATCC 29935	Meat	Negative
Cronobacter sakazakii	GT1483		ATCC 29544	Human	Negative
Edwardsiella hoshinae	GT1710		ATCC 33379	Bird	Negative
Edwardsiella tarda	GT569		ATCC 15947	Feces	Negative
Enterobacter aerogenes	GT1487		ATCC 29940	Human	Negative
Enterobacter amnigenus	GT1482		ATCC 33072	Soil	Negative
Enterobacter cloacae	GT1481		ATCC 29941		Negative
Enterobacter intermedia	GT1480		ATCC 33110		Negative
Escherichia blattae	GT1460		CDC		Negative

Escherichia coli	GT1214		ATCC 12038		Negative
Escherichia fergusonii	GT1459		ATCC 35473	Feces	Negative
Escherichia hermannii	GT1216		ATCC 33650	Human	Negative
Hafnia alvei	GT241		ATCC 29927	Human	Negative
Klebsiella oxytoca	GT1503		ATCC 13182	Human	Negative
Klebsiella planticola	GT1478		ATCC 33531	Radish	Negative
Klebsiella pneumoniae subsp. *ozaenae*	GT1499		ATCC 11296		Negative
Kluyvera ascorbata	GT3600		ATCC 33433	Human	Negative
Morganella morganii	GT303		ATCC 25830	Human	Negative
Pantoea agglomerans	GT1467		ATCC 29917		Negative
Pasteurella multocida	GT358		ATCC 19427		Negative
Proteus mirabilis	GT1493		ATCC 25933	Human	Negative
Proteus myxofaciens	GT366		ATCC 19692		Negative
Proteus penneri	GT367		ATCC 33519		Negative
Proteus vulgaris	GT368		ATCC 13315		Negative
Providencia alcalifaciens	GT371		ATCC 9886	Feces	Negative
Providencia rettgeri	GT373		ATCC 29944		Negative
Providencia rustigiani	GT374		ATCC 33673		Negative
Pseudomonas aeruginosa	GT1909		ATCC 27853	Blood	Negative
Serratia marcescens	GT392		ATCC 29937	Human	Negative
Serratia rubidae	GT1713		ATCC 15338		Negative

[a] Centers for Disease Control and Prevention, Atlanta, GA.
[b] American Type Culture Collection, Manassas, VA.

Table 3. Results and probability of detection calculations for the Reveal 2.0 Group D1 *Salmonella* presumptive and confirmed assays (raw shell eggs, poultry feed, and chicken carcass rinse) (1)

Food type	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	Reveal presumptive result			Reveal confirmed result			$dPOD_{CP}$[f]	95% CI[g]
				X[c]	POD_{CP}[d]	95% CI	X	POD_{CC}[e]	95% CI		
Raw Shell Eggs	*S*. Enteritidis 207	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.45, 0.45
		0.94 (0.48, 1.7)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	-0.14, 0.14
		43 (9.8, 19)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.45, 0.45
Raw Shell Eggs[h]	*S*. Enteritidis ATCC13076	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.45, 0.45
		0.20 (0.11, 0.33)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0	-0.14, 0.14
		2.4 (1.0, 5.5)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.45, 0.45
Poultry Feed	*S*. Enteritidis GT5188	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.1 (0.23, 4.6)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		6.0 (1.7, 22)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Chicken Carcass Rinse	*S*. Enteritidis GT5180	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		2.8 (0.54, 13)	20	16	0.80	0.58, 0.92	15	0.75	0.53, 0.89	0.05	-0.21, 0.30
		7.2 (1.3, 30)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] X = Number of positive test portions.
[d] POD_{CP} = Candidate method presumptive positive outcomes.
[e] POD_{CC} = Candidate method presumptive positive outcomes confirmed positive.
[f] $dPOD_{CP}$ = Difference between the candidate method presumptive and candidate method confirmed POD values.
[g] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h] Trial performed by independent laboratory.

Table 4. Results and probability of detection calculations for the Reveal 2.0 Group D1 *Salmonella* confirmed and FDA/BAM or USDA/MLG reference culture methods (raw shell eggs, poultry feed, and chicken carcass rinse) (1)

Food type	Inoculum strain	Inoc. level (CFU/portion)[a]	N[b]	Reveal confirmed result			Reference method result[c]			dPOD$_C$[g]	95% CI[h]
				X[d]	POD$_C$[e]	95% CI	X	POD$_R$[f]	95% CI		
Raw Shell Eggs	S. Enteritidis 207	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.45, 0.45
		0.94 (0.48, 1.7)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	-0.14, 0.14
		43 (9.8, 19)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.45, 0.45
Raw Shell Eggs[i]	*S.* Enteritidis ATCC 13076	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.45, 0.45
		0.20 (0.11, 0.33)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0	-0.14, 0.14
		2.4 (1.0, 5.5)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.45, 0.45
Poultry Feed	*S.* Enteritidis GT5188	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		1.1 (0.23, 4.6)	20	15	0.75	0.53, 0.89	9	0.45	0.26, 0.66	0.30	0, 0.54
		6.0 (1.7, 22)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43
Chicken Carcass Rinse	*S.* Enteritidis GT5180	0	5	0	0	0, 0.43	0	0	0, 0.43	0	-0.43, 0.43
		2.8 (0.54, 13)	20	15	0.75	0.53, 0.89	15	0.75	0.53, 0.89	0	-0.26, 0.26
		7.2 (1.3, 30)	5	5	1	0.57, 1	5	1	0.57, 1	0	-0.43, 0.43

[a] Determined by most probable number analysis.
[b] N = Number of test portions.
[c] FDA/BAM method (3) for raw shell eggs and poultry feed, USDA/MLG method (4) for chicken carcass rinse.
[d] X = Number of positive test portions.
[e] POD$_C$ = Candidate method confirmed positive outcomes.
[f] POD$_R$ = Reference method confirmed positive outcomes.
[g] dPOD$_C$ = Difference between the candidate method and reference method POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i] Trial performed by independent laboratory.

REFERENCES CITED

1. Mozola, M., Biswas, P., Viator, R., Feldpausch, E., Fori, D., Li, L., Le, Q.N., Alles, S., and Rice, J., Evaluation of the Validation of the Reveal® 2.0 Group D1 *Salmonella* Test for Detection of *Salmonella* Enteritidis in Raw Shell Eggs and Poultry Associated Matrices, AOAC® *Performance Tested*^{SM} certification number 041602.
2. US FDA (2014) *Bacteriological Analytical Manual*, chapter 5 http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm
3. USDA-FSIS (2014) *Microbiology Laboratory Guidebook*, chapter 4.08 http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES