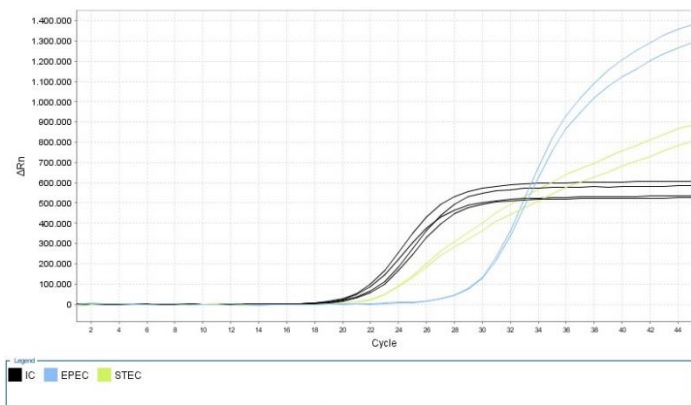
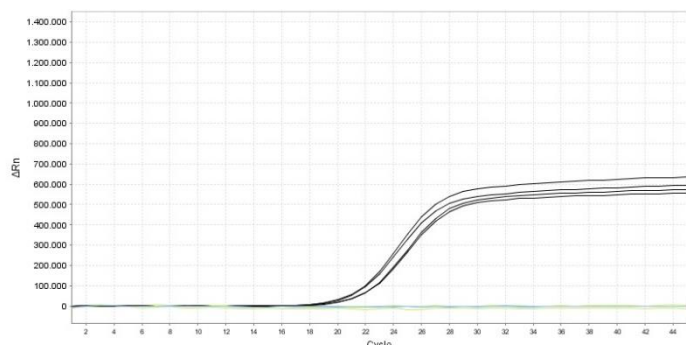


Positive Control –



Negative Control –



11. SPECIFICITY/INCLUSIVITY

- None of the 37 strains of closely related organisms or organisms occurring in the same habitat were detected (Table 1). No statistically significant differences were obtained between the performances of small variations in method parameters, which proves that the SUPREME Real Time Detection Kit *E. coli* is robust.
- For inclusivity, 66 *E. coli* strains containing *stx1* and/or *stx2* and *eae* genes (such as O157, O26, O103, O111, and O145) of 67 were correctly detected by the corresponding gene target (Table 2). The strains used were mainly from culture collections, proficiency tests and local isolates. Each strain was enriched in mTSB+N at 37 ± 1°C for 18–24 h per ISO/TS 13136:2012.

12. PERFORMANCE CHARACTERISTICS

A detection limit of 1 to 10 Cells per 25g of food sample can be achieved after enrichment. In the AOAC validation study, the SUPREME Real Time Detection Kit *E. coli* showed no statistical difference detected compared to the reference method (ISO/TS 13136:2012) for the foods tested, raw ground beef, orange juice, salad (green, purple lettuce and coriander) and cream cheese.

Note: In the context of the AOAC validated method, a positive result is considered presumptive positive and it is recommended to further confirm.

13. CONFIRMATION

Samples producing positive results for SUPREME Real Time Detection Kit *E. coli* can be confirmed according to the ISO/TS 13136: 2012 (or most current ISO reference procedure). EPEC/STEC strains can be isolated from the enrichment by streaking onto tryptone bile x-glucuronide agar as described in Annex F of ISO/TS 13136: 2012. Real-time PCR is conducted directly from isolated colonies using the primers and probes as described in Annex E of ISO/TS 13136:2012.

AOAC-RI VALIDATION

SUPREME Real Time Detection Kit *E. coli* has been validated by AOAC-Research Institute under the *Performance Tested Method*SM Program for detection of pathogenic *E. coli* associated with pathotypes EPEC, STEC and the sub-group EHEC associated with the combination of the virulence genes *stx1* and/or *stx2* and *eae* from raw ground beef, orange juice, salad (green, purple lettuce and coriander) and cream cheese. A positive result with SUPREME Real Time Detection Kit *E. coli* should be considered presumptive and it is recommended to confirm according to standard reference methods.

Certificate no. 081902.



SUPREME

REAL TIME DETECTION KIT

Escherichia coli

Detection of Enteropathogenic (EPEC) & Shiga-Toxin (STEC)

Ref: BIOPSFS-0002

1. PATHOGEN DESCRIPTION

Enteropathogenic *E. coli* (EPEC) was the first recognized pathogenic group, and presently, continues to be a leading cause of diarrhoea among infants from developing countries worldwide. The locus of enterocyte effacement (LEE) is necessary for the pathogenicity and intimin, one of the resultant products, is coded by the *eae* gene. The main pathogenic property of Shiga toxin-producing *E. coli* (STEC) strains is the production of Shiga toxins, coded by *stx* genes. Illnesses associated with STEC are usually watery diarrhoea and are particularly serious in children. Numerous outbreaks have been attributed to STEC strains of serotype O157:H7, but non-O157 serogroups, most commonly O26, O55, O103, O111, O117 O145, O146 and O191 have been shown to cause infections in the EU over recent years. The focus on *E. coli* O157 instead of other serotypes has been further enhanced by the ease of isolation of *E. coli* O157. In contrast, the other serotypes have been sub-diagnosed due to the lack of suitable methods to isolate those strains. EHEC were originally defined as a subset of STEC, that were associated with watery diarrhoea, haemorrhagic colitis, haemolytic-uremic syndrome, and that in addition to the *stx*-encoding genes, usually carry the attaching and effacing gene (*eae*; intimin-coding). EHEC strains are typically isolated from cases of severe disease but are poorly defined because there is no commonly accepted definition of EHEC.

2. INTENDED USE

SUPREME Real Time Detection Kit *E. coli* is a kit for the detection of pathogenic *E. coli* associated with the pathotypes EPEC, STEC and the subgroup EHEC associated with the combination of the virulence genes *stx1* and/or *stx2* and *eae*. Within Enterobacteriaceae, other species may also contain these virulence genes, therefore, the kit also detects species like *E. albertii*, *Shigella boydii* or *S. sonnei*, when target genes are present. The kit enables a qualitative detection of target genes in food samples by Real Time PCR, after a selective enrichment step, based on ISO/TS 13136:2012. The test may be used with the following matrixes: raw ground beef, orange juice, salad (green, purple lettuce and coriander) and cream cheese. The kit includes an Internal Control (IC). The IC is used for the evaluation of PCR inhibitors in the sample, or for the evaluation of problems that occurred during PCR preparation/amplification. The kit includes Master mix for the target and IC, primers and TaqMan® probes, labelled with non-fluorescent quenchers. The signal for the detection of the target is in the FAM channel, and the one for the IC is in the ROX channel. The kit contains Uracil-DNA Glycosylase (UDG), preventing DNA contamination with PCR products. Included in the kit is also a positive control, allowing an evaluation of the primers and probes used for the detection of the targets and a negative control (nuclease-free water) to confirm the integrity of the kit reagents. The kit was validated for use with the ABI PRISM® 7500 Fast. The kit is compatible with all thermocyclers working in **FAM** and **ROX** channels. The detection kit must not be used for diagnostic procedures. For Food use only.

The procedure includes the following main steps:



3. TRADEMARKS AND DISCLAIMER

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. BPMR kit handbooks and user manuals can be requested from BPMR or your local distributor.

4. LIMITED LICENSE AGREEMENT

Use of this product signifies the agreement of the following terms: The kit must be used solely in accordance with the respective Instructions for Use. BPMR grants no license under any of its intellectual property to use or

** High DNA concentration of the target in the sample can lead to a reduced or absent fluorescence signal of the IC.

Table 1: Exclusivity of the SUPREME Real Time Detection Kit *E. coli*

ID	Strain	SUPREME Kit Result	
		stx1/2	eae
NCTCa 11351	<i>Campylobacter jejuni subsp jejuni</i>	NEG	NEG
DSMb 20171	<i>Brochothrix thermosphacta</i>	NEG	NEG
DSM 756	<i>Clostridium perfringens</i>	NEG	NEG
CECTc 4022	<i>Lactobacillus paracasei subsp paracasei</i>	NEG	NEG
NCTC 11994	<i>Listeria monocytogenes</i>	NEG	NEG
NCTC 6571	<i>Staphylococcus aureus</i>	NEG	NEG
DSM 16636	<i>Citrobacter rodentium</i>	NEG	NEG
MB 198	<i>Citrobacter sp.</i>	NEG	NEG
MB 118	<i>Cronobacter sakazakii</i>	NEG	NEG
MB 490	<i>Enterobacter cloacae</i>	NEG	NEG
MB 20	<i>Klebsiella oxytoca</i>	NEG	NEG
MB 9	<i>Pseudomonas aeruginosa</i>	NEG	NEG
DSM 13772	<i>Salmonella bongori</i>	NEG	NEG
MB 164	<i>S. enterica subsp. enterica serovar Enteritidis</i>	NEG	NEG
NCTC 74	<i>S. enterica subsp. enterica serovar Typhimurium</i>	NEG	NEG
MB 168	<i>Shigella flexneri</i>	NEG	NEG
DSM 7532	<i>Shigella boydii</i>	NEG	NEG
DSM 5570	<i>Shigella sonnei</i>	NEG	NEG
534-1715343	<i>Shigella sonnei</i>	NEG	NEG
MB 249	<i>Yersinia enterocolitica</i>	NEG	NEG
MF 22	<i>Aspergillus flavus</i>	NEG	NEG
MF 101	<i>S. cerevisiae</i>	NEG	NEG
534-1715344	<i>Escherichia coli K12</i>	NEG	NEG
NCTC 9007	<i>Escherichia coli O7</i>	NEG	NEG
DSM 1103	<i>Escherichia coli O6</i>	NEG	NEG
MB 189	<i>Escherichia coli</i>	NEG	NEG
MB 190	<i>Escherichia coli</i>	NEG	NEG
MB 239	<i>Escherichia coli</i> - Hemolytic	NEG	NEG
MB 607	<i>Escherichia coli</i> - Hemolytic	NEG	NEG
MB 264	<i>Escherichia coli BLSE+</i>	NEG	NEG
NCTC 7464	<i>Bacillus cereus</i>	NEG	NEG
NCTC 11366	<i>Campylobacter coli</i>	NEG	NEG
NCTC 11352	<i>Campylobacter lari</i>	NEG	NEG
MB 282	<i>Legionella pneumophila</i>	NEG	NEG
MB 116	<i>Listeria innocua</i>	NEG	NEG
NCTC 11348	<i>Vibrio cholerae</i>	NEG	NEG
MB 135	<i>Vibrio parahaemolyticus</i>	NEG	NEG

Table 2: Inclusivity of the SUPREME Real Time Detection Kit *E. coli*

ID	Strain	stx1 gene	stx2 gene	eae gene	SUPREME Kit Result		Result comparison
					stx1/2	eae	
LMVa_E_2	<i>Escherichia coli O26</i>	+	+	+	POS	POS	Matched
LMV_E_3	<i>Escherichia coli O111</i>	+	+	+	POS	POS	Matched
LMV_E_4	<i>Escherichia coli O145</i>	+	-	+	POS	POS	Matched
LMV_E_5	<i>Escherichia coli O157</i>	+	+	+	POS	POS	Matched
LMV_E_6	<i>Escherichia coli O157</i>	+	-	+	POS	POS	Matched
LMV_E_7	<i>Escherichia coli O103</i>	-	+	+	POS	POS	Matched
MB E111	<i>Escherichia coli O103</i>	-	+	+	POS	POS	Matched
MB LM21	<i>Escherichia coli O157</i>	+	+	+	POS	POS	Matched
NTCCd 12079	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
CECTe 4267	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
CECT 4783	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
CECT 4782	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
CCCf-1-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-3-12	<i>Escherichia coli O26</i>	+	+	+	POS	POS	Matched
CCC-5-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-7-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-10-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-11-12	<i>Escherichia coli O157</i>	+	-	+	NEG	POS	Not Matched
CCC-12-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-13-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-14-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-15-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-16-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-18-12	<i>Escherichia coli O157</i>	-	+	+	POS	POS	Matched
CCC-20-12	<i>Escherichia coli O26</i>	+	-	+	POS	POS	Matched
CCC-21-12	<i>Escherichia coli O26</i>	+	-	+	POS	POS	Matched
CCC-22-12	<i>Escherichia coli O26</i>	+	-	+	POS	POS	Matched
00760EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
00960EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
01068EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
01076EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
01144EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
01320EO2A6H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
01511EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
01863EO1A6H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
02171EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
02172EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
02264EO1A6H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
02269EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
02270EO1A24H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
02306EO1A6H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
02309EO1A6H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
02450EO1A24H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
02871EO1A6H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
02922EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
02942EO1A6H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
03084EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
03085EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
03220EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
03222EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
03321EO1C6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
03322EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
03650EO1A6H	<i>Escherichia coli O157:H7</i>	+	+	+	POS	POS	Matched
03804EO1A6H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
03878EO1A6H	<i>Escherichia coli O157:H7</i>	-	+	+	POS	POS	Matched
MSUh TW14960	<i>Escherichia coli O111</i>	+	+	+	POS	POS	Matched
MSU DEC8D	<i>Escherichia coli O111</i>	+	+	+	POS	POS	Matched
PSUJ 5.0959	<i>Escherichia coli O111</i>	+	+	+	POS	POS	Matched
PSU 7.1686	<i>Escherichia coli O111</i>	+	+	+	POS	POS	Matched
PSU 7.1711	<i>Escherichia coli O145</i>	+	+	+	POS	POS	Matched
PSU 10.0707	<i>Escherichia coli O145</i>	+	+	+	POS	POS	Matched
MSU TW09153	<i>Escherichia coli O145</i>	+	+	+	POS	POS	Matched
MSU TW07596	<i>Escherichia coli O145</i>	+	+	+	POS	POS	Matched
MSU TW11239	<i>Escherichia coli O103</i>	+	+	+	POS	POS	Matched
MSU TW07697	<i>Escherichia coli O103</i>	+	+	+	POS	POS	Matched
PSU 5.1658	<i>Escherichia coli O103</i>	+	+	+	POS	POS	Matched
PSU 7.1691	<i>Escherichia coli O103</i>	+	+	+	POS	POS	Matched



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