CERTIFICATION

AOAC Research Institute

Performance Tested Methods^{SM}

Certificate No.

011103

The AOAC Research Institute hereby certifies the method known as:

Reveal® 2.0 *E. coli* O157:H7 Test System

manufactured by

Neogen Corporation

620 Lesher Place

Lansing, Michigan 48912

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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

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METHOD NAME
Reveal® 2.0 *E. coli* O157:H7 Test System

CATALOG NUMBER
9734

INDEPENDENT LABORATORY
Richter International
1730 Atlas Street, Columbus, OH
Columbus, Ohio
USA

APPLICABILITY OF METHOD
Target organism – *Escherichia coli* serotypes O157:H7 and O157:NM.

Matrixes – Raw beef trim, raw ground beef (65g and 375g)

Performance claims – Sensitivity relative to the reference method: 88-275%; specificity 100%.

REFERENCE METHOD

USDA-FSIS (2008) *Microbiology Laboratory Guidebook* (2)

ORIGINAL CERTIFICATION DATE
January 01, 2011

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
1. **November 2018 Level 1**
2. **November 2019 Level 1**
3. **December 2023 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial changes.**
2. **Editorial changes.**
3. **Editorial changes.**

Under this AOAC *Performance Tested Methods*^{SM} License Number, 011103 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)
The Reveal *E. coli* 2.0 test is a lateral-flow format, immunodiagnostic test that facilitates rapid and accurate detection of *Escherichia coli* O157:H7 in raw beef samples. A portion (200 μL) of the final enrichment culture is treated with promoter reagent to enhance antigen availability and introduced to the Reveal *E. coli* 2.0 device. The sample is wicked through a reagent zone, which contains specific antibodies conjugated to colloidal gold particles. If *E. coli* O157:H7-specific antigens are present in the sample, they will bind to the colloidal gold conjugated antibodies. This antigen-antibody complex then leaves the reagent zone and travels through the nitrocellulose membrane, which contains a zone of anti-*E. coli* O157:H7 antibodies. The immune complex with colloidal gold conjugate is captured and aggregates in this zone, displaying a visible line. The remainder of the sample continues to migrate to the end of the membrane, where it will eventually be deposited into a waste pad. The reagent zone also contains a colloidal gold conjugate of a second antigen, which is also eluted by the sample. The colloidal gold-conjugated control indicator migrates through the membrane to the negative control capture zone (containing antibody to the second antigen), where it is captured and aggregated to form a visible line. Regardless of the presence of *E. coli* O157:H7 antigen, the control line will form in the control zone, ensuring that the test is working properly. Positive assay results must be confirmed by standard culture methods.

DISCUSSION OF THE VALIDATION STUDY (1)
Results of the internal and independent laboratory studies reported here show that the Reveal *E. coli* 2.0 method demonstrates comparable sensitivity to that of the USDA-FSIS reference culture method for detection of *E. coli* O157:H7 in raw beef trim and ground beef samples. In one trial, the Reveal method detected significantly more positives than the USDA-FSIS method after both 12 h and 20 h enrichment, despite the fact that 375-g test portions were analyzed by the Reveal method and 65-g test portions by the reference procedure. In all other trials, performance of the Reveal and reference methods was statistically equivalent. There were some instances where *E. coli* O157:H7 was isolated from test portions that produced negative Reveal results. All but one of these additional plating positives came from 375-g samples where the initial levels of target and competing bacteria are less favorable in comparison to smaller samples. In the internal trials, additional positives were also obtained from USDA-FSIS enrichments after IMS and plating to CHROMagar, compared to results obtained from plating to Rainbow agar alone as called for in the USDA-FSIS procedure. Analysts performing the internal and independent laboratory studies commented alike that presumptive *E. coli* O157:H7 isolates were more distinct and the background flora more greatly suppressed on CHROMagar as compared with Rainbow agar. Plating to CHROMagar following enrichment and IMS proved to be an extremely sensitive method, with both the Reveal and reference culture procedures demonstrating some false negative results in comparison.

There were no false positive results obtained in any portion of the method comparison study. This supports the exceptional specificity of the Reveal *E. coli* 2.0 method as initially demonstrated by the results of inclusivity and exclusivity testing. Aside from reactions with certain strains of *E. coli* O157:H38 and O157:H43, the test is specific for O157:H7 and O157:NM serotypes of *E. coli*.

The Reveal *E. coli* 2.0 method also offers maximum flexibility in laboratory workflow. A single enrichment medium is used and samples may be tested at any point after 12 h and up to 20 h of enrichment.

						Reveal Result	
Strain #	**ATCC #**	**Description**	**Notes**	**Source (if known)**	**Origin (if known)**	**1:100 dilution**	**1:1000 dilution**
A110	35150	*E. coli* O157:H7			human feces	Positive	Positive
A141	43888	*E. coli* O157:H7	SLT-1 and SLT-2 negative	CDC	human feces	Positive	Positive
A143	43890	*E. coli* O157:H7	SLT-1 only	CDC	human feces	Positive	Positive
A160	43895	*E. coli* O157:H7	EDL933 genome strain		hamburger	Positive	Positive
A142	43889	*E. coli* O157:H7	SLT-2 only	CDC	human feces	Positive	Positive
125		*E. coli* O157:H7		USDA		Positive	Positive
126		*E. coli* O157:H7		USDA		Positive	Negative
127		*E. coli* O157:H7		CDC	meat	Positive	Positive
128		*E. coli* O157:H7		USDA		Positive	Positive
130		*E. coli* O157:H7		CDC		Positive	Positive
133		*E. coli* O157:H7		CDC		Positive	Positive
134		*E. coli* O157:H7		CDC		Positive	Positive
136		*E. coli* O157:H7		USDA		Positive	Positive
137		*E. coli* O157:H7		CDC		Positive	Positive
138		*E. coli* O157:H7		USDA		Positive	Positive
139		*E. coli* O157:H7		USDA		Positive	Positive
140		*E. coli* O157:H7		USDA		Positive	Positive
141		*E. coli* O157:H7		CDC		Positive	Positive
GT5121		*E. coli* O157:H7		T. Whittam	human	Positive	Positive
GT5122		*E. coli* O157:H7		T. Whittam	human	Positive	Positive
GT5123		*E. coli* O157:H7	sorbitol positive	T. Whittam	human	Positive	Positive
GT5129		*E. coli* O157:H7		T. Whittam	human	Positive	Positive
GT5139		*E. coli* O157:H7	Sakai genome strain	T. Whittam		Positive	Positive
GT5140		*E. coli* O157:H7		T. Whittam	hamburger	Positive	Positive
GT5141		*E. coli* O157:H7		T. Whittam	human	Positive	Positive
GT4132		*E. coli* O157:H7		USDA	veal kidney	Positive	Positive
GT4133		*E. coli* O157:H7		USDA	veal kidney	Positive	Positive
GT4134		*E. coli* O157:H7		USDA	veal kidney	Positive	Positive
GT4139		*E. coli* O157:H7		USDA	veal kidney	Positive	Positive
GT4140		*E. coli* O157:H7		USDA	veal kidney	Positive	Positive
GT4135		*E. coli* O157:H7		GENE-TRAK Systems	beef brisket	Positive	Positive
GT632		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT633		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT634		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT635		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT636		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT637		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT638		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT639		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT641		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT642		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT643		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT644		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT645		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT646		*E. coli* O157:H7		Mass. State Lab		Positive	Positive
GT5120		*E. coli* O157:H-	EHEC	T. Whittam	human	Positive	Positive
GT5125		*E. coli* O157:NM	EHEC	T. Whittam	human	Positive	Positive
GT5130		*E. coli* O157:NM	EHEC	T. Whittam	human	Positive	Positive
GT5131		*E. coli* O157:NM	EHEC	T. Whittam	human	Positive[a]	Positive[a]
GT5137		*E. coli* O157:NM	EHEC	T. Whittam	human	Positive	Positive
GT5138		*E. coli* O157:NM	EHEC	T. Whittam	human	Positive	Positive

Table 1. Results of inclusivity testing for the Reveal *E. coli* 2.0 method (1)

[a] Poor growth, tested at 1:10 and 1:100 dilutions.

Table 2. Results of exclusivity testing for the Reveal *E. coli* 2.0 method (1)

Strain #	ATCC #	Organism	Description	Source (if known)	Origin (if known)	Reveal Result ~ 10^9 cfu/mL	~ 10^8 cfu/mL
GT1720	25922	*E. coli*		FDA	human	Negative	Negative
GT1740	15597	*E. coli*				Negative	Negative
GT1723	14948	*E. coli*				Negative	Negative
GT1721	8677	*E. coli*				Negative	Negative
GT4137		*E. coli* O157:H16		USDA	pork sausage	Negative	Negative
GT5126		*E. coli* O157:H16		T. Whittam	human	Negative	Negative
164		*E. coli* O157:H19		CDC		Negative	Negative
GT4138		*E. coli* O157:H38		USDA	ground beef	Positive	Positive
A164		*E. coli* O157:H38		Neogen Corp.		Positive	Positive
GT5127		*E. coli* O157:H42	UPEC	T. Whittam	human	Negative	Negative
GT4136		*E. coli* O157:H43		USDA	pork sausage	Positive	Positive
GT5124		*E. coli* O157:H43	ETEC	T. Whittam	swine	Negative	Negative
GT5128		*E. coli* O157:H45	UPEC	T. Whittam	human	Negative	Negative
GT5136		*E. coli* O157:H45	EPEC	T. Whittam	cattle	Negative	Negative
166		*E. coli* O157:H45		CDC		Negative	Negative
GT5133		*E. coli* O26:H11	EHEC	T. Whittam	human	Negative	Negative
GT5132		*E. coli* O55:H7	STEC	T. Whittam	human	Negative	Negative
GT4684	33780	*E. coli* O111:H-			human	Negative	Negative
GT4685	43887	*E. coli* O111:NM		CDC	human	Negative	Negative
GT5134		*E. coli* O111:H8	EHEC	T. Whittam	human	Negative	Negative
GT5135		*E. coli* O111:H8	EHEC	T. Whittam	human	Negative	Negative
GT5150		*E. coli* O45	EHEC	T. Whittam		Negative	Negative
GT5151		*E. coli* O103	EHEC	T. Whittam		Negative	Negative
GT5152		*E. coli* O121	EHEC	T. Whittam		Negative	Negative
GT5153		*E. coli* O145	EHEC	T. Whittam		Negative	Negative
GT1485	25405	*Citrobacter amalonaticus*			feces	Negative	Negative
GT1475	27126	*Citrobacter diversus*				Negative	Negative
GT1477	33128	*Citrobacter freundii*			urine	Negative	Negative
GT1476	29935	*Citrobacter youngae*			meat	Negative	Negative
GT1487	29940	*Enterobacter aerogenes*			human	Negative	Negative
GT1481	29941	*Enterobacter cloacae*				Negative	Negative
GT1216	33650	*Escherichia hermannii*			human	Negative	Negative
GT241	29927	*Hafnia alvei*			human	Negative	Negative
GT1503	13182	*Klebsiella oxytoca*			human	Negative	Negative
GT1500	13883	*Klebsiella pneumoniae*				Negative	Negative
GT4361	27155	*Pantoea agglomerans*			chicken liver	Negative	Negative
GT1493	25933	*Proteus mirabilis*			human	Negative	Negative
GT368	13315	*Proteus vulgaris*				Negative	Negative
GT371	9886	*Providencia alcalifaciens*			feces	Negative	Negative
GT1909	27853	*Pseudomonas aeruginosa*			blood	Negative	Negative
GT392	29937	*Serratia marcescens*			human	Negative	Negative

Table 3. Results of beef trim and ground beef testing with the Reveal *E. coli* 2.0 method at 12 hours (1)

Sample	Size	Strain	MPN/g	MPN/portion	No.Samples	Reveal Presumptive[a]	Reveal Confirmed[b]	USDA Presumptive[c]	USDA Confirmed[d]	Sensitivity (%)[e]	Specificity (%)[f]	$\chi^{2\,g}$
Beef trim	65 g	ATCC 43895	0.0074	0.48	20	11	11[h]	10	7[i]	157	-	1.58
				0.00	0.00	5	0	0	0	0	-	100	-
	375 g	ATCC 43895		0.48	20	11	11[j]			157	-	1.58
				0.00	5	0	0			-	100	-
Beef trim[m]	65 g	ATCC 43895	0.023	1.50	20			16	16			
				0.00	0.00	5			0	0	-	100	-
	375 g	ATCC 43895		1.50	20	15	15[k]			94	-	0.14
				0.00	5	0	0			-	100	-
Ground beef	65 g	ATCC 35150	0.0033	0.21	20	9	9	4	4[l]	225	-	2.78
				0.00	0.00	5	0	0	0	0	-	100	-
	375 g	ATCC 35150		0.21	20	11	11			275	-	5.10
				0.00	5	0	0			-	100	-

[a] Number of samples positive by Reveal assay not considering subsequent culture confirmation.
[b] Number of samples positive by Reveal assay and confirmed by plating to from enrichment cultures to Chromagar.
[c] Number of samples positive by lateral flow immunoassay screening test not considering subsequent culture confirmation.
[d] Number of samples positive by lateral flow immunoassay screening test and confirmed by plating from enrichment cultures to Rainbow agar.
[e] Sensitivity of the Reveal method relative to that of the USDA method (Reveal+ / USDA+).
[f] Specificity of the Reveal assay. Only calculated for uninoculated control samples.
[g] Chi square by Mantel-Haenszel formula [6]. $\chi^2 > 3.84$ indicates a significant difference at $\rho < 0.05$.
[h] There was 1 Reveal- negative, plating-positive sample.
[i] There were 6 additional positive samples by plating to CHROMagar from the reference method enrichment cultures.
[j] There were 4 lateral flow screen-negative, plating-positive samples.
[k] There were 4 Reveal-negative, plating-positive samples.
[l] There were 3 additional positive samples by plating to CHROMagar from the reference method enrichment cultures
[m] Trial performed by independent laboratory.

Table 4. Results of beef trim and ground beef testing with the Reveal *E. coli* 2.0 method at 20 hours (1)

Sample	Size	Strain	MPN/g	MPN/portion	No.Samples	Reveal Presumptive[a]	Reveal Confirmed[b]	USDA Presumptive[c]	USDA Confirmed[d]	Sensitivity (%)[e]	Specificity (%)[f]	$\chi^{2\,g}$
Beef trim	65 g	ATCC 43895	0.0074	0.48	20	11	11[h]	10	7[i]	157	-	1.58
			0.00	0.00	5	0	0	0	0	-	100	-
	375 g	ATCC 43895		0.48	20	12	12[j]			171	-	2.44
				0.00	5	0	0			-	100	-
Beef trim[m]	65 g	ATCC 43895	0.023	1.50	20			16	16			
				0.00	5			0	0			
	375 g	ATCC 43895		1.50	20	14	14[k]			88	-	0.52
				0.00	5	0	0			-	100	-
Ground beef	65 g	ATCC 35150	0.0033	0.21	20	9	9	4	4[l]	225	-	2.78
				0.00	5	0	0	0	0	-	100	-
	375 g	ATCC 35150		0.21	20	11	11			275	-	5.10
				0.00	5	0	0			-	100	-

[a] Number of samples positive by Reveal assay not considering subsequent culture confirmation.
[b] Number of samples positive by Reveal assay and confirmed by plating to from enrichment cultures to Chromagar.
[c] Number of samples positive by lateral flow immunoassay screening test not considering subsequent culture confirmation.
[d] Number of samples positive by lateral flow immunoassay screening test and confirmed by plating from enrichment cultures to Rainbow agar.
[e] Sensitivity of the Reveal method relative to that of the USDA method (Reveal+ / USDA+).
[f] Specificity of the Reveal assay. Only calculated for uninoculated control samples.
[g] Chi square by Mantel-Haenszel formula [6]. $\chi^2 > 3.84$ indicates a significant difference at $\rho < 0.05$.
[h] There was 1 Reveal- negative, plating-positive sample.
[i] There were 6 additional positive samples by plating to CHROMagar from the reference method enrichment cultures.
[j] There were 3 lateral flow screen-negative, plating-positive samples.
[k] There were 4 Reveal-negative, plating-positive samples.
[l] There were 3 additional positive samples by plating to CHROMagar from the reference method enrichment cultures
[m] Trial performed by independent laboratory.

REFERENCES CITED

1. Hoerner, R., Feldpausch, J., Gray, R.L., Curry, S., Lewis, P., Tolan, J., Goldy, T., Klein, F., Neiditch, B., Hosking, E., Norton, P., Rice, J., and Mozola, M., Evaluation of Reveal® *E. coli* 2.0 Method for Detection of *Escherichia coli* O157:H7 in Raw Beef, AOAC *Performance Tested Methods*[SM] certification number 011103.
2. USDA-FSIS (2008) *Microbiology Laboratory Guidebook*, www.fsis.usda.gov/PDF/MLG_5_04.pdf