



# Summary of Results

## Shiga toxin-producing *Escherichia coli* Scheme

### External Quality Assessment for Food Microbiology

Distribution Number: STX6  
Sample Numbers: STX011 & STX012

Distribution Date:	7 January 2019
Results Due:	1 February 2019
Report Date:	27 February 2019
Samples prepared and quality control tested by:	Thomas Harper Zak Prior
Test analysed by:	Nita Patel Zak Prior Manchari Rajkumar
Report compiled by:	Nita Patel Zak Prior Manchari Rajkumar
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The data in FEPTU reports is confidential

**Overview:**

This Scheme provides external quality assessment samples for laboratories that examine foods products for Shiga toxin-producing *Escherichia coli* in accordance with European legislation specified in Regulation (EC) 2073/2005 Microbiological Criteria for Foodstuffs associated with Regulation (EC) 852/2004 and subsequent amendments such as 209/2013 (microbiological criteria for sprouts and the sampling rules for poultry carcasses and fresh poultry meat).

This proficiency testing scheme challenges laboratories in detection of the major virulence genes associated with *Escherichia coli* serogroups O157, O111, O26, O103, O145 and O104:H4 (STEC). The scheme focuses on detection of *stx*-coding genes in *E. coli* cultures, for their identification as STEC. The determination of the presence of the intimin-coding gene *eae* is also included, since it is considered a hallmark of STEC strains pathogenic to humans.

The samples are prepared using killed STEC micro-organisms therefore the enrichment part of the test process is not included in the scheme design and cannot be assessed.

**FEPTU Quality Control:**

The samples were tested in a PHE reference laboratory prior to distribution. LENTICULE® discs selected randomly from a batch were examined using TaqMan™ real-time polymerase chain reaction (RT-PCR) method from Applied Biosystems™ RapidFinder™ STEC Screening Assay.

FEPTU used the following Bio-Rad kits to examine the samples:

iQ-Check™ STEC SerO (Real-time PCR detection of 7 major serogroups in Shiga Toxin Producing *E. coli*) and iQ-Check™ STEC VirX (Real-time PCR detection of virulence genes in Shiga Toxin Producing *E. coli*)

To demonstrate homogeneity of the sample for presence/absence of *stx* and *eae* genes, a minimum of 10 LENTICULE® discs, selected randomly from a batch, are tested in FEPTU.

To demonstrate stability of the sample for presence/absence of *stx* and *eae* genes, a minimum of nine LENTICULE discs, selected randomly from a batch, are examined throughout the distribution period in FEPTU.

The results letters provide guidance for participants regarding the intended result.

**Guidelines and general advise:**

If you experience difficulties with any of the examinations please refer to section 17.0 of the Scheme Guide <https://www.gov.uk/government/publications/food-and-water-proficiency-testing-schemes-scheme-guide>

All participants are reminded that reporting an incorrect or incomplete identification of pathogens from food samples could have serious public health implications.

**Please contact FEPTU staff for advice and information:**

<b>Repeat samples</b>	Carmen Gomes or Kermin Daruwalla	<b>Tel: +44 (0)20 8327 7119</b>
<b>Data analysis</b>	Zak Prior or Nita Patel	<b>Fax: +44 (0)20 8200 8264</b>
<b>Microbiological advice</b>	Zak Prior or Nita Patel	<b>E-mail: foodeqa@phe.gov.uk</b>
<b>General comments and complaints</b>	Zak Prior or Nita Patel	<b><a href="#">FEPTU's website</a></b>
<b>Scheme Consultant</b>	Charles Fuller	
<b>Scheme Advisors</b>	Marie Chattaway <sup>i</sup> & Frieda Jorgensen <sup>ii</sup>	
<b>Scheme Co-ordinator</b>	Nita Patel	

**Accreditation:** PHE Food EQA Scheme for Shiga toxin-producing *Escherichia coli* is accredited by the United Kingdom Accreditation Service (UKAS) to ISO/IEC 17043:2010.



A total of 28 participants were sent this distribution, of which 25 examined the samples, three did not return any results.

<sup>i</sup> Pathogen Lead for *Salmonella* Services, Gastrointestinal Bacteria Reference Unit, Public Health England

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Shiga toxin-producing *Escherichia coli* (STEC) have been identified as a worldwide cause of serious human gastrointestinal disease and the life-threatening haemolytic uraemic syndrome (HUS). The most common serotype implicated is *E. coli* O157:H7, but infections involving various non-O157 serotypes have been found with increasing frequency in many countries. Food-borne outbreaks caused by STEC can affect large numbers of people and cause serious morbidity, making the bacteria one of the most important emerging pathogens<sup>1</sup>.

As there is no specific treatment of the disease currently available<sup>2-3</sup>, there is an urgent need for effective preventive measures in identifying STEC contaminated foods before they reach the market and a detailed understanding of infectious epidemiology<sup>4-5</sup>. Such measures will also be dependent on the availability of rapid, sensitive, and simple procedures for the detection of the pathogens both in human samples and in samples of nonhuman origin such as food<sup>6</sup>.

#### Incidence in the European Union (EU):

There has been a statistically significant increase in the EU for STEC from 2008–2012, from approximately 3000 to 6000 reported cases<sup>7</sup>. This was probably due to the implementation of rapid techniques and increasing awareness of non-O157 STEC organisms in addition to strains of STEC O157 in testing laboratories. This trend spiked in 2011 due to a large outbreak.

On 21 May 2011, Germany reported an ongoing outbreak of STEC, serotype O157:H7. There were approximately 3842 cases of illness caused by the strain with 855 cases presenting HUS, and 53 deaths being reported to the European Centre for Disease and Control (ECDC). Consumption of sprouted fenugreek seeds was identified as the most likely origin<sup>8</sup>.

On 20 October 2011 the European Food Safety Authority (EFSA) adopted a scientific opinion that the contamination of dry seeds with bacterial pathogens, such as STEC, is the most likely initial source of sprout-associated outbreaks<sup>9</sup>.

#### Legislation:

Commission Regulation (EU) No 209/2013 amends Commission Regulation (EU) 2073/2005 on microbiological criteria for sprouts to include STEC detection. It stipulates that microbiological criteria should be considered for six sero-groups that are recognised as causing most cases of HUS: O157, O26, O111, O103, O145 and O104:H4.

The legislation refers to ISO/TS 13136:2012<sup>10</sup> as the analytical method that must be followed. In addition to the considerations of the six sero-groups, it advises that organisms that are potentially highly pathogenic to humans usually show the presence of the virulence factors; Shiga toxins genes (*stx1* and *stx2*) and intimin adhesin gene (*eae*).

#### References:

1. Karmali MA. Prospects for preventing serious systemic toxemic complications of Shiga toxin-producing *Escherichia coli* infections using Shiga toxin receptor analogues. *Journal of Infectious Diseases*. 2004 Feb 1;189 (3):355-9.
2. World Health Organization. Zoonotic non-O157 Shiga toxin-producing *Escherichia coli* (STEC). World Health Organisation; 1998.
3. Grisaru S. Management of hemolytic-uremic syndrome in children. *International journal of nephrology and renovascular disease*. 2014; 7:231.
4. Behravesh CB, Williams IT, Tauxe RV. Emerging foodborne pathogens and problems: expanding prevention efforts before slaughter or harvest. In: Institute of Medicine (US). *Improving Food Safety Through a One Health Approach: Workshop Summary*. Washington (DC): National Academies Press (US); 2012. A14. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK114501/>
5. World Health Organization. *Foodborne disease outbreaks: guidelines for investigation and control*. World Health Organization; 2008.
6. Karch H, Bielaszewska M, Bitzan M, Schmidt H. Epidemiology and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Diagnostic microbiology and infectious disease*. 1999 Jul 31; 34 (3):229-43.

<sup>10</sup> ISO/TS 13136:2012 Microbiology of food and animal feed -- Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens - Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

7. Bartels C, Beaute J, Fraser G, de Jong B, Urtaza JM, Nicols G. Annual epidemiological report 2014: food-and waterborne diseases and zoonoses. Stockholm: ECDC. 2014 Oct 10.
8. Muniesa M, Hammerl JA, Hertwig S, Appel B, Brüssow H. Shiga toxin-producing *Escherichia coli* O104: H4: a new challenge for microbiology. *Applied and environmental microbiology*. 2012 Jun 15;78(12):4065-73.
9. EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2011. Scientific Opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA Journal* 2011;9(11):2424, 101 pp. doi:10.2903/j.efsa.2011.2424

Example Report

**Sample: STX011****Sample type:** Simulated food**Request:** Examine sample for STEC**Contents:** *Escherichia coli* O157:H7; *stx 2*, *stx1/2* and *eae* positive ( $>1.0 \times 10^4$ ) (NCTC 12080)  
*Citrobacter braakii* ( $1 \times 10^4$ ) (wild strain), *Enterococcus faecalis* ( $1 \times 10^4$ ) (wild strain), and *Morganella morganii* ( $1 \times 10^4$ ) (wild strain)

All levels presented are colony forming units per mL

**A summary of the results returned by 25 laboratories is shown in the table below:**

Examination	Expected result	Total participants reporting	Total participants reporting correctly	Percentage of correct results
<b>stx 1</b>	Not detected	16	15	94
<b>stx 2</b>	Detected	16	16	100
<b>stx 1 and 2</b>	Detected	13	13	100
<b>eae</b>	Detected	23	23	100
<b>Serogroup</b>	<i>E. coli</i> O157 – detected	20	9	95
	<i>E. coli</i> O26 – not detected	11	11	100
	<i>E. coli</i> O103 – not detected	11	11	100
	<i>E. coli</i> O104 – not detected	5	5	100
	<i>E. coli</i> O111 – not detected	11	11	100
	<i>E. coli</i> O145 – not detected	11	11	100
	<b>Serotype</b>	H7 – detected	6	6

**Your results reported**

Examination	Expected result	Your result	PHE score	Z-score
<b>stx 1</b>	Not detected			
<b>stx 2</b>	Detected			
<b>stx 1 and 2</b>	Detected			
<b>eae</b>	Detected			
<b>Serogroup</b>	<i>E. coli</i> O157 – detected			
	<i>E. coli</i> O26 – not detected			
	<i>E. coli</i> O103 – not detected			
	<i>E. coli</i> O104 – not detected			
	<i>E. coli</i> O111 – not detected			
	<i>E. coli</i> O145 – not detected			
	<b>Serotype</b>	H7 – detected		

Interpretation of results for sample STX011 is shown on pages 20 - 22. The table also summarises any comments about the conclusion provided on the sample.

Five laboratories reported a not detected result for serogroup O45 and four for serogroup O121.

**Sample: STX012****Sample type:** Simulated food**Request:** Examine sample for STEC**Contents:** *Escherichia coli* O103:H2; *stx 1*, *stx 1/2* and *eae* positive ( $>1.0 \times 10^4$ ) (NCTC 13782)  
*Candida tropicalis* ( $1 \times 10^5$ ) (wild strain) and *Proteus mirabilis* ( $1 \times 10^5$ ) (wild strain)

All levels presented are colony forming units per mL

**A summary of the results returned by 25 laboratories is shown in the table below:**

Examination	Expected result	Total participants reporting	Total participants reporting correctly	Percentage of correct results
<b>stx 1</b>	Detected	16	10	63
<b>stx 2</b>	Not detected	16	16	100
<b>stx 1 and 2</b>	Detected	13	6	46
<b>eae</b>	Detected	23	12	52
<b>Serogroup</b>	<i>E. coli</i> O157 – not detected	18	18	100
	<i>E. coli</i> O26 – not detected	10	10	100
	<i>E. coli</i> O103 – detected	11	9	82
	<i>E. coli</i> O104 – not detected	7	7	100
	<i>E. coli</i> O111 – not detected	11	11	100
	<i>E. coli</i> O145 – not detected	12	*10	83
<b>Serology</b>	H2 - detected	-	-	-

\* Assay used by participant does not differentiate O103/O145

**Your results reported**

Examination	Expected result	Your result	PHE score	Z-score
<b>stx 1</b>	Detected			
<b>stx 2</b>	Not detected			
<b>stx 1 and 2</b>	Detected			
<b>eae</b>	Detected			
<b>Serogroup</b>	<i>E. coli</i> O157 – not detected			
	<i>E. coli</i> O26 – not detected			
	<i>E. coli</i> O103 – detected			
	<i>E. coli</i> O104 – not detected			
	<i>E. coli</i> O111 – not detected			
	<i>E. coli</i> O145 – not detected			
<b>Serology</b>	H2 - detected			

Interpretation of results for sample STX012 is shown on pages 20 – 22. The table also summarises any comments about the conclusion provided on the sample.

Five laboratories reported a not detected result for serogroup O45 and four for serogroup O121.

Several laboratories reported a not detected result for the *stx 1*, *stx 1/2* and *eae* genes. Further investigation suggests that this may be due to the level of organisms being too low and therefore being at the limit of detection for assays used. Laboratories reporting a not detected result are advised as part of their own internal investigation to read their run again at a different threshold – this sometimes changes the reading of a very late Ct value to a positive one.

Laboratories reporting a 'not detected' result for the genes have been excluded from scoring.

### **General comments on sample design**

Participants are informed that due to the safety classification of the STEC organisms the scheme design does not allow stages prior to the extraction process to be assessed. This is currently a limitation of the scheme design; the samples do not contain viable STEC organisms as the initial liquid broth culture has been inactivated using a low concentration of formalin. This allows samples to be handled in containment level 2 facilities whilst wearing the appropriate personal safety equipment.

This process of preparing the samples using formalin allows the micro-organisms to remain intact so that in principle the DNA extraction part of the process can be assessed with this proficiency testing scheme.

### **General comments on methods**

Participants should have a comprehensive understanding of the assays they use as well as an understanding of the limitations of assays. This should include knowing the impact on results obtained regarding volumes used from enrichment broth, DNA extraction, reagent ratios, cycle runs etc.

This scheme may not be suitable for rapid techniques other than those based on RT-PCR. Participants should contact the organisers to confirm suitability.

### **Scoring information**

The samples in this distribution have been scored using the following scoring criteria.

#### **Presence/absence results**

Participants' correct results for detection are allocated scores up to a maximum of two points as follows:

Fully correct result for the intended result	2
False positive / false negative	0

#### **Non-return of results**

Participants who do not return a result by the specified date are allocated a PHE score of zero for all tests.

### **General comments**

Participants are reminded that if you do not examine a specific parameter you must return your results as 'not examined'.

Participants should follow the instruction sheet and should contact the Organisers if clarification is required.

Summary of participants results STX011 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
	Detected	28.71	Not examined		Not examined		Detected	29.31	O157	29	O26 O103 O111 O157 O4 O21		H7	27.34	Bio-Rad iQ-Check® VirX lysis Reagent Bio-Rad, IQ-Check STEC SerO; Bio-Rad IQ-Check® STEC VirX Qiagen Rotor-Gene Q
	Detected	30.26	Not detected		Detected	30.17	Detected	28.7	O157	29.76	O26 O103 O104 O111 O145				Promega Maxwell® 16 Cell DNA purification kit Applied Biosystems™ RapidFinder™ STEC; Applied Biosystems™ Taqman™ custom assays Applied Biosystems® 7500 Fast Real-Time PCR System
	Not examined		Not detected		Detected	29.07	Detected	29.07							BIOTECON foodproof® StarPrep One Kit BIOTECON diagnostics Foodproof® STEC screening LyoKit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection Systems
			Not detected		Detected		Not examined								Roche Diagnostics MagNA Pure Compact Nucleic Acid Isolation Kit Roche Diagnostics TIB Molbiol Stx 1 and EHEC Roche Diagnostics LightCycler® 480
			Detected		Detected		Detected		O157						



Summary of participants results STX011 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
	Detected	29.9	Not detected		Detected	30.03	Detected	28.19	O157	29.71	O26 O103 O104 O111 O145				Promega Maxwell® 16 Cell DNA purification kit Applied Biosystems™ RapidFinder™ STEC Applied Biosystems® 7500 Fast Real-Time PCR System
	Detected	30.7	Not detected		Detected	32.5	Detected	30.9	O157	30.4	O26 O103 O104 O111 O145 O45 O121		H7	33.6	Pall Corporation Extraction Pack Food 1 Pall Corporation GeneDisc® Technologies - Pall GeneDisc E. coli O104 and Pall GeneDisc Top 7 STEC Pall Corporation GeneDisc® Cyclor
	Detected	31.49	Not Detected		Detected	30.73	Detected	30.25	O157	28.87					Promega Maxwell® 16 Cell DNA purification kit Applied Biosystems™ MicroSEQ™ E. coli O157:H7 Detection kit Applied Biosystems™ RapidFinder™ STEC Screening Kits Applied Biosystems™ STEC Serotype Kit Applied Biosystems® 7500 Fast Real-Time PCR System
			Not detected		Detected	27.9	Detected	27							ThermoFisher™ Primerdesign custom assay Applied Biosystems® StepOnePlus™ Real-Time PCR System

Summary of participants results STX011 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used	
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT		
	Detected	28.57	Not examined		Not examined		Detected	27.82	O157	26.59		O103 O111 O145			<p>CONGEN Biotechnologie GmbH SureFast® STEC Screening PLUS, SureFast STEC 4plex and SureFast <i>Escherichia coli</i> eae</p> <p>Roche Diagnostics LightCycler® 96 System</p>	
	Detected	33.28				Detected	35.37	O157	25.79	O26 O103 O111 O145 O45 O121		O104	H7		<p>Qiagen DNeasy Blood &amp; Tissue Kits</p> <p>Bio-Rad IQ-Check® STEC VirX</p> <p>Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection Systems</p>	
	Detected	38.2	Not detected		Detected	33.31	Detected	33.1	O157	32.95	O26 O103 O111 O145		O104	H7	32.21	<p>DuPont Qualicon BAX® System</p> <p>Applied Biosystems® QuantStudio™ 6 Flex Real-Time PCR System</p>
			Not detected		Detected	2.12	Detected	33.91	O157	34.42						<p>Promega, Maxwell® 16 Cell DNA purification kit</p>
	Detected	29,64				Detected	31,44	O157	29.53							<p>Bio-Rad iQ-Check® VirX lysis Reagent</p> <p>Bio-Rad, IQ-Check STEC SerO; Bio-Rad IQ-Check® STEC VirX</p>

Summary of participants results STX011 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
															Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected	27,99	Not examined		Not examined		Detected	30,49	O157	27,94		O26 O103 O104 O111 O145	H7	27,94	Bio-Rad iQ-Check® VirX lysis Reagent Bio-Rad IQ-Check® STEC VirX; Bio-Rad IQ-Check O157:H7 Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected	27.739	Not examined		Not examined		Detected	29.054	O157	21.274		O26 O103 O111 O145	H7	31.274	Bio-Rad IQ-check® VirX Lysis Reagent Bio-Rad, IQ-Check STEC SerO ; Bio-Rad IQ-Check® STEC VirX.
	Not examined		Not detected		Detected	28.52	Detected	28,5	O157	28,8		O26 O103 O111 O145	O104		Qiagen QIAamp DNA Mini Kit Roche Diagnostics LightCycler® 2.0
	Non-return of results														
	Non-return of results														
	Not examined		Not detected		Detected	27.66	Detected	28.11	O157			O26 O103 O104 O111 O145 O45			BIOTECON Foodproof® Starprep One Kit BIOTECON diagnostics Foodproof® STEC Screening and Identification Lyokits Roche Diagnostics LightCycler® 96 System

Summary of participants results STX011 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
	Detected	26					Detected	26	O157	27		O26 O103 O104 O111 O145			Applied Biosystems™ PrepSEQ™ Rapid Spin Sample Preparation Kit Applied Biosystems™ MicroSEQ™ E.coli O157:H7 Detection kit Agilent Technologies Mx3005P qPCR System
Non-return of results															
	Not applicable		Not detected		Detected	24.69	Detected	24.84							BIOCONTROL Assurance GDS® MPX for Top 7 STEC BIOCONTROL Assurance GDS® MPX Top 7 STEC BIOCONTROL Assurance GDS Rotor-Gene®
	Detected	36.8					Detected	35.1			O157 O26 O103 O111 O145 O121 O45	O104			bioMérieux VIDAS® UP E. coli Serogroups (ESPT) bioMérieux GENE-UP® EHEC Solution bioMérieux GENE-UP®
	Not examined		Not detected		Detected	21.83	Detected	19.72	O157	21.44		O26 O103 O104 O111 O145			BIOCONTROL Assurance GDS® MPX for Top 7 STEC BIOCONTROL Assurance GDS® MPX Top 7 STEC BIOCONTROL Assurance GDS Rotor-Gene®
	Detected	24.28					Detected	23.96	O157	28.77			H7	29.29	Applied Biosystems™ PrepSEQ™ Rapid Spin Sample Preparation Kit

Summary of participants results STX011 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
															Applied Biosystems™ MicroSEQ™ <i>E.coli</i> O157:H7 Detection kit ThermoFisher™ SureTect™ <i>E.coli</i> O157:H7 PCR Assay Applied Biosystems® 7500 Fast Real-Time PCR System
			Not detected		Detected		Detected		O157	27.43	O26 O103 O11 O145 O45 O121	O104			In-house Applied Biosystems® 7500 Fast Real-Time PCR System
	Not examined		Not detected		Detected	27,27	Detected	50.7	O157	30.07	O26 O103 O104 O111 O145				Qiagen, DNeasy Blood & Tissue Kits Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
									O157				H7		

Example Report

Summary of participants results STX012 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
	Detected	35	Not examined		Not examined		Detected	34	O103 O145		O26 O111 O45 O111	O103			Bio-Rad iQ-Check® VirX lysis Reagent Bio-Rad, IQ-Check STEC SerO; Bio-Rad IQ-Check® STEC VirX Qiagen Rotor-Gene Q
	Not detected		Not detected		Not detected		Not detected		O103	36.89	O117 O26 O104 O111 O145				Promega Maxwell® 16 Cell DNA purification kit Applied Biosystems™ RapidFinder™ STEC; Applied Biosystems™ Taqman™ custom assays Applied Biosystems® 7500 Fast Real-Time PCR System
	Not examined		detected	33.33	Not detected		Detected	32.88							BIOTECON foodproof® StarPrep One Kit BIOTECON diagnostics Foodproof® STEC screening LyoKit Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection Systems
			Detected	31.28	Not detected		Not examined								Roche Diagnostics MagNA Pure Compact Nucleic Acid Isolation Kit Roche Diagnostics TIB Molbiol Stx 1 and EHEC Roche Diagnostics LightCycler® 480
			Not detected		Not detected		Not detected				O157				
	Not detected		Not detected		Not detected		Detected	34.15				O157			Promega Maxwell® 16 Cell DNA purification kit

Summary of participants results STX012 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
												O26 O103 O104 O111 O145			Applied Biosystems™ RapidFinder™ STEC Applied Biosystems® 7500 Fast Real-Time PCR System
	Detected	38.1	Detected	36	Not detected		Detected	35.9	O103	39.1		O157 O2 O104 O111 O145 O45 O121	H7 – not detected		Pall Corporation Extraction Pack Food 1 Pall Corporation GeneDisc® Technologies - Pall GeneDisc E. coli O104 and Pall GeneDisc Top 7 STEC Pall Corporation GeneDisc® Cyclor
	Not detected		Not detected		Not detected		Not detected					O157 O26 O103 O104 O111 O145			Promega Maxwell® 16 Cell DNA purification kit Applied Biosystems™ MicroSEQ™ E. coli O157:H7 Detection kit Applied Biosystems™ RapidFinder™ STEC Screening Kits Applied Biosystems™ STEC Serotype Kit Applied Biosystems® 7500 Fast Real-Time PCR System
			Detected	34	Not detected		Detected	33.9							ThermoFisher™ Primerdesign custom assay Applied Biosystems® StepOnePlus™ Real-Time PCR System

Summary of participants results STX012 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
	Not detected	37,15	Not examined		Not examined		Not detected	>45			O157	O26 O103 O104 O111 O145			CONGEN Biotechnologie GmbH SureFast® STEC Screening PLUS, SureFast STEC 4plex and SureFast <i>Escherichia coli</i> eae Roche Diagnostics LightCycler® 96 System
	Not detected		Not examined		Not examined		Not detected				O157 O111 O145 O45 O121	O104			Qiagen DNeasy Blood & Tissue Kits Bio-Rad, IQ-Check STEC SerO ; Bio-Rad IQ-Check® STEC VirX Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection Systems
	Not detected		Not detected		Not detected		Not detected				O157 O26 O103 O104 O111 O145				DuPont Qualicon BAX® System Applied Biosystems® QuantStudio™ 6 Flex Real-Time PCR System
			Not detected		Not detected		Not detected				O157				Promega, Maxwell® 16 Cell DNA purification kit
	Detected	35,78					Detected	37,01							Bio-Rad iQ-Check® VirX lysis Reagent Bio-Rad, IQ-Check STEC SerO ; Bio-Rad IQ-Check® STEC VirX Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System



Summary of participants results STX012 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
	Detected	34,96	Not examined		Not examined		Detected	29,97			O157	O26 O103 O104 O111 O145			Bio-Rad iQ-Check® VirX lysis Reagent Bio-Rad IQ-Check® STEC VirX; Bio-Rad IQ-Check O157:H7 Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
	Detected	33.21 2	Not examined		Not examined		detected	34.042	O103 O145	31.591	O157 O103 O104 O111 O45 O121				Bio-Rad IQ-check® VirX Lysis Reagent Bio-Rad, IQ-Check STEC SerO ; Bio-Rad IQ-Check® STEC VirX.
	Not examined		Detected	24.58	Not detected		Detected	33,6	O103	33,1	O157 O26 O111 O145	O104			Qiagen QIAamp DNA Mini Kit Roche Diagnostics LightCycler® 2.0
	Non-return of results														
	Non-return of results														
	Not examined		Detected	31.95	Not detected		Not detected				O157 O26 O103 O104 O111 O145 O45				BIOTECON Foodproof® StarPrep One Kit BIOTECON diagnostics Foodproof® STEC Screening and Identification Lyokits Roche Diagnostics LightCycler® 96 System

Summary of participants results STX012 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
	Detected	32					Detected	30			O157	O103			Applied Biosystems™ PrepSEQ™ Rapid Spin Sample Preparation Kit Applied Biosystems™ MicroSEQ™ E. coli O157:H7 Detection kit Agilent Technologies Mx3005P qPCR System
	Non-return for result														
	Not examined		Detected	30.8	Not detected		Not detected								BIOCONTROL Assurance GDS® MPX for Top 7 STEC BIOCONTROL Assurance GDS® MPX Top 7 STEC BIOCONTROL Assurance GDS Rotor-Gene®
	Not detected						Not detected		O103		O157 O26 O111 O145 O45 O121	O104			bioMérieux VIDAS® UP E. coli Serogroups (ESPT) bioMérieux GENE-UP® EHEC Solution bioMérieux GENE-UP®
	Not examined		Detected	26.74	Not detected		Detected	25.49			O157	O26 O103 O104 O111 O145			BIOCONTROL Assurance GDS® MPX for Top 7 STEC BIOCONTROL Assurance GDS® MPX Top 7 STEC BIOCONTROL Assurance GDS Rotor-Gene®
	Detected	30.11					Detected	29.01	O103	31.1	O157				Applied Biosystems™ PrepSEQ™ Rapid Spin Sample Preparation Kit

Summary of participants results STX012 (incorrect results are shown in red)

Lab	stx 1/2		stx 1		stx 2		eae		Serogroup				Serotype		Extraction Assay Platform used
	Result	CT	Result	CT	Result	CT	Result	CT	Detected	CT	Not detected	Not examined	Result	CT	
											O26 O104 O111 O145				Applied Biosystems™ MicroSEQ™ <i>E.coli</i> O157:H7 Detection kit ThermoFisher™ SureTect™ <i>E.coli</i> O157:H7 PCR Assay Applied Biosystems® 7500 Fast Real-Time PCR System
			Detected		Not detected		Not detected		O103		O157 O26 O111 O145 O45 O121	O104			In-house Applied Biosystems® 7500 Fast Real-Time PCR System
	Not examined		Detected	33,11	Not detected		Detected	32,33	O103	31,26	O157 O26 O104 O111 O145				Qiagen, DNeasy Blood & Tissue Kits Bio-Rad CFX96 Touch™ Deep Well RT-PCR Detection System
									O103 O145						

Example Report

**Interpretation of results for sample STX011 and STX012 based on those shown in ISO/TS 13136:2012 and participant reported results**

Where more than one interpretation has been reported by the participant, the one highlighted in green is the interpretation that should be selected based on the results reported.

If a conclusion reported by participants is incorrect based on the results reported this is highlighted in red.

If a conclusion reported by participants should be a different based on the results reported this is shown in the column labeled 'Comments by FEPTU'.

Laboratory	Interpretation by laboratory for STX011	Comments by FEPTU (based on results obtained for STX011)	Interpretation by laboratory for STX012	Comments by FEPTU (based on results obtained for STX012)
	Presumptive detection of STEC in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	STEC not detected in the test portion of x g or x ml	
	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml		Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	
	Presumptive detection of STEC in the test portion of x g or x ml		Presumptive detection of STEC in the test portion of x g or x ml	
	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml		STEC not detected in the test portion of x g or x ml	
	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml		Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	
	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	STEC not detected in the test portion of x g or x ml	
	Presumptive detection of STEC in the test portion of x g or x ml	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	Presumptive detection of STEC in the test portion of x g or x ml	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml
	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	STEC not detected in the test portion of x g or x ml	
	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	STEC not detected in the test portion of x g or x ml	

Laboratory	Interpretation by laboratory for STX011	Comments by FEPTU (based on results obtained for STX011)	Interpretation by laboratory for STX012	Comments by FEPTU (based on results obtained for STX012)
	x g or x ml			
	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml		STEC not detected in the test portion of x g or x ml	
	Presumptive detection of STEC in Lenticule. The stx gene and the eae gene present in the serogroups O157, O111, O26, O103, O145 are detected	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	Presumptive detection of STEC of O157 serogroup in Lenticule. The stx gene is not detected however eae gene and rfbE gene are detected.	STEC not detected in the test portion of x g or x ml
	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml		Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml
	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml		Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	
	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml		Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml
	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml		Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	
	Non-return of results			
	Non-return of results			
	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml		STEC not detected in the test portion of x g or x ml	Presumptive detection of STEC in the test portion of x g or x ml
	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	
	Non-return of results			

Laboratory	Interpretation by laboratory for STX011	Comments by FEPTU (based on results obtained for STX011)	Interpretation by laboratory for STX012	Comments by FEPTU (based on results obtained for STX012)
	OTHER - Detected Interpretation guidance from PCR programme		OTHER - Not Detected Interpretation guidance from PCR programme	
	Not examined / Not applicable		Not examined / Not application	
	Presumptive detection of STEC in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	Presumptive detection of STEC in the test portion of x g or x ml	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml
	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml		Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	
	Presumptive detection of STEC in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	STEC not detected in the test portion of x g or x ml
	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml	Presumptive detection of STEC causing the attaching and effacing lesion in the test portion of x g or x ml	Presumptive detection of STEC of xx serogroup in the test portion of x g or x ml

Example Report

### Questionnaire results:

Please note that not all participants provided the relevant information. FEPTU are aware that processes are different and therefore have not attempted to categorise the information into specific groups such as automation versus manual etc.

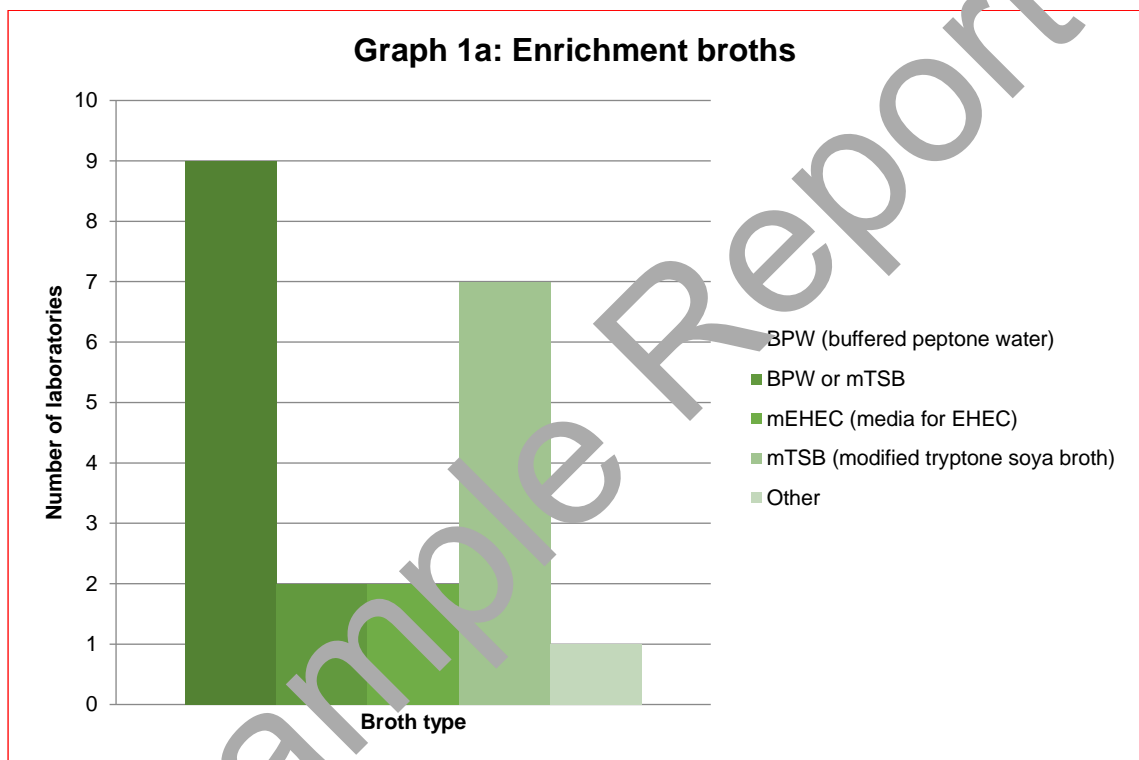
The data shown below is for information only. It does not evaluate or associate the data with a failure with PT to a method/process used nor does it attempt to compare performance of the various molecular kits/processes with each other.

#### 1. The use of ISO/TS 13136:2012<sup>ii</sup>

- 12/23 (52%) of participants stated they follow the recommended ISO method

#### 2. Enrichment process

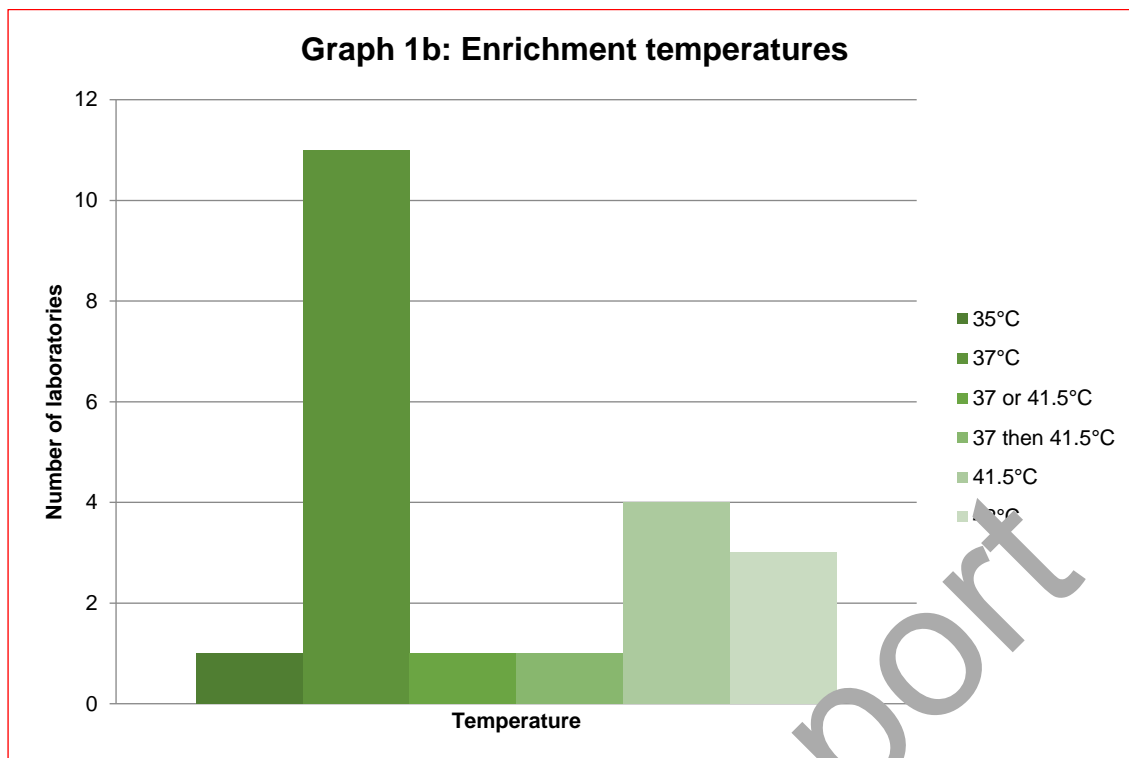
- The majority of participants would use Buffered Peptone Water (BPW) and/or modified Tryptone Soya Broth (mTSB) for enriching viable STEC organisms (Graph 1a)



The majority of participants would use 37° C to incubate their broths (Graph 1b). Participants that use higher temperatures should be aware that although 41 - 42 °C is preferable for selection, the exact temperature is critical as poor growth of O157 has been observed above 42 °C<sup>iii</sup>.

<sup>ii</sup> Microbiology of food and animal feed -- Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens - Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups

<sup>iii</sup> Raghubeer EV, Matches JR. Temperature range for growth of *Escherichia coli* serotype O157: H7 and selected coliforms in *E. coli* medium. Journal of clinical microbiology. 1990 Apr 1;28(4):803-5.



### 3. DNA extraction

- The majority of participants reported using a commercial extraction kit shown in table below (n=21).

Extraction assay used	Number of laboratories
Applied Biosystems™ PrepSEQ™ Rapid Spin Sample Preparation Kit	2
BIOCONTROL Assurance GDS® MPX for Top 7 STEC	2
bioMérieux VIDAS® UP <i>E. coli</i> Serogroups (ESPT)	1
Bio-Rad iQ-Check® VirX lysis kit	1
Bio-Rad IQ-check® VirX Lysis Reagent	3
BIOTECON foodproof® S. enteritidis One Kit	2
DuPont Qualicon BAX® System	1
Pall Corporation Extraction Pack Food 1	1
Promega Maxwell® 16 Cell DNA purification kit	4
Qiagen DNeasy Blood & Tissue Kits	2
Qiagen QIAamp DNA Mini Kit	1
Roche Diagnostics MagNA Pure Compact Nucleic Acid Isolation Kit	1



#### 4 Type of molecular test

- 19/23 (83%) reported using a RT-PCR
- 1/23 (4%) reported using both a RT-PCR and conventional
- 1/23 (4%) reported using a conventional PCR
- 1/23 (4%) reported using a PCR with IMS
- 1/23 (4%) reported using a RT-PCR with VIDAS® UP *E. coli* Serogroups (ESPT)

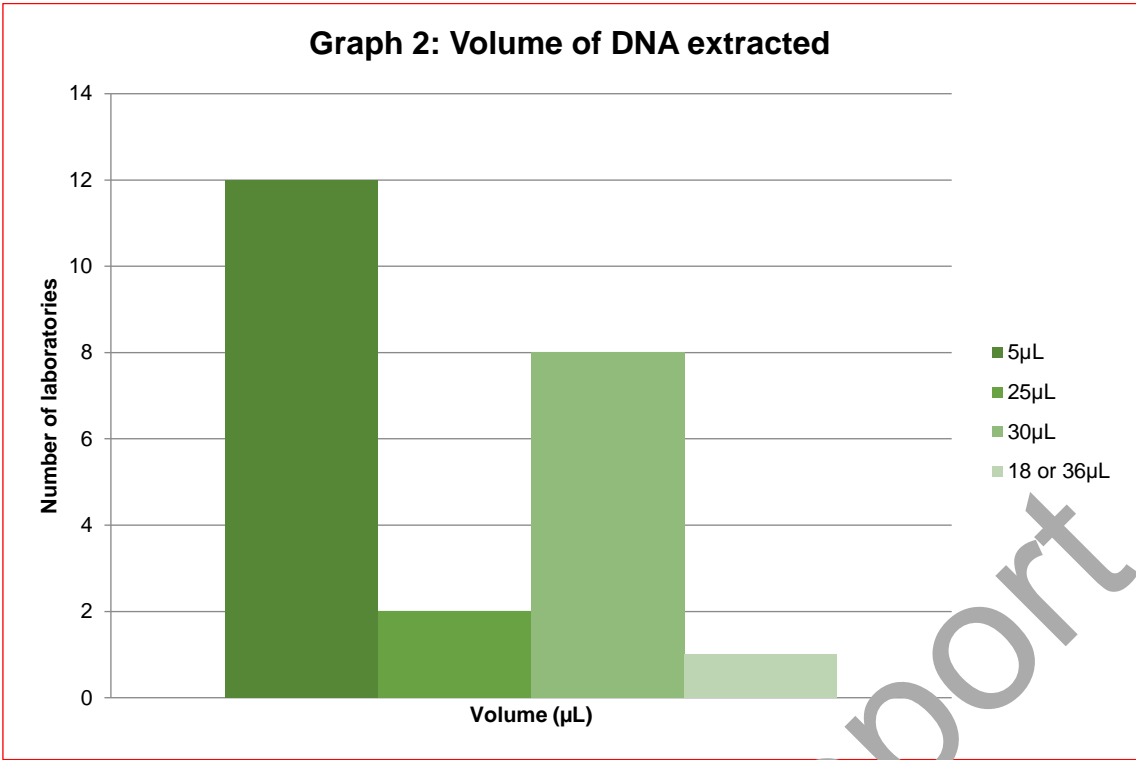
#### 5 Assays used by participants

- Some participants used more than one assay as part of their testing procedures.
- The majority of participants used a commercial assay for their RT-PCR.
- There was a large variation in commercial assays used by participants as shown in the table below.

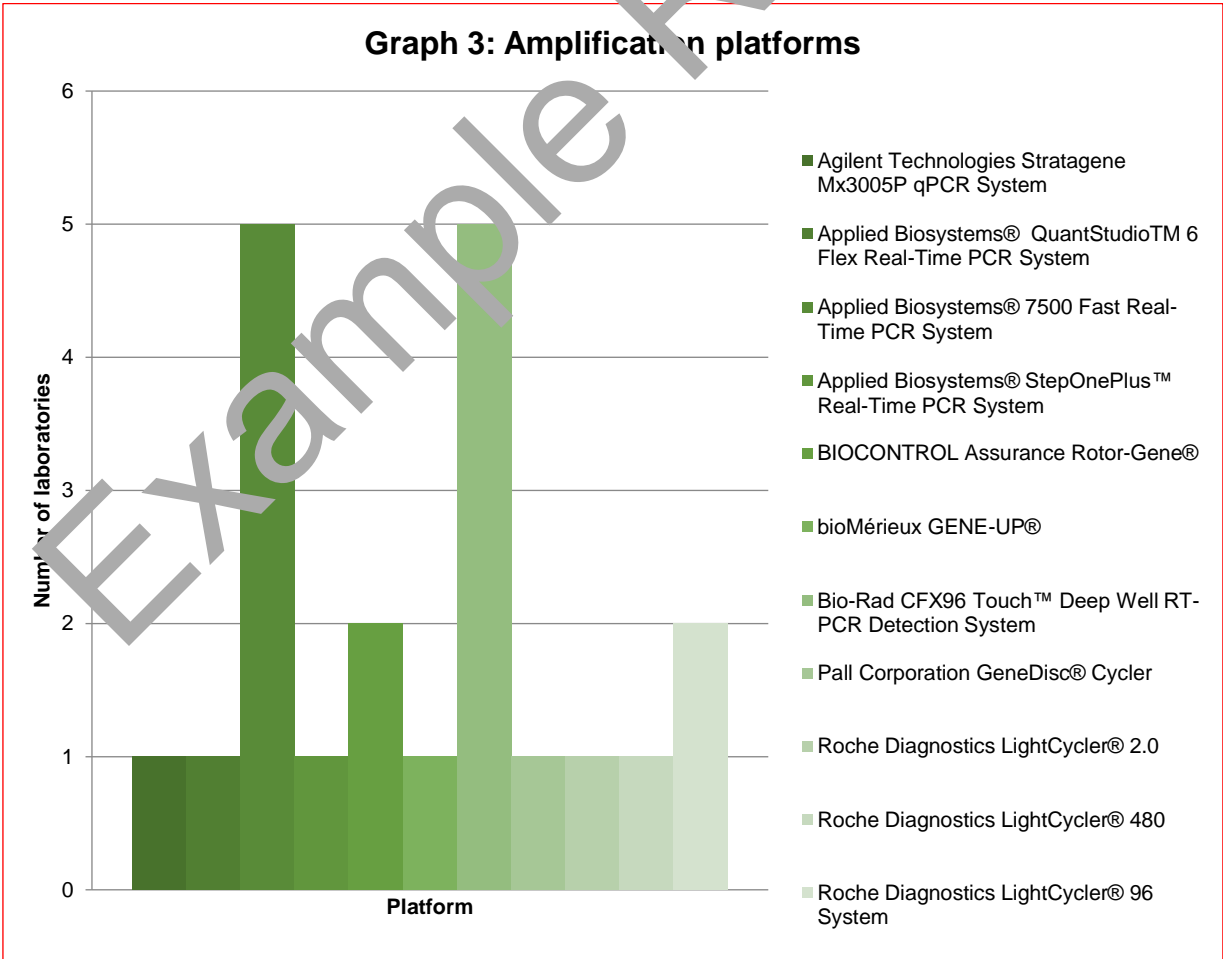
Commercial assays used	Number of laboratories
Applied Biosystems™ RapidFinder™ STEC	2
Applied Biosystems™ Taqman™ custom assays	1
Applied Biosystems™ MicroSEQ™ <i>E.coli</i> O157:H7 Detection kit	3
Applied Biosystems™ RapidFinder™ STEC Serotype Kit	1
BIOCONTROL Assurance GDS® MPX Top 7 STEC	2
bioMérieux GENE-UP® EHEC Solution	1
Bio-Rad, IQ-Check® STEC SerO	2
Bio-Rad, IQ-Check® STEC VirX	4
Bio-Rad IQ-Check® O157:H7	1
BIOTECON diagnostics, Foodproof® STEC Screening Lyokit	2
BIOTECON diagnostics, Foodproof® STEC Identification Lyokit	1
CONGEN Biotechnologie GmbH SureFast® STEC Screening PLUS	1
CONGEN Biotechnologie GmbH SureFast STEC 4plex Kit	1
CONGEN Biotechnologie GmbH SureFast <i>Escherichia coli eae</i>	1
In-house	1
ThermoFisher™ SureTect™ <i>E.coli</i> O157:H7 PCR Assay	1
ThermoFisher™ primerdesign custom assay	1

#### 6. Volume of extracted DNA used in assays

- Participants used between 2 - 36 µL of extracted DNA (Graph 2).
- The majority used 5 µL.



7. Amplification platform used is shown in graph 3.



## 8. PCR cycle information

### a) Initial denaturation temperature and time

- All the participants used a denaturation temperature of 95 °C (14 responses).

### b) Cycling

- Participants used between x35 - 50 cycles:
  - 1/17 (6%) used 35 cycles
  - 6/17 (35%) used 40 cycles
  - 3/17 (18%) used 45 cycles
  - 7/17 (41%) used 50 cycles

16 laboratories provided more information on their cycle, this is shown in the table below.

Lab ID	Step 1 temp (°C)	Step 1 hold	Step 2 temp (°C)	Step 2 hold	Step 3 temp (°C)	Step 3 hold	Step 4 temp (°C)	Step 4 hold
	95	00:00:03	60	00:00:30				
	95	00:00:05	60	00:00:05				
	95	00:00:05	60	00:00:15	72	00:00:15		
	95	00:00:03	60	00:00:30				
	95	00:02:00	95	00:02:00	60	00:30:00		
	95	00:00:10	60	00:01:00				
	95	00:00:15	60	00:00:30				
	95	00:00:15	58	00:00:20	72	00:00:30		
	95	00:00:15	58	00:00:20	72	00:00:30		
	95	00:00:15	58	00:00:20	72	00:00:30		
	95	00:00:15	58	00:00:32	72	00:00:30		
	95	00:00:10	60	00:00:30	72	00:00:01		
	95	00:00:10	60	00:01:00				
	95	00:00:05	60	00:00:30				
	95	00:00:15	59	00:01:00	95	00:00:15	59	00:01:00
	95	00:00:15	60	00:01:00				

Those highlighted in yellow were incorrect recording by the participants, these participants are advised to re-visit the information reported.

**End of report.**