FOOD BIOLOGICAL CONTAMINANTS

Validation of the Thermo Scientific SureTect *Escherichia coli* O157:H7 Real-Time PCR Assay for Raw Beef and Produce Matrixes

***Performance Tested Method*^SM 021501**

Abstract

The Thermo Scientific™ SureTect™ *Escherichia coli* O157:H7 Assay is a new real-time PCR assay which has been validated through the AOAC Research Institute (RI) *Performance Tested Methods*^SM program for raw beef and produce matrixes. This validation study specifically validated the assay with 375 g 1:4 and 1:5 ratios of raw ground beef and raw beef trim in comparison to the U.S. Department of Agriculture, Food Safety Inspection Service, *Microbiology Laboratory Guidebook* (USDS-FSIS/MLG) reference method and 25 g bagged spinach and fresh apple juice at a ratio of 1:10, in comparison to the reference method detailed in the International Organization for Standardization 16654:2001 reference method. For raw beef matrixes, the validation of both 1:4 and 1:5 allows user flexibility with the enrichment protocol, although which of these two ratios chosen by the laboratory should be based on specific test requirements. All matrixes were analyzed by Thermo Fisher Scientific, Microbiology Division, Vantaa, Finland, and Q Laboratories Inc, Cincinnati, Ohio, in the method developer study. Two of the matrixes (raw ground beef at both 1:4 and 1:5 ratios) and bagged spinach were additionally analyzed in the AOAC-RI controlled independent laboratory study, which was conducted by Marshfield Food Safety, Marshfield, Wisconsin. Using probability of detection statistical analysis, no significant difference was demonstrated by the SureTect kit in comparison to the USDA FSIS reference method for raw beef matrixes, or with the ISO reference method for matrixes of bagged spinach and apple juice. Inclusivity and exclusivity testing was conducted with 58 *E. coli* O157:H7 and 54 non-*E. coli* O157:H7 isolates, respectively, which demonstrated that the SureTect assay was able to detect all isolates of *E. coli* O157:H7 analyzed. In addition, all but one of the nontarget isolates were correctly interpreted as negative by the SureTect Software. The single isolate giving a positive result was an *E. coli* O157:NM isolate. Nonmotile isolates of *E. coli* O157 have been demonstrated to still contain the H7 gene; therefore, this result is not unexpected. Robustness testing was conducted to evaluate the performance of the SureTect assay with specific deviations to the assay protocol, which were outside the recommended parameters and which are open to variation. This study demonstrated that the SureTect assay gave reliable performance. A final study to verify the shelf life of the product, under accelerated conditions was also conducted.

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The method was independently tested, evaluated, and certified by the AOAC Research Institute as a *Performance Tested Method*^SM. *See* http://www.aoac.org/testkits/steps.html for information on certification.

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Scope of Method

(**a**) *Target organism.*—*Escherichia coli* O157:H7.

(**b**) *Matrixes.*—375 g raw ground beef (1:4 and 1:5 ratios),

Table 1. Inclusivity of the Thermo Scientific SureTect *Escherichia coli* O157:H7 assay

Organism	ID Number	Source	SureTect result	Organism	ID Number	Source	SureTect result
E. coli O157:H7	TW00116[a]	Clinical	Positive	*E. coli* O157:H7	QL2-705[a]	Beef trim	Positive
E. coli O157:H7	TW00975	Clinical	Positive	*E. coli* O157:H7	DEC3B[a]	Clinical	Positive
E. coli O157:H7	TW02302	Hamburger	Positive	*E. coli* O157:H7	DEC3C	Clinical	Positive
E. coli O157:H7	TW04863	Clinical	Positive	*E. coli* O157:H7	DEC3D	Clinical	Positive
E. coli O157:H7	TW05356	Clinical	Positive	*E. coli* O157:H7	QL2-370	Beef trim	Positive
E. coli O157:H7	TW07587	Clinical	Positive	*E. coli* O157:H7	DEC4A	Cow	Positive
E. coli O157:H7	QL2-710	Beef Trim	Positive	*E. coli* O157:H7	DEC4B	Clinical	Positive
E. coli O157:H7	QL2-207	Ground beef	Positive	*E. coli* O157:H7	ATCC®[b] BAA-460	Clinical	Positive
E. coli O157:H7	NCTC™[c] 13125	Clinical	Positive	*E. coli* O157:H7	DEC4D	Cow	Positive
E. coli O157:H7	NCTC 13126	Clinical	Positive	*E. coli* O157:H7	DEC4E	Clinical	Positive
E. coli O157:H7	NCTC13127	Clinical	Positive	*E. coli* O157:H7	ATCC 35150	Clinical	Positive
E. coli O157:H7	NCTC 13128	Clinical	Positive	*E. coli* O157:H7	QL2-202	Ground beef	Positive
E. coli O157:H7	QL164673	Ground beef	Positive	*E. coli* O157:H7	QL2-203	Ground beef	Positive
E. coli O157:H7	ATCC 43888	Clinical	Positive	*E. coli* O157:H7	ATCC 51659	Clinical	Positive
E. coli O157:H7	ATCC 43889	Clinical	Positive	*E. coli* O157:H7	QL2-205	Ground beef	Positive
E. coli O157:H7	ATCC 43890	Clinical	Positive	*E. coli* O157:H7	QL2-206	Ground beef	Positive
E. coli O157:H7	ATCC 43894	Clinical	Positive	*E. coli* O157:H7	NCTC 12900	Clinical	Positive
E. coli O157:H7	ATCC 43895	Raw hamburger	Positive	*E. coli* O157:H7	QL2-214	Beef trim	Positive
E. coli O157:H7	ATCC 51657	Clinical	Positive	*E. coli* O157: H7	DEC3E	Clinical	Positive
E. coli O157:H7	ATCC 51658	Clinical	Positive	*E. coli* O157:H7	QL2-701	Beef trim	Positive
E. coli O157:H7	QL2-204	Ground beef	Positive	*E. coli* O157: H7	QL2-704	Beef trim	Positive
E. coli O157:H7	ATCC 700531	Clinical	Positive	*E. coli* O157: H7	DEC3A	Clinical	Positive
E. coli O157:H7	ATCC 700599	Salami	Positive	*E. coli* O157: H7	QL2-706	Beef trim	Positive
E. coli O157:H7	ATCC 700927	Unknown	Positive	*E. coli* O157: H7	QL2-707	Beef trim	Positive
E. coli O157:H7	DEC4C	Buffalo	Positive	*E. coli* O157: H7	QL2-708	Beef trim	Positive
E. coli O157:H7	AT0070	Meat	Positive	*E. coli* O157: H7	AT0085	Jack in the Box	Positive
E. coli O157:H7	AT0076	Meat	Positive	*E. coli* O157: H7	AT0126	Food	Positive
E. coli O157:H7	AT0083	Salami	Positive	*E. coli* O157: H7	AT0130	Ground beef	Positive
E. coli O157:H7	AT0084	Apple cider	Positive	*E. coli* O157: H7	AT0248	Clinical	Positive

[a] TWO, QL, DEC, and AT strains – Q Laboratories Inc., culture collection, Cincinnati, OH.

[b] ATCC – American Type Culture Collection, Manassas, VA.

[c] NCTC – National Collection of Type Cultures, Public Health, London, UK.

375 g raw beef trim (1:4 and 1:5 ratios) and 25 g (1:10 ratio) bagged spinach and apple juice.

(**c**) *Performance claims.*—For raw ground beef and beef trim following enrichment for 9–24 h: Performance equivalent to the U.S. Department of Agriculture, Food Safety Inspection Service, *Microbiology Laboratory Guidebook* (USDA-FSIS/MLG 5.08) Detection, Isolation, and Identification of *E. coli* O157:H7 from Meat Products and Carcass and Environmental samples (1). For apple juice and spinach matrixes, following enrichment for 8–24 h: Performance equivalent to the International Organization for Standardization (ISO) horizontal method for the detection of *E. coli* O157, ISO 16654:2001 (2).

General Information

Escherichia coli O157:H7 is a Gram-negative bacillus, causing mild gastroenteritis to more often serious and life-threatening disease with sequel such as hemorrhagic colitis, hemolytic uremic syndrome and kidney failure. *E. coli* O157:H7 was first implicated as a human pathogen in the United States in the early 1980s, and since that time, this serotype of *E. coli* has spread globally to become one of the most common pathogenic bacteria in food microbiology after *Salmonella* and *Listeria monocytogenes*. This serotype commonly contaminates cattle and dairy herds, which act as a reservoir for infection and produce, where it is has most commonly been isolated from leafy greens and to a lesser extent fruit juices. Like other serotypes of *E. coli*, O157:H7 is a robust organism, often surviving acidic and other hostile conditions, facilitating its environmental

Table 2. Exclusivity of the Thermo Scientific SureTect *Escherichia coli* O157:H7 Assay

Organism	ID Number	Source	SureTect result	Organism	ID Number	Source	SureTect result
Alcaligenes faecalis	ATCC®[a] 8750™	Unknown	Negative	*E. coli* O145:H-	NCTC™[b] 10279	Clinical	Negative
Bacillus cereus	ATCC 11778	Unknown	Negative	*E. coli* O145	TW07596[c]	Clinical	Negative
Candida albicans	ATCC 10231	Clinical	Negative	*E. coli* O145	TW01664	Clinical	Negative
Citrobacter freundii	ATCC 8090	Unknown	Negative	*E. coli* O146:H21	NCTC 10677	Clinical	Negative
Edwardsiella tarda	ATCC 15947	Clinical	Negative	*E. coli* O26	TW04270	Clinical	Negative
Enterobacter aerogenes	ATCC 13048	Clinical	Negative	*E. coli* O26	TW07814	Clinical	Negative
Escherichia blattae	ATCC 29907	Cockroach	Negative	*E. coli* O26	DEC9F[c]	Clinical	Negative
E. coli O163	NCTC11021	Clinical	Negative	*E. coli* O26	TW00971	Clinical	Negative
E. coli O103	TW08101	Clinical	Negative	*E. coli* O26:H11	DEC10E	Cow	Negative
E. coli O103	TW07971	Clinical	Negative	*E. coli* O45	TW07947	Clinical	Negative
E. coli O103	NCTC 8196	Unknown	Negative	*E. coli* O45	TW14003[c]	Clinical	Negative
E. coli O103	TW07697	Clinical	Negative	*E. coli* O45	TW10121	Clinical	Negative
E. coli O103	TW11239	Clinical	Negative	*E. coli* O55	TW00588	Clinical	Negative
E. coli O111	TW07926	Clinical	Negative	*E. coli* O55:H6	DEC1A	Clinical	Negative
E. coli O111	DEC8D	Clinical	Negative	*E. coli* O91:H-	NCTC 9091	Clinical	Negative
E. coli O111:H12	DEC6A	Clinical	Negative	*E. coli* O157:NM	ATCC 700376	Clinical	Positive
E. coli O111:H8	DEC6C	Clinical	Negative	*E. coli* O157	Ad525[d]	Clinical	Negative
E. coli O113:K75:H21	NCTC 9113	Clinical	Negative	*E. coli* O157	Ad527	Clinical	Negative
E. coli O115	NCTC 10444	Calf	Negative	*E. coli* O157:H-	Ad535		Negative
E. coli O117:H4	NCTC 9117	Calf	Negative	*E. fergusonii*	ATCC 35469	Clinical	Negative
E. coli O118:H-	NCTC 9118	Calf	Negative	*E. hermannii*	ATCC 33650	Clinical	Negative
E. coli O121	TW07614	Clinical	Negative	*E. vulneris*	ATCC 29943	Clinical	Negative
E. coli O121	TW08023	Clinical	Negative	*Hafnia alvei*	ATCC 51815	Milk	Negative
E. coli O121:H10	NCTC 9121	Calf	Negative	*Klebsiella pneumoniae* subsp. *pneumoniae*	ATCC 4352	Cow's milk	Negative
E. coli O142	NCTC 10089	Clinical	Negative	*Microbacterium testaceum*	ATCC 15829	Rice paddy	Negative
E. coli O145	TW09356	Clinical	Negative	*Pseudomonas aeruginosa*	ATCC 9027	Clinical	Negative
E. coli O26	TW04284	Clinical	Negative	*Salmonella choleraesuis*	ATCC 10708	Unknown	Negative

[a] ATCC – American Type Culture Collection, Manassas, VA.

[b] NCTC – National Collection of Type Cultures, Public Health, London, UK.

[c] TWO, DEC, and TWI strains – Q Laboratories Inc., culture collection, Cincinnati, OH.

[d] Ad strains –ADRIA Développement culture collection, Quimper, France.

spread and survival in animal slurries, which are increasingly used as agricultural fertilizers and soil improvers, unfortunately potentially leading to the contamination of vegetables and fruits.

The largest source of human illness caused by *E. coli* O157:H7 is through the consumption of undercooked beef products, especially those originating from ground/minced beef (e.g., hamburgers) and "rare" steaks. Cross-contamination between uncooked beef products and poor hygiene standards in food preparation and storage areas have led to both regional and countrywide outbreaks of illness. In recent years, there has been an increased focus on testing for *E. coli* O157:H7 and preventing the transport of contaminated meat products from abattoirs and raw meat processing facilities before contaminated products arrive in consumers' shopping baskets. Within the United States, the first legal requirements put in place declared *E. coli* O157 as a food adulterant, mandating the testing of all raw beef for the pathogen.

The SureTect *Escherichia coli* O157:H7 assay is a rapid and accurate real-time PCR method designed to detect the presence of *E. coli* O157:H7 in raw beef and produce samples within a total time of 9.5 to 25.5 h, depending on the matrix and enrichment protocol.

Principle

The Thermo Scientific SureTect *Escherichia coli* O157:H7 PCR assay is a real-time test intended to be used in conjunction with the Thermo Scientific PikoReal™ Real-Time PCR Instrument and SureTect Software for the detection of *E. coli* O157:H7 in human foods.

The SureTect system has been developed to be a user-friendly method which greatly reduces hands-on time and assay steps that routinely impact end-user costs and reduce laboratory workflow. The assay is supplied as a kit containing all necessary

Table 3. Thermo Scientific SureTect *Escherichia coli* O157:H7 Assay presumptive PCR result versus confirmed result by latex test – POD analysis

				SureTect method presumptive result			SureTect method confirmation (latex[c])			dPOD CP,	
Matrix	Strain	MPN[a]/test portion	N[b]	X[d]	POD_{CP}[e]	95% CI	X	POD_{CC}[f]	95% CI	CC[g]	95% CI[h]
Raw ground beef 20% fat	*E. coli* O157:H7	NA[i]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 9 h	ATCC 35150	0.60 (0.33,1.00)	20	11	0.55	(0.34,0.74)	10	0.50	(0.30,0.70)	0.05	(–0.28, 0.28)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 24 h	ATCC 35150	0.60 (0.33,1.00)	20	10	0.50	(0.30,0.70)	10	0.50	(0.30,0.70)	0.00	(–0.28, 0.28)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 9 h	ATCC 35150	0.60 (0.33,1.00)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(–0.28, 0.28)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 24 h	ATCC 35150	0.60 (0.33,1.00)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(–0.28, 0.28)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
375 g, 1:4 ratio, 9 h	ATCC 43895	0.49 (0.25, 0.85)	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	–0.05	(–0.32, 0.23)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 24 h	ATCC 43895	0.49 (0.25, 0.85)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(–0.28, 0.28)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 9 h	ATCC 43895	0.49 (0.25, 0.85)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(–0.28, 0.28)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 24 h	ATCC 43895	0.49 (0.25, 0.85)	20	10	0.50	(0.30, 0.70)	9	0.45	(0.26, 0.66)	0.05	(–0.24, 0.33)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Bagged spinach	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 8 h	OCC 897	0.88 (0.02, 1.50)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(–0.28, 0.28)
		0.73 (0.38, 1.39)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Bagged spinach	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 24 h	OCC 897	0.88 (0.02, 1.50)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(–0.28, 0.28)
		0.73 (0.38, 1.39)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Pasteurized apple juice	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 8 h	OCC1983	2.56 (1.52, 4.29)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(–0.27, 0.27)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Pasteurized apple juice 2	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
5 g, 1:10 ratio, 24 h	OCC1983	2.56 (1.52, 4.29)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(–0.27, 0.27)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Bagged spinach[j]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 8 h	ATCC 700599™	0.28 (0.12, 0.75)	20	3	0.15	(0.05, 0.36)	3	0.15	(0.05, 0.36)	0.00	(–0.23, 0.23)
		0.58 (0.33, 1.6)	5	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(–0.43, 0.43)
Bagged spinach[j]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 24 h	ATCC 700599	0.28 (0.12, 0.75)	20	3	0.15	(0.05, 0.36)	3	0.15	(0.05, 0.36)	0.00	(–0.23, 0.23)
		0.58 (0.33, 1.6)	5	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(–0.43, 0.43)

Table 3. (*continued*)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect method presumptive result			SureTect method confirmation (latex[c])			dPOD CP,	
				X[d]	POD_{CP}[e]	95% CI	X	POD_{CC}[f]	95% CI	CC[g]	95% CI[h]
Raw ground beef 20% fat[i]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 24 h	ATCC 35150	1.1 (0.58, 2.3)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(–0.27, 0.27)
		3.0 (1.3, 7.0)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat[i]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 24 h	ATCC 35150	1.1 (0.58, 2.3)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(–0.27, 0.27)
		3.0 (1.3, 7.0)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)

[a] MPN = Most probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

[b] N = Number of test portions.

[c] Both latex kits (Oxoid *Escherichia coli* O157 latex kit and the Wellcolex *E. coli* O157:H7 kit) gave identical results when testing for the O157 antigen.

[d] X = Number of positive test portions.

[e] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of portions.

[f] POD_{CC} = Candidate confirmation method positive outcomes divided by the total number of portions.

[g] $dPOD_{CP}$ = Difference between the candidate presumptive result and the candidate method confirmed result 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] NA = Not applicable.

[j] Independent laboratory study.

reagents to conduct the sample lysis, including pre-filled Lysis Tubes and lyophilized PCR pellets, containing all necessary PCR reagents (target-specific primers, dye-labeled probes and PCR master mix components) to easily conduct PCR analysis. PCR probes are short oligonucleotides with a quencher molecule at one end that, when not bound to target DNA, greatly reduces fluorescence from the dye label at the opposite end of the probe molecule. The oligonucleotides target unique DNA sequences found in *E. coli* O157:H7, which if present are amplified, and as a result the increasing fluorescent signals generated are detected by the PikoReal Real-Time instrument and interpreted by the SureTect Software. In addition to detection of any target DNA, the SureTect *Escherichia coli* O157:H7 PCR assay pellets contain probe, primers, and DNA templates for an internal amplification control (IAC). During PCR cycling, the IAC template is amplified whether any DNA from *E. coli* O157:H7 is present or not. The probe used for the IAC uses a different colored fluorescent dye than that of the probes used within the assay to detect target DNA and so can be detected by the PikoReal Instrument through a separate dye channel. The result is that after a successful PCR run, the instrument will detect amplification of the IAC DNA sequence. In the absence of any target DNA being detected by the assay, the presence of the IAC amplification curve confirms that the PCR process occurred successfully.

The assays used in the SureTect System are based on Solaris™ qPCR technology. The PCR probes have a molecule called Minor Groove Binder attached to one end, which enhances the probe-template DNA bond and yields a better S/N by lowering the background fluorescence. Results from this assay system are achieved in 1 h and 20 min of loading the prepared sample into the PikoReal Instrument and are displayed on the attached personal computer screen as simple positive or negative symbols with PCR amplification plots that are easily accessible for review. All results interpreted by the SureTect Software can be stored, printed, or downloaded by the user.

Definitions

(**a**) *Probability of Detection (POD).*—POD is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration-dependent. Several POD measurements can be calculated: POD_R (reference method POD), POD_C (candidate method POD), POD_{CP} (candidate method presumptive result POD), and POD_{cc} (candidate method confirmation result POD).

(**b**) *Difference of Probabilities of Detection (dPOD).*—dPOD is the difference between any two POD values. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Materials and Methods

Test Kit Information

(**a**) *Kit name.*—Thermo Scientific SureTect *Escherichia coli* O157:H7 PCR Assay.

(**b**) *Cat. No.*—PT0400A.

(**c**) *Ordering information.*—(*1*) *USA.*—Remel Inc., part of Thermo Fisher Scientific, Santa Fe Dr, Lenexa, KS, 66215; Tel: (800) 255-6730.

(*2*) *Europe.*—Oxoid Ltd, part of Thermo Fisher Scientific, Wade Rd, Basingstoke, Hampshire, RG24 8PW, UK. Tel: +44 1256 841144.

(*3*) *Asia/Pacific*-China.—Thermo Fisher Scientific, Thermo Fisher Biochemicals (Beijing) Ltd, 3rd Fl, 28 Yuhua Rd, Area B, Tianzhu Airport Industrial Zone, Beijing 101312, China.

Table 4. Thermo Scientific SureTect *Escherichia coli* O157:H7 Assay confirmation result versus reference AOAC Official Method confirmation result – POD analysis

				SureTect method confirmation result (latex[c])			Reference confirmation result (CC2)				
Matrix	Strain	MPN[a]/test portion	N[b]	X[d]	POD_{CP}[e]	95% CI	X	POD_{CC2}[f]	95% CI	dPOD CP, CC2[g]	95% CI[h]
Raw ground beef 20% fat	*E. coli* O157:H7	NA[i]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 9 h	ATCC 35150	0.60 (0.33,1.00)	20	10	0.50	(0.30,0.70)	10	0.50	(0.30,0.70)	0.00	(–0.28, 0.28)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat	*E. coli* O157:H	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 24 h	ATCC 35150	0.60 (0.33,1.00)	20	10	0.50	(0.30,0.70)	10	0.50	(0.30,0.70)	0.00	(–0.28, 0.28)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 9 h	ATCC 35150™	0.60 (0.33,1.00)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(–0.28, 0.28)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 24 h	ATCC 35150	0.60 (0.33,1.00)	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(–0.28, 0.28)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 9 h	ATCC 43895	0.49 (0.25, 0.85)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(–0.28, 0.28)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 24 h	ATCC 43895	0.49 (0.25, 0.85)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(–0.28, 0.28)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 9 h	ATCC 43895	0.49 (0.25, 0.85)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(–0.28, 0.28)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 24 h	ATCC 43895	0.49 (0.25, 0.85)	20	9	0.45	(0.26, 0.66)	9	0.45	(0.26, 0.66)	0.00	(–0.28, 0.28)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Bagged spinach	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 8 h	OCC 897	0.88 (0.02, 1.50)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(–0.28, 0.28)
		0.73 (0.38, 1.39)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Bagged spinach	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 24 h	OCC 897	0.88 (0.02, 1.50)	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(–0.28, 0.28)
		0.73 (0.38, 1.39)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Pasteurized apple juice	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 8 h	OCC1983	2.56 (1.52, 4.29)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(–0.27, 0.27)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Pasteurized apple juice	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 24 h	OCC1983	2.56 (1.52, 4.29)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(–0.27, 0.27)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Bagged spinach[j]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 8 h	ATCC 700599	0.28 (0.12, 0.75)	20	3	0.15	(0.05, 0.36)	3	0.15	(0.05, 0.36)	0.00	(–0.23, 0.23)
		0.58 (0.33, 1.6)	5	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(–0.43, 0.43)
Bagged spinach[j]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 24 h	ATCC 700599	0.28 (0.12, 0.75)	20	3	0.15	(0.05, 0.36)	3	0.15	(0.05, 0.36)	0.00	(–0.23, 0.23)
		0.58 (0.33, 1.6)	5	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(–0.43, 0.43)

Table 4. (*continued*)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect method confirmation result (latex[c]) X[d]	POD_{CP}[e]	95% CI	Reference confirmation result (CC2) X	POD_{CC2}[f]	95% CI	dPOD CP, CC2[g]	95% CI[h]
Raw ground beef 20% fat[i]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 24 h	ATCC 35150	1.1 (0.58, 2.3)	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(–0.27, 0.27)
		3.0 (1.3, 7.0)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat[i]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 24 h	ATCC 35150	1.1 (0.58, 2.3)	20	6	0.30	(0.15, 0.52)	6	0.30	(0.15, 0.52)	0.00	(–0.27, 0.27)
		3.0 (1.3, 7.0)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)

[a] MPN = Most probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

[b] N = Number of test portions.

[c] Both latex kits (Oxoid *E. coli* O157 latex kit and the Wellcolex *E. coli* O157:H7 kit) gave identical results when testing for the O157 antigen.

[d] X = Number of positive test portions.

[e] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of portions.

[f] POD_{CC2} = Candidate confirmation method positive outcomes divided by the total number of portions.

[g] $dPOD_{CP,CC2}$ = Difference between the candidate method confirmation result and the reference method confirmation result 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] NA = Not applicable.

[j] Independent laboratory study.

(*4*) *Australia.*—Thermo Fisher Scientific Australia Pty Ltd, 20 Dalgleish St, Thebarton, Adelaide, South Australia, 5031.

Test Kit Components

(**a**) *SureTect Lysis Reagent 1.*—96 pre-filled, sealed tubes (in 12 × 8 format). Each tube contains 170 µL of Lysis Reagent.

(**b**) *Proteinase K.*—One tube containing 1.1 mL.

(**c**) *SureTect Escherichia coli O157:H7 PCR assay tubes.*—12 × 8 pre-filled, sealed strips. Each tube contains one SureTect *Escherichia coli* O157:H7 assay PCR pellet.

(**d**) *SureTect Lysis Tube caps.*—12 strips of 8 caps.

(**e**) *SureTect PCR Tube caps.*—12 strips of 8 caps.

Additional supplies and reagents

(**a**) *Modified Tryptone Soya Broth (mTSB).*—Oxoid Cat. No. CM0989, or equivalent.

(**b**) *Sorbitol MacConkey Agar supplemented with Cefixime and Tellurite (CT-SMAC).*—Ready prepared media available from Thermo Fisher Scientific-Microbiology. In United States, Remel Inc., Cat. No. R110241; in Europe, Oxoid Ltd, Cat. No. PO0702A, or equivalent.

(**c**) *Oxoid Escherichia coli O157 Latex Test kit.*— Thermo Fisher Scientific-Microbiology, Cat. No. DR0620M, a latex agglutination test kit for the confirmation of presumptive positive colonies directly from CT-SMAC Agar. The test confirms the presence of the O157 antigen.

(**d**) *Remel Wellcolex™ E. coli O157:H7 kit.*—Cat. No. R30959601, a latex agglutination test kit for the confirmation of presumptive positive colonies for the O157 antigen directly from CT-SMAC Agar and for the H7 antigen from either blood agar or TSB.

(**e**) *Dynabeads™ anti-E. coli O157 magnetic beads.*—Available from Life Technologies, part of Thermo Fisher Scientific 71003 or 71004.

Apparatus

Items (**a**) and (**c**) to (**i**) are available as a complete SureTect Starter Pack, which is product code PT0600.

(**a**) *Thermo Scientific PikoReal qPCR System.*—PT0600, available as part of the SureTect starter pack.

(**b**) *Personal Computer.*—Available from Thermo Fisher Scientific, Microbiology (as part of the SureTect starter pack PT0600).

(**c**) *Dry bed heating block.*—Two heaters required to perform the lysis protocol. PT0611 (115V model) or PT0620 (230V model), available from Thermo Fisher Scientific, Microbiology.

(**d**) *Heater block insert.*—PT0612, one Boekel Scientific block insert is required for each dry bed heater to hold lysis tubes. Available from Thermo Fisher Scientific, Microbiology.

(**e**) *Thermo Scientific SureTect Software.*—PT0500W, available from Thermo Fisher Scientific, Microbiology.

(**f**) *Thermo Scientific CapEase™ tool.*—For capping and decapping lysis tubes. PT0621, available from Thermo Fisher Scientific, Microbiology.

(**g**) *PCR Tube rack-for holding lysis and PCR tubes.*—Thermo Fisher Scientific, Microbiology.

(**h**) *Pipets for transferring reagents and samples.*—Two adjustable mechanical pipets covering 5–50 µL and 20–200 µL and one multichannel adjustable 5–50 µL pipet covering eight wells. All pipets should be calibrated to deliver the required volumes within 10%.

(**i**) *Suitable DNA free filtered pipet tips.*—PT0609, available from Thermo Fisher Scientific, Microbiology.

(**j**) *Incubators.*—For incubating enrichments at 41.5 ± 1°C and confirmation plates at 37 ± 1°C.

(**k**) *Stomacher®.*—Seward model 400 or equivalent for

Table 5. Thermo Scientific SureTect *Escherichia coli* O157:H7 Assay confirmed result versus reference method result – POD analysis

				SureTect method confirmed result (C)			Reference method (R)			dPOD C,	
Matrix	Strain	MPN[a]/test portion	N[b]	X[c]	POD_C[d]	95% CI	X	POD_R[e]	95% CI	R[f]	95% CI[g]
Raw ground beef 20% fat	*E. coli* O157:H7	NA[h]	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 9 h	ATCC 35150	0.60 (0.33,1.00)	20	10	0.50	(0.30,0.70)	8	0.40	(0.22, 0.61)	0.10	(–0.19, 0.37)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 24 h	ATCC 35150	0.60 (0.33,1.00)	20	10	0.50	(0.30,0.70)	8	0.40	(0.22,0.61)	0.10	(–0.19, 0.37)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 9 h	ATCC 35150	0.60 (0.33,1.00)	20	11	0.55	(0.34, 0.74)	8	0.40	(0.22, 0.61)	0.15	(–0.15, 0.41)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw ground beef 20% fat	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 24 h	ATCC 35150	0.60 (0.33,1.00)	20	11	0.55	(0.34, 0.74)	8	0.40	(0.22, 0.61)	0.15	(–0.15, 0.41)
		4.38 (1.72,11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 9 h	ATCC 43895	0.49 (0.25, 0.85)	20	6	0.30	(0.15, 0.52)	7	0.35	(0.18, 0.57)	–0.05	(–0.32, 0.23)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 24 h	ATCC 43895	0.49 (0.25, 0.85)	20	7	0.35	(0.18, 0.57)	7	0.35	(0.18, 0.57)	0.00	(–0.28, 0.28)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 9 h	ATCC 43895	0.49 (0.25, 0.85)	20	9	0.45	(0.26, 0.66)	7	0.35	(0.18, 0.57)	0.10	(–0.19, 0.37)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Raw beef trim	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g 1:5 ratio 24 h	ATCC 43895	0.49 (0.25, 0.85)	20	9	0.45	(0.26, 0.66)	7	0.35	(0.18, 0.57)	0.10	(–0.19, 0.37)
		4.38 (1.72, 11.15)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Bagged spinach	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 8 h	OCC 897	0.88 (0.02, 1.50)	20	10	0.50	(0.30, 0.70)	11	0.55	(0.34, 0.74)	–0.05	(–0.33, 0.24)
		0.73 (0.38, 1.39)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Bagged spinach	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 24 h	OCC 897	0.88 (0.02, 1.50)	20	10	0.50	(0.30, 0.70)	11	0.55	(0.34, 0.74)	–0.05	(–0.33, 0.24)
		0.73 (0.38, 1.39)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Pasteurized apple juice	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 8 h	OCC 1983	2.56 (1.52, 4.29)	20	14	0.70	(0.48, 0.85)	18	0.90	(0.70, 0.97)	–0.20	(–0.43, 0.05)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Pasteurized apple juice	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 24 h	OCC 1983	2.56 (1.52, 4.29)	20	14	0.70	(0.48, 0.85)	18	0.90	(0.70, 0.97)	–0.20	(–0.43, 0.05)
		4.37 (1.71, 11.19)	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(–0.43, 0.43)
Bagged spinach[i]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 8 h	ATCC 700599	0.28 (0.12, 0.75)	20	3	0.15	(0.05, 0.36)	5	0.25	(0.11, 0.47)	–0.05	(–0.29, 0.20)
		0.58 (0.33, 1.6)	5	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(–0.43, 0.43)
Bagged spinach[i]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
25 g, 1:10 ratio, 24 h	ATCC 700599	0.28 (0.12, 0.75)	20	3	0.15	(0.05, 0.36)	5	0.25	(0.11, 0.47)	–0.05	(–0.29, 0.20)
		0.58 (0.33, 1.6)	5	3	0.60	(0.23, 0.88)	3	0.60	(0.23, 0.88)	0.00	(–0.43, 0.43)

Table 5. (*continued*)

Matrix	Strain	MPN[a]/test portion	N[b]	SureTect method confirmed result (C) X[c]	POD_C[d]	95% CI	Reference method (R) X	POD_R[e]	95% CI	dPOD C, R[f]	95% CI[g]
Raw ground beef 20% fat[i]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:4 ratio, 24 h	ATCC 35150	1.1 (0.58, 2.3)	20	14	0.70	(0.48, 0.85)	8	0.40	(0.22, 0.61)	0.30	(–0.01, 0.54)
		3.0 (1.3, 7.0)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 1.00)	0.20	(–0.27, 0.62)
Raw ground beef 20% fat[i]	*E. coli* O157:H7	NA	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(–0.43, 0.43)
375 g, 1:5 ratio, 24 h	ATCC 35150	1.1 (0.58, 2.3)	20	6	0.30	(0.15, 0.52)	8	0.40	(0.22, 0.61)	–0.10	(–0.36, 0.18)
		3.0 (1.3, 7.0)	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 0.61)	0.20	(–0.27, 0.62)

[a] MPN = Most probable number is based on the POD of the reference method test portions using the Least Cost Formulations MPN calculator with 95% confidence interval.

[b] N = Number of test portions.

[c] X = Number of positive test portions.

[d] POD_C = Candidate method confirmed positive outcomes divided by the total number of portions.

[e] POD_R = Reference method positive outcomes divided by the total number of portions.

[f] $dPOD_{C,R}$ = Difference between the candidate confirmed positive result and the reference method confirmed result 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] NA = Not applicable.

[i] Independent laboratory study.

preparing food samples, except those containing hard particles such as bone, pepper corns, etc.

Media and Materials for the Reference Methods

(**a**) *Modified Tryptone Soya Agar (mTSA).*—Oxoid, CM0989 or equivalent.

(**b**) *Casein hydrolysate (acid).*—Oxoid, LP0041B.

(**c**) *Novobiocin supplement.*—Oxoid, SR0181E or equivalent.

(**d**) *CT-SMAC.*—Prepared media, Remel R110241 or equivalent.

(**e**) *Modified Rainbow® Agar.*—Biolog Inc., Hayward, CA.

(**f**) *Nutrient agar.*

(**g**) *TSA supplemented with 5% sheep blood.*

(**h**) *Dynal anti-E. coli O157 antibody-coated paramagnetic beads.*—Thermo Fisher Scientific, 71004.

(**i**) *Remel RIM E. coli O157:H7 Latex Test Kit.*—Remel, R24250.

(**j**) *Remel microID™,* AOAC Official Method **989.12**.—Remel, R38145.

Safety Precautions

Laboratory.—E. coli O157:H7 has a very low infective dose, in the region of 10–100 cells. All enrichments and cultures must be handled using the appropriate laboratory containment facility required according to national legislation.

Enrichment broths.—All hazardous microbiological waste, including enrichment broths, should be disposed of by sterilization according to local guidelines, even if it is shown that the sample is negative for *E. coli* O157:H7.

PCR kits.—It is important that following the complete or partial completion of a PCR run, that PCR tubes are not opened, because amplified DNA (amplicon) in the tubes could easily contaminate equipment and the surrounding laboratory environment, potentially leading to false results in future experiments. The reagents should not pose a safety problem when used as directed in the SureTect method instructions. Dispose of waste lysate and PCR tubes according to local guidelines.

Refer to the SureTect Real-Time PCR Instrument Manual for guidelines on cleaning equipment and handling possible amplicon contamination.

For disposal of uninoculated culture media or any of the reagents and materials included in the SureTect *Escherichia coli* O157:H7 assay and associated tests, refer to the manufacturer's material safety data sheets and apply appropriate local guidelines.

General Preparation

(**a**) Prepare all media and use all confirmation kits according to the manufacturer's instructions (3).

(**b**) Turn on lysis heating blocks and ensure that the block insert temperatures are at 37 and 95°C.

(**c**) Allow tubes of SureTect Lysis Reagent and PCR tubes to reach room temperature before use by placing on the laboratory bench about 10 min before they are required.

(**d**) When using a short enrichment protocol, it is essential to confirm that the enrichment broth being used has reached the required temperature (41.5°C) before adding food samples. The pre-warming step should be validated according to the heating efficiency of the laboratory incubator used. Ideally, fan-assisted incubators should be used to facilitate heating.

Table 6. Percentage activity of target 1 in stored SureTect *Escherichia coli* O157:H7 PCR tablets compared to time zero

Storage temp., °C	*E. coli* O157:H7 DNA: copies/reaction	3 months	6 months	9 months
+5	10^4	99%	98%	102%
	10^3	100%	97%	101%
+25	10^4	100%	99%	98%
	10^3	100%	97%	96%
+37	10^4	101%	99%	88%
	10^3	99%	94%	81%

SureTect Method

(**a**) For raw beef matrixes, weigh 375 g of the food sample to be tested into a Stomacher bag and add either a 1:4 ratio (1125 mL) or 1:5 ratio (1500 mL) of sterile pre-warmed (41.5°C) mTSB. For produce samples (bagged spinach and apple juice) add 25 g of the sample to be tested to a Stomacher bag and add 225 mL (1:10 ratio) of sterile pre-warmed (41.5°C) mTSB.

(**b**) Homogenize all samples thoroughly for 30 s to 1 min using a Stomacher set to 230 rpm, or by hand for samples containing hard particles, such as bone.

(**c**) Incubate raw beef trim and raw ground/minced beef samples for 9–24 h (depending on the total viable count (TVC) level. Samples suspected of having an aerobic TVC level exceeding 10^3 CFU/g should be incubated for 24 h, whereas samples having a TVC level of 10^3 or less can be incubated using the short enrichment protocol of 9 to 24 h at 41.5 ± 1°C, and apple juice and bagged spinach for 8 to 24 h at 41.5 ± 1°C. Ensure that prepared samples are not left at room temperature and that the delay between the end of the pre-warming step and incubation is not longer than 45 min.

(**d**) Remove the enriched sample from the incubator and mix the liquid in the Stomacher bag by hand for a few seconds. Allow any food particles to settle, then open the bag and remove 1 mL of the enrichment using a pipet with an extra-long filtered tip and dispense into a microfuge tube. Close the cap until ready to process the sample. If not processed immediately it is possible to store the aliquoted enrichments at 2–8°C for up to 72 h.

Sample Lysis

Care should be taken to ensure that incubation temperatures and times are closely adhered to during the sample lysis steps.

(**a**) Take the required number of SureTect Lysis Reagent 1 Tubes from the kit box and place into a suitable rack. Tap the rack of tubes onto the bench or flick your wrist while holding the tubes to remove any liquid from the cap area and to collect the reagent at the bottom of the tubes. Before starting analysis, it is important to ensure that the reagents are at room temperature.

(**b**) Add 10 µL of Proteinase K to each of the required Lysis Tubes. Avoid contamination by using a fresh filtered tip every time Proteinase K is withdrawn from the stock tube.

(**c**) Add 10 µL of the aliquoted enriched samples to the Lysis Tubes. Ensure when adding the sample that the pipet tip reaches the bottom of the Lysis Tube to facilitate complete mixing of the sample with the Lysis reagent. Repeat this step for each of test samples.

(**d**) Seal the Lysis Tubes with SureTect Lysis Caps using the CapEase tool and incubate in the heating block at 37 ± 1°C for 10 min. Immediately transfer the tubes to the 95 ± 1°C heating block and incubate for 5 min. Immediately remove the tubes from the heating block and allow to cool at ambient temperature for 2 min before starting the PCR set-up.

The prepared lysates can be stored at 2–8°C for a maximum of 24 h, allowing repeat analysis, if required. Lysates should not be stored for longer than 24 h. If storage of samples for longer than 24 h is required, an aliquot of the enrichment broth can be kept at 2–8°C for up to 72 h. Stored enrichments will need to be analyzed, starting from the lysis steps.

Analysis

PCR Set up:

(**a**) Create the plate setup for the PikoReal Instrument, according to instructions detailed in the SureTect Software Manual (4).

(**b**) Remove the required number of SureTect *Escherichia coli* O157:H7 assay PCR Tubes from the packaging and place into a suitable rack. One tube is required to analyze each sample. Tap the rack of PCR Tubes gently on the bench to ensure that any PCR pellets that may have become stuck inside the caps or toward the top of the tubes are dislodged and located at the bottom of the tubes. Allow the PCR Tubes to come to room temperature by leaving them on the bench for 5 min before opening.

(**c**) Open the Lysis Tubes using the CapEase tool and remove 20 µL of the lysate. Ensure the lysate is removed from the top half of the liquid, taking care not to disrupt the particles which settle at the bottom of the Lysis Tubes. If the particles become disturbed, leave the tube for 1–2 min to allow the particles to re-settle. Particular care should be taken to ensure that no particles are transferred into the PCR Tubes. The presence of these particles during the PCR process may inhibit PCR from occurring.

(**d**) Transfer the 20 µL of lysate into the opened PCR Tube to rehydrate the SureTect PCR pellet. The pipet tip should not touch the PCR pellet when adding the lysate.

(**e**) Seal the PCR Tubes by hand with the flat optical caps provided.

(**f**) Open the drawer of the PikoReal Instrument and load the PCR Tubes according to the plate setup created on the software. Close the drawer and start the run. Refer to the SureTect Software Manual for detailed directions.

Interpretation and Test Result Report

When the run is completed, remove the processed samples from the PikoReal Instrument and review the results on the computer screen. The SureTect software will automatically interpret where PCR amplification has taken place for the target analyte and a positive result, indicated by a red "+" symbol will be displayed on the plate layout. Where there has been no PCR amplification for the target analyte, a negative result, indicated by a green "–" symbol will be displayed. In all cases, it is possible to check that a PCR amplification run was successful by reviewing the IAC result which should be displayed as a positive result (red "+" symbol). A yellow "!" symbol means that the run has failed and should be repeated,

Table 7. Percentage activity of target 2 in stored SureTect *Escherichia coli* O157:H7 PCR tablets compared to time zero

	E. coli O157:H7			
Storage temp., °C	DNA: copies/reaction	3 months	6 months	9 months
+5	10^4	99%	97%	101%
	10^3	100%	97%	100%
+25	10^4	99%	99%	98%
	10^3	100%	98%	98%
+37	10^4	101%	101%	88%
	10^3	100%	99%	84%

starting from either the stored lysate or enrichment broth. The SureTect Software Manual has a help section to assist with result interpretation.

Validation Studies

The validation study was conducted according to the AOAC Research Institute *Performance Tested Methods*SM program and the *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces* (5). Method developer studies were conducted by Thermo Fisher Scientific, Vantaa, Finland, and Q Laboratories Inc., Cincinnati, Ohio, for the matrix studies. The Independent laboratory study was conducted by Marshfield Food Safety, Marshfield, Wisconsin, where a matrix study was undertaken on two of the claimed food matrixes.

Inclusivity Testing

A total of 58 different isolates of *E. coli* O157:H7 were analyzed with the SureTect *Escherichia coli* O157:H7 assay. All of the isolates were part of the Q Laboratories Inc., culture collection.

Isolates were removed from storage at –80°C and inoculated into mTSB, before incubation at 41.5°C for 18–24 h. Following incubation, cultures were diluted in phosphate buffered water (BPW) to a level of 10 to 100 times the LOD_{50} of the SureTect assay, which for the purposes of this study was estimated to be 10^4 CFU.

Diluted cultures were processed as detailed in the SureTect assay instructions for use (3) by adding 10 µL of each diluted broth culture to tubes of prepared Lysis Reagent 1.

All 58 of the *E. coli* O157:H7 isolates tested were detected by the SureTect *Escherichia coli* O157:H7 assay (Table 1).

Exclusivity Testing

A total of 54 different isolates of nontarget organisms, comprising the AOAC Research Institute (AOAC-RI) O157 exclusivity panel guide were analyzed with the SureTect *Escherichia coli* O157:H7 assay. Fifty-one of the isolates were part of the Q Laboratories Inc., culture collection and were prepared by inoculating isolates previously stored at –80°C into 10 mL sterile TSB. Cultures were incubated under appropriate conditions to produce optimal growth before being analyzed undiluted at the growth level achieved with the SureTect assay. A further three isolates were part of the ADRIA Développement culture collection and analyzed during the AFNOR Certification EN ISO 16140 validation study. These three isolates were prepared by inoculating cultures previously stored at –80°C into 10 mL Brain Heart Infusion (BHI) broth and incubating for 18–20 h at 37°C. Isolates were then diluted in BPW to enable the inoculation of approximately 10^5 CFU into 225 mL BPW. Enrichments were incubated for 24 h at 37°C before being analyzed with the SureTect assay.

With the exception of one isolate which was an *E. coli* O157:NM strain, the other 53 exclusivity isolates all gave negative results with the SureTect assay, demonstrating that the SureTect assay has excellent exclusivity. The positive result for the nonmotile *E. coli* O157 isolate was not unexpected since a number of studies (6–8) have previously demonstrated that some isolates of *E. coli* O157 fail to give serologically positive reactions for the H7 antigen, while still harboring the *fliC* gene which is often detected by molecular assays (7). Although outside the scope of this exclusivity study, Sowers et al. (6) previously demonstrated that *E. coli* O157 isolates initially classified as being nonmotile can be made to express the H7 antigen after multiple passages on nonselective semi-solid media. A further 3 H7 negative isolates of *E. coli* O157 were analyzed by ADRIA Développement during the AFNOR Certification ISO 16140 study, and all gave negative results with the SureTect assay (Table 2).

Food Matrix Testing

All food matrixes were obtained from local supermarkets and were pre-screened using either the Romer Labs™ RapidChek™ O157 lateral flow kit or the USDA FSIS reference method to ensure that samples were not naturally contaminated with *E. coli* O157:H7. During the method developer study, 325 g samples of raw beef and 25 g samples of produce were pre-screened, while in the AOAC-RI independent laboratory study, 25 g samples of raw beef were pre-screened for natural contamination with *E. coli* O157:H7.

Sample Preparation.—Due to the differences in enrichment broths between the reference methods and the SureTect method, this was an unpaired study. All samples were therefore analyzed using either the SureTect method (3), or depending on the matrix, the ISO reference method detailed in ISO 16654:2001 for produce or the USDA MLG 5.07 reference method for raw beef matrixes. All enrichments, without respect to presumptive result by the candidate method, were subject to culture confirmation according to an AOAC *Official Methods of Analysis* (OMA) confirmation method as part of the reference method confirmation procedure, as detailed in the approved study protocol. Where colonies typical for *E.coli* O157 were present, at least one colony was confirmed using biochemical (API® 20E, VITEK GN or microID kits) and serological (commercial latex agglutination tests for O157 and H7 antigens) tests.

In addition to these confirmation methods, all candidate method enrichments were also confirmed using the confirmation procedure detailed in the SureTect assay instructions for use, which consisted of an immunocapture step on 1 mL of the SureTect enrichment broth with anti-*E. coli* O157 magnetic beads and streaking 50 µL of the processed beads onto a plate of CT-SMAC Agar. If present, at least one presumptive positive

Table 8. Robustness study results: SureTect *Escherichia coli* O157:H7 Assay

Run No.	Sample volume to lysis, µL	Lysate volume to PCR tablet, µL	Lysis temp., °C	Positive *E. coli* O157:H7 samples	Positive non-*E. coli* O157:H7 samples
Normal protocol	10	20	37	3/10	0/5
1	8	18	35	4/10	0/5
2	8	18	39	2/10	0/5
3	8	22	35	7/10	0/5
4	8	22	39	3/10	0/5
5	12	18	35	7/10	0/5
6	12	18	39	7/10	0/5
7	12	22	35	4/10	0/5
8	12	22	39	4/10	0/5

colony (colorless to straw-colored colonies) was confirmed using the Oxoid *Escherichia coli* O157 latex test (to confirm the presence of the O157 antigen) or the Wellcolex *E. coli* O157:H7 latex kit to confirm the presence of both the O157 and H7 antigens. The Wellcolex kit required the subculture of presumptive positive colonies from CT-SMAC Agar onto blood agar.

Preparation of spiked food samples.—Due to the large amount of food analyzed in each of the food runs, individual packages of foods were aseptically combined in a sterile large Stomacher bag or other suitable sterile container and mixed to prepare a "master" sample. Master samples were subsequently divided into three test portions: a negative (unspiked) control and two additional bulk samples that were spiked with isolates of *E. coli* O157:H7 (grown from –80°C isolates) cultured for 18–24 h on nonselective media and inoculated into nonselective broth. Nonselective broths were incubated for 18–24 h before serially diluting in Maximum Recovery Diluent (MRD) to give contamination levels of approximately 0.2–2 CFU/25 g test portion for low spiked samples and approximately 5 CFU/25 g test portion in the high spiked samples. For low spiked samples, the aim was to achieve fractional positive test results, with one of the two methods (candidate or reference). Therefore, actual spiking levels were adjusted to meet the AOAC-RI validation requirements detailed in the study protocol.

Once prepared, both unspiked and spiked bulk food samples were stored at 2–8°C for 2–4 days before analysis to stress the inoculated organism within the food matrix.

An additional set of test portions was prepared from both the unspiked and spiked bulk portions of each matrix to allow the estimation of the spike levels by most probable number (MPN) analysis. For MPN analysis of produce samples during the method developer study, five test portions of high spiked food at 5, 25, and 100 g, and five test portions of 5 and 100 g, and the twenty 25 g test portions of the low spiked samples were prepared and analyzed using the reference method. For raw beef matrixes, analyzed during the method developer study, five test portions of 650 g, 20 portions of 325 g, and five portions of 130 g for both the high and low spiked samples were prepared and analyzed using the reference method. In the independent laboratory study, five test portions of 10 g, five portions of 25 g, and five portions of 50 g of both the low and high spiked samples were prepared and analyzed using the reference method. All results for the MPN analysis were calculated using the Least Cost Formulations MPN calculator program (9).

An additional sample of each unspiked matrix was also stored alongside the spiked bulk samples to run a TVC test.

Data analysis.—All test results were analyzed by POD statistical analysis to 95% confidence intervals. POD analysis of the study data was conducted as detailed in the AOAC INTERNATIONAL validation guidelines. All data from the POD analysis are detailed in Tables 3–5.

Results and Discussion

The results from the method developer and independent studies performed to validate the performance of the SureTect *Escherichia coli* O157:H7 assay with two key raw beef matrixes at both 1:4 and 1:5 ratios and representative foods for two produce matrixes are summarized in Tables 3–5. This study demonstrated that there were no statistical differences by POD statistical analysis in the performance of the SureTect method to those of the USDA-FSIS/MLG 5.08 method, for raw beef matrixes of ground beef and beef trim having a low aerobic TVC, or to the reference method detailed in ISO 16654:2001 for the representative produce matrixes of bagged spinach and apple juice with the enrichment protocols detailed. As detailed in Table 3, there was no statistical difference in the presumptive results obtained with the SureTect PCR assay and the corresponding results for the SureTect confirmation method for any of the matrixes analyzed in both the method developer and independent laboratory studies.

The SureTect confirmation method of immunomagnetic separation, followed by plating onto CT-SMAC Agar and confirmation with either the Oxoid *E. coli* O157 or Wellcolex *Escherichia coli* O157:H7 latex kits, was compared to several OMA approved confirmation tests, which formed the reference confirmation method (Table 4). The actual OMA method used during this study depended on the method utilized by each of the laboratories taking part in this study and consisted of either the Remel microID test, bioMerieux VITEK GN kit, or the bioMerieux API 20E biochemical micro gallery kit. POD statistical analysis at 95% confidence levels demonstrated that there was no difference in the SureTect or approved OMA confirmation tests for all the food matrixes analyzed during the validation study.

The results comparing the confirmed SureTect method result and (depending on the matrix) the ISO or USDA MLG reference method result (Table 5) showed that the SureTect methods analyzed were equivalent to those of the relevant reference

method, by POD statistical analysis at 95% confidence levels for all four of the matrixes analyzed during both the method developer and the independent laboratory studies.

The TVC of background flora present in the raw beef matrixes tested during the method developer study was 1.2×10^3 CFU/g for the beef trim and 2.0×10^3 for the raw ground beef. These TVC levels were far less than the TVC of the raw ground beef tested during the independent laboratory study, which was found to be $\geq 10^5$ CFU/g. Therefore, claims for the performance of the SureTect assay with samples having a TVC level of greater than 10^3 CFU/g cannot be made based on the results generated during the independent laboratory study.

The assay detected 100% of the *E. coli* O157:H7 inclusivity isolates tested, and with the exception of the *E. coli* O157:NM isolate, all exclusivity isolates were correctly interpreted as negative. As previously discussed within this paper, although the initial serological status of the nonmotile *E. coli* O157 isolate included in the exclusivity study was documented from previous analysis to be H7 negative, it is unclear without DNA sequencing to confirm that this isolate truly did not have the $fliC_{H7}$ gene within its genome. Previously published studies have shown that even though isolates appear to give serologically negative results with H7 antisera, the $fliC_{H7}$ gene is actually present but not expressed unless induced, which can often require multiple passages of the isolate on nonselective semi-solid agar media.

Lot-to-Lot Testing and Stability Studies

The lot-to-lot and real time stability test studies are currently on-going, and data were not available at the time this study took place. Therefore, accelerated stability testing was performed to assess the stability of the assay (Tables 6 and 7).

Previous studies (10, 11) have detailed the stability performance of the SureTect Lysis Reagent 1, which is common to all of the SureTect assays. These studies have demonstrated that according to the Arrhenius equation, under accelerated test conditions the reagent is stable for at least 728 days or 2 years. This data supports the one year shelf life claimed for the SureTect *Escherichia coli* O157:H7 assay.

Accelerated Stability Testing Method, SureTect *Escherichia coli* O157:H7 PCR tablets:

(**a**) PCR tablets from kits manufactured according to the manufacturing process were stored at test temperatures of 5, 25, and 37°C, in their original packaging.

(**b**) At each time point, stability of the PCR tablets was evaluated by performing three parallel PCR reactions with tablets maintained at each of the storage temperatures. After enriching minced beef (prepared by adding 25 g of minced beef to 225 mL of mTSB and incubating at 37°C) for 8 h, genomic DNA from an isolate of *E. coli* O157:H7 (Oxoid Culture Collection 1983, Oxoid Ltd/Thermo Fisher Scientific, Basingstoke, UK) was added to give concentrations of 10^2, 10^3, and 10^4 copies/reaction. Samples were then lysed according to the SureTect lysis protocol.

(**c**) A 20 µL aliquot of the prepared minced beef/genomic *E. coli* DNA lysate was added to PCR tubes containing stored PCR tablets, in triplicate.

(**d**) All samples for PCR analysis were then loaded into the PikoReal Instrument and analyzed using the SureTect software, and all Cq values were recorded.

At 37°C, based on the results for target 1, the SureTect *Escherichia coli* O157:H7 PCR assay tablets were shown to be stable under accelerated conditions for at least 3 months. The recommend storage temperature for the SureTect kit is 5°C. Therefore, using the Arrhenius equation (predicted stability = accelerated stability $\times 2^{\Delta T/10}$) as an indicator of product stability under increased temperature, the predicted stability of the kit is at least 827 days or 2.26 years. Since $Qn = 90 \times 2^{(37\text{-}5)/10)}$, when the Q_{10} rule of the Arrhenius equation is applied to the current accelerated stability testing data, the current shelf life of one year is supported.

Results and discussion.—The current accelerated stability data for the PCR tablets reinforces the shelf life that has currently been assigned to the SureTect *Escherichia coli* O157:H7 assay, bearing in mind that previous validation studies (10, 11) have demonstrated that the stability of the liquid lysis reagent under accelerated test conditions is equivalent to at least 2 years according to the stated storage instructions.

Robustness Testing

A series of three variables that could be altered by an end user and have the potential to impact the performance of the assay were evaluated. The variables evaluated were: (*1*) sample volume dispensed into the lysis tube (8 and 12 µL); (*2*) lysis temperature (35 and 39°C); and (*3*) lysate volume added to the PCR tablet (18 and 22 µL). These variables were chosen and reviewed by AOAC-RI and set out within the study protocol. PCR cycling parameters and result interpretation were not selected, since these parameters are controlled by the SureTect Software and are not able to be modified by a user.

Methodology

(**a**) Test isolates of *E. coli* O157:H7 TCC 913 and *Citrobacter freundii* TCC 1917 were prepared and inoculated into either mTSB for the target organism or BHI for the non-target organism and incubated for 8 h at 41.5°C.

(**b**) Following incubation, cultures of the target analyte were diluted in sterile MRD to achieve fractional positive results. Nontarget *E. coli* cultures were tested undiluted.

(**c**) Diluted samples were then processed according to the combinations of different robustness conditions, which are detailed along with the results in Table 8.

Results and discussion.—The results from the different combinations of robustness testing are detailed in Table 5.

All three of the variations to the standard SureTect protocol which were tested during the robustness study gave similar levels of fractional positive results, demonstrating that there was no effect on the performance on the assay from the standard protocol detailed in the product insert by either varying the lysis temperature, the sample, or lysate volumes.

Conclusions

The results in this validation study support the product claims that the SureTect *Escherichia coli* O157:H7 assay is an effective method for the detection of *E. coli* O157:H7 and is statistically comparable to the USDA FSIS reference method for raw ground and trimmed beef and the ISO reference method for spinach and apple juice at the validated enrichment times. The reduced

handling and pipetting steps and rapid lysis protocols of the SureTect protocol assist laboratory workflow and enable users to obtain faster results, compared to the traditional culture-based methods detailed in the reference methods.

References

(1) U.S. Department of Agriculture, Food Safety and Inspection Service, *Microbiology Laboratory Guidebook,* 5.08 (2014) *Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat products and Carcass and Environmental Sponges*. http://www.fsis.usda.gov/wps/wcm/connect/51507fdb-dded-47f7-862d-ad80c3ee1738/MLG-5.pdf?MOD=AJPERES

(2) *ISO 16654:2001: Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the Detection of Escherichia coli O157*, International Organization for Standardization, Brussels, Belgium

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(4) Thermo Scientific SureTect Software Manual. Version 1.1

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