Rapid Screening and Identification Method for STEC in Meat Samples

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Background

Shiga-toxin producing *Escherichia coli* (STEC or VTEC) are known to cause diarrhea, hemorrhagic colitis (HC), and the potentially fatal hemolytic uremic syndrome (HUS). STEC are most commonly transmitted through raw ground beef, raw or inadequately pasteurized milk, sprouts, and vegetables.

BIOTECON Diagnostics has developed a rapid 8-hour enrichment protocol for fresh raw beef and had it compared to the USDA reference method.

The **food**proof® STEC Screening LyoKit and the **food**proof® STEC Identification LyoKit are in accordance with ISO/TS 13136. The rapid 8-hour enrichment protocol for fresh raw beef samples received AOAC RI Performance Tested Methods℠ (PTM) Certification (No. 102004) in October 2020.

Following DNA extraction with the **food**proof® StarPrep Three Kit, the **food**proof® STEC Screening LyoKit detects *stx1*, *stx2*, and *eae*. Positive samples can be further analyzed with the **food**proof® STEC Identification LyoKit, which identifies the eight most important STEC serogroups O26, O45, O103, O104, O111, O121, O145, and O157 in one single PCR test using melting curve analysis.

Methods

In the matrix study, the **food**proof® STEC method was compared to the reference method "U.S. Department of Agriculture-Food Safety and Inspection Service MLG 5C.00". To each 375 g test portion 1,125 ml of modified tryptone soya broth (mTSB) (1:4) and to each 25 g sample 225 ml of mTSB (1:10) was added. Samples were incubated at 42.0 ± 1°C and analyzed following the **food**proof® STEC procedure after 12 h and 20-24 h incubation time for the 375 g samples and after 8 h and 20-24 h incubation time for the 25 g samples. Five different real-time PCR instruments were included in the study. In comparison to the isolation and confirmation procedure of the reference method the undiluted column eluate was plated onto modified rainbow agar (mRBA) and for confirmation a bacterial colony was taken from a selective agar (mRBA) and not from non-selective agar (SBA).

Probability of detection (POD) was calculated as the number of positive outcomes divided by the total number of trials. The POD was calculated for the candidate presumptive results (POD_{CP}), the confirmatory results (POD_{CC}), the difference in the candidate presumptive and confirmatory results ($dPOD_{CP}$), presumptive candidate results that confirmed positive for 375 g test portions (POD_C) and presumptive candidate results for 25 g test portions (POD_{CP}), the reference method results (POD_R), and the difference in the presumptive candidate results that confirmed positive (375 g) or the presumptive candidate results (25 g) and reference method results (dPO_{DC} / $dPOD_{CP}$).

For inclusivity, a total of 446 strains were analyzed. For exclusivity, 184 strains of closely related bacteria, non-STEC organisms and STEC strains of known serogroups other than the eight major STEC O groups were tested.

Variations in method parameters, e.g., sample volume for PCR or incubation time during extraction, were evaluated as part if the robustness study.

Results

Within the method comparison study, the **food**proof® STEC method demonstrated no significant differences between presumptive and confirmed results or between candidate and reference method results for 375 g test portions following 12 h and 20-24 h enrichment time and for 25 g test portions following 8 h and 20-24 h enrichment time. The POD analysis between the presumptive and the confirmed results of the **food**proof® STEC method (data not shown) as well as the POD analysis between the **food**proof® STEC method and the reference method (table 1) showed no significant differences at the 5% level.

All used PCR devices provided identical results.

In comparison to the isolation and confirmation procedure of the reference method, the alternative confirmation procedure provides increased sensitivity by plating the undiluted column eluate onto mRBA and improved specificity by confirming a bacterial colony from a selective agar (e.g., mRBA) rather than a non-selective agar (SBA) as in the reference method.

Inclusivity strains were correctly included, while exclusivity strains were excluded.

The **food**proof® STEC method withstands small variations in method parameters.

Conclusion

Compared to the USDA reference method, the **food**proof® STEC method was shown to be an effective alternative method for detection and confirmation of STEC in raw ground beef and raw beef trim. An advantage are the rapid enrichment protocols (8-20 h and 12-20 h), which have been validated for 25 g test portions and 375 g test portions, respectively. Furthermore, using the enrichment medium mTSB without the addition of any antibiotics saves costs. The alternative confirmation procedure provides increased sensitivity and improved specificity as well as a reduction in time to result and in material costs. Finally, the STEC screening and identification method with its open platform has been successfully tested on several cyclers (LightCycler® 480, LightCycler® 96, AriaMx, CFX96, ABI 7500 fast).

For the best convenience, safety, and sensitivity, the PCR reagents are lyophilized.

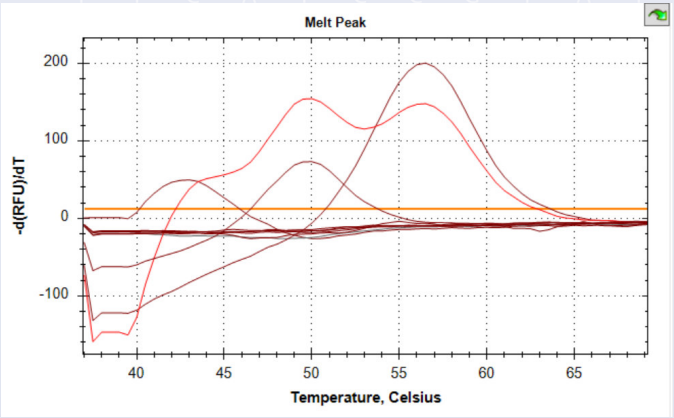

Figure 1: Example of Melting Curve Analysis
Three samples are shown, the control template (red line), and negative control (no melting curve peaks), analyzed with the **food**proof STEC Identification LyoKit on the CFX instrument. For the three samples, melting curve peaks can be seen in ROX channel at 42 - 47 °C (O157), 48 - 53 °C (O111) and 57 - 62 °C (O145).

Matrix	Strain	MPN[a] /test portion	N[b]	Incubation Time (h)	BIOTECON Diagnostics STEC presumptive			Reference method[e]			dPOD$_{CP}$[g]	95% CI[h]
					x[c]	POD$_{CP}$[d]	95% CI	x	POD$_R$[f]	95% CI		
Samples analyzed by BIOTECON Diagnostics GmbH												
Fresh Raw Ground Beef (25 g)	*E. coli* O157 JLU[j] No. 1433	N/A[i]	5	8	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				22 ± 2	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.13 (0.59, 2.16)	20	8	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
				22 ± 2	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	-0.13, 0.13
		3.46 (1.75, 6.83)	5	8	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
				22 ± 2	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Fresh Raw Beef Trim (25 g)	*E. coli* O26 JLU 413/89 - 1	N/A	5	8	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
				22 ± 2	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		1.83 (0.94, 3.55)	20	8	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
				22 ± 2	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.13, 0.13
		2.91 (1.54, 5.50)	5	8	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
				22 ± 2	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47
Samples analyzed by the independent laboratory												
Fresh Raw Beef Trim (25 g)	*E. coli* O26 ATCC[k] BAA-1653	N/A	5	8 and 22 ± 2	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.47, 0.47
		0.61 (0.33, 1.02)	20		9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0.00	-0.13, 0.13
		1.97 (0.91, 4.27)	5		5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.47, 0.47

Table 1: Example Data from the Method Comparison Study
All used PCR devices provided identical results. Samples analyzed by BIOTECON Diagnostics GmbH were tested on the LightCycler® 480 Instrument II, the LightCycler® 96 system, and the Agilent AriaMx real-time PCR system. Samples analyzed by the independent laboratory were tested on the ABI 7500 Fast and the CFX96 Touch real-time PCR system.
a) MPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
b) N = Number of test potions.
c) x = Number of positive test portions.
d) POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
e) Reference method = MLG 5C.00. Reference method portions were tested at 25 g.
f) POD_R = Reference method positive outcomes divided by the total number of trials.
g) $dPOD_{CP}$ = Difference between the candidate method presumptive POD values 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) N/A = Not applicable.
j) JLU = Justus Liebig University, Gießen, Germany.
k) ATCC = American Type Culture Collection, Manassas, VA.