FEPTU
Food & Environmental Proficiency Testing Unit
Operated by UK Health Security Agency

Summary of Results
Shiga toxin-producing *Escherichia coli* Scheme

External Quality Assessment for Food Microbiology

Distribution Number: **STX17**
Sample Numbers: **STX033 & STX034**

Distribution Date:	**27 January 2025**
Results Due:	**21 February 2025**
Report Date:	**11 March 2025**
Samples prepared and quality control tested by:	**Divya George** **Zak Prior**
Data analysed by:	**Joanna Donn** **Nita Patel**
Report compiled by:	**Joanna Donn** **Nita Patel**
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The data in FEPTU reports is confidential

Lab Number:

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 carcases 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 strains selected were tested in a UKHSA reference laboratory prior to preparation. 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-CheckTM STEC SerO (Real-time PCR detection of 7 major serogroups in Shiga Toxin Producing *E. coli*) and iQ-CheckTM 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 six 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
Scheme Guide - Food and Environmental Proficiency Testing Unit
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:

Repeat samples	Carmen Gomes or Kermin Daruwalla	**Tel: +44 (0)20 8327 7119**
Data analysis	Zak Prior or Nita Patel	
Microbiological advice	Zak Prior or Nita Patel	**E-mail: foodeqa@ukhsa.gov.uk**
General comments and complaints	Zak Prior or Nita Patel	**FEPTU's website**
Scheme Consultant	Charles Fuller	
Scheme Advisors	Marie Chattaway[i] & Frieda Jorgensen[ii]	
Scheme Co-ordinator	Nita Patel	

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

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A total of 36 participants were sent this distribution, of which 35 examined the samples, one did not return any results.

[i] Pathogen Lead for *Salmonella* Services, Gastrointestinal Bacteria Reference Unit, UK Health Security Agency
[ii] Senior Scientist and National Training Officer, Food Water and Environmental Microbiology Laboratory, UK Health Security Agency

Introduction

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[1].

As there is no specific treatment of the disease currently available[2-3], 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[4-5]. 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[6].

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[7]. 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 O104:H4. 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[8].

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[9].

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[i] as the analytical method that must be followed. In addition to the considerations of the six serogroups, it advises that organisms that are potentially highly pathogenic to humans usually show the presence of the virulence factors; Shiga toxins genes (*stx*1 and *stx*2) 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.

[i] 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

Sample: STX033

Sample type: Simulated food

Request: Examine sample for STEC

Contents: *Escherichia coli* O26:H11, *stx*1, *stx*2, *stx*1/2 and *eae* detected (>1.0x10^4) (NCTC 13733), *Enterococcus faecalis* (6.4x10^3) (NCTC 5957), *Escherichia coli* (2.8x10^3) (wild strain) and *Lactococcus lactis* (5.3x10^3) (wild strain)

All levels presented are colony forming units per disc

A summary of the results returned by 35 laboratories is shown in the table below:

Examination	Expected result	Total participants reporting	Total participants reporting correctly	Percentage of correct results
***stx* 1**	Detected	19	18	94.7
***stx* 2**	Detected	19	17	89.5
***stx* 1 and 2**	Detected	19	18	94.7
eae	Detected	34	33	97.1
Serogroup	*E. coli* O157 – not detected	23	*23	100
	E. coli O26 – detected	19	19	100
	E. coli O103 – not detected	16	16	100
	E. coli O104 – not detected	5	5	100
	E. coli O111 – not detected	16	16	100
	E. coli O145 – not detected	16	16	100
Serotype	H11 – detected	-	-	-

Your results reported

Examination	Expected result	Your result	Ct value	UKHSA score	Z-score
***stx* 1**	Detected				
***stx* 2**	Detected				
***stx* 1 and 2**	Detected				
eae	Detected				
Serogroup	*E. coli* O157 – not detected				
	E. coli O26 – detected				
	E. coli O103 – not detected				
	E. coli O104 – not detected				
	E. coli O111 – not detected				
	E. coli O145 – not detected				
Serotype	H11 – detected				
Additional serogroups reported					

*One laboratory reported a weak signal was obtained for this serogroup, however the result has been interpreted as 'not detected'.

Sample: STX034

Sample type: Simulated food

Request: Examine sample for STEC

Contents: *Escherichia coli* O145:H25, *stx*1 not detected, *stx*2, *stx*1/2 and *eae* detected (>1.0x10^4) (NCTC 13797), *Citrobacter freundii* (9.6x10^3) (wild strain) and *Escherichia coli* (3.2x10^4) (wild strain) and *Enterococcus faecium* (2.3x10^3) (wild strain)
All levels presented are colony forming units per disc

A summary of the results returned by 35 laboratories is shown in the table below:

Examination	Expected result	Total participants reporting	Total participants reporting correctly	Percentage of correct results
***stx* 1**	Not detected	18	17	94.4
***stx* 2**	Detected	18	15	83.3
***stx* 1 and 2**	Detected	19	18	94.7
eae	Detected	34	33	97.1
Serogroup	*E. coli* O157 – not detected	23	23	100
	E. coli O26 – not detected	17	17	100
	E. coli O103 – not detected	16	16	100
	E. coli O104 – not detected	7	7	100
	E. coli O111 – not detected	16	16	100
	E. coli O145 – detected	18	17	94.4
Serology	H25 - detected	-	-	-

Your results reported

Examination	Expected result	Your result	Ct value	UKHSA score	Z-score
***stx* 1**	Not detected				
***stx* 2**	Detected				
stx* 1 and *2	Detected				
eae	Detected				
Serogroup	*E. coli* O157 – not detected				
	E. coli O26 – not detected				
	E. coli O103 – not detected				
	E. coli O104 – not detected				
	E. coli O111 – not detected				
	E. coli O145 – detected				
Serology	H25 - detected				
Additional serogroups reported					

Your method/kit details

DNA extraction platform	
Commercial kit	
Amplification platform	

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 Real-time 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 UKHSA 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'.

New website

We are pleased to announce the launch of our new website: https://www.feptu.org.uk/. Please refer to this website to obtain the latest information for your proficiency testing.

Information of importance

To understand more about the proficiency testing schemes, please use the following links for information on:

1. Report format explained: Annotated report

2. Performance rating: Performance-over-time and Scheme guide (section 16.0)

3. Scoring and statistics used: Scoring information and stats

4. Homogeneity and stability: Scheme guide (section 9.0)

5. Complaints and appeal process: Scheme guide (section 20.0 and 21.0)

For further information about the operation of the service including confidentiality and terms of participation, please refer to the Scheme Guide: Scheme guide

Interpretation of results for sample STX033 and STX034 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 should be a different based on the results reported this is shown in the column labelled 'Comments by FEPTU'.

Laboratory	Interpretation by laboratory for STX033	Comments by FEPTU (based on results obtained for STX033)	Interpretation by laboratory for STX034	Comments by FEPTU (based on results obtained for STX034)
	O26 detected	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 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 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 causing the attaching and effacing lesion in the test portion of x g or x ml	
	Serogroup 026 detected per ml. Stx and eae detected	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		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	
	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	

Laboratory	Interpretation by laboratory for STX033	Comments by FEPTU (based on results obtained for STX033)	Interpretation by laboratory for STX034	Comments by FEPTU (based on results obtained for STX034)
	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 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 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 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 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 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	

Laboratory	Interpretation by laboratory for STX033	Comments by FEPTU (based on results obtained for STX033)	Interpretation by laboratory for STX034	Comments by FEPTU (based on results obtained for STX034)
	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 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 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 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 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		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	
	Presence of STEC highly pathogenic to humans in the test portion - 026 - STX1/STX2/EAE Detected		Presence of STEC highly pathogenic to humans in the test portion - 0145 - STX2/EAE Detected	
	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

Laboratory	Interpretation by laboratory for STX033	Comments by FEPTU (based on results obtained for STX033)	Interpretation by laboratory for STX034	Comments by FEPTU (based on results obtained for STX034)
	STEC not detected in the test portion of x g or xml		STEC not detected in the test portion of x g or xml	
	Presumptive detection of STEC in the test portion of x g or x ml		STEC not detected in the test portion of x g or xml	
	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 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 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 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	

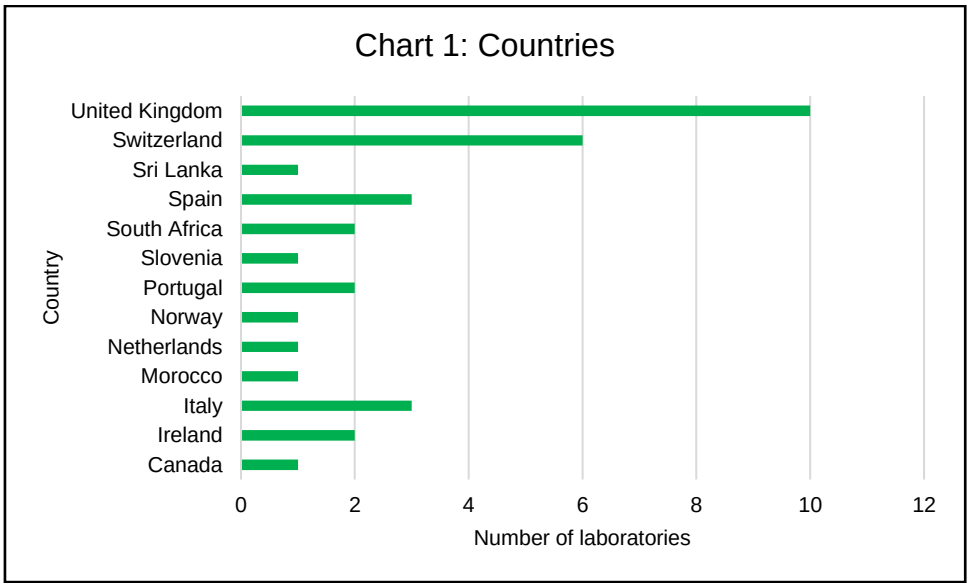
Questionnaire results:

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.

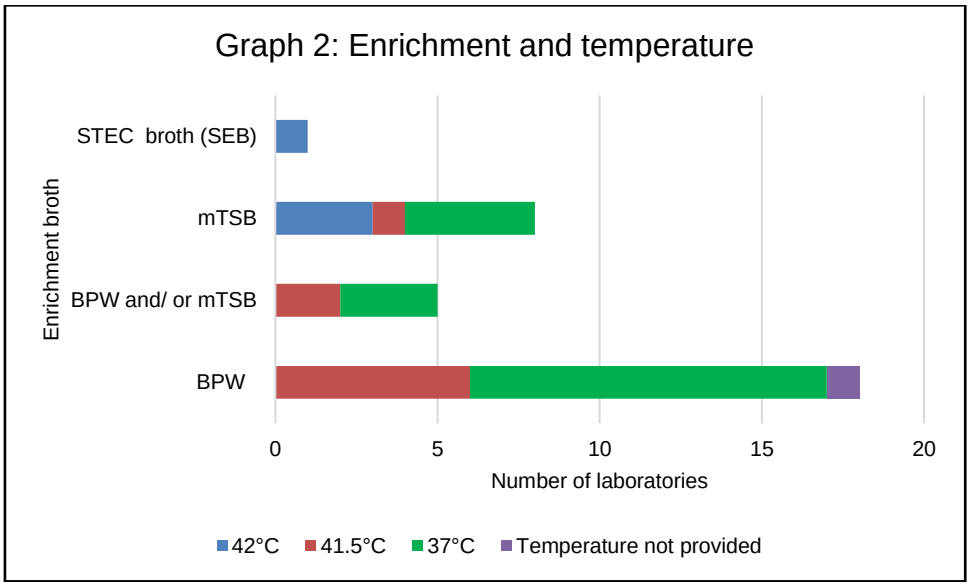
34 laboratories returning a result provided information additional information. However the total numbers will not always correspond to 34 as some laboratories did not provide information on all the questions and some questions allowed for more than one option to be selected.

Graph 1 shows the countries taking part in this PT exercise (n=34).



1. **The use of ISO/TS 13136:2012[ii]**
   - 20/33 (60.6%) 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) at 37°C for 24 hours to enrich viable STEC organisms (Graph 2)(n=32)



[ii] 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

- 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[iii].

3. DNA extraction

- The majority of participants reported using a commercial extraction kit shown in table below (n=31). Some laboratories use more than one kit. Where extraction kit information was incomplete this is not included in the table below.

Extraction assay used	**Number of laboratories**
Applied Biosystems™, PrepSEQ™ Rapid Spin Sample Preparation Kit	2
Bio-Rad iQ-Check® VirX lysis Kit	6
Bio-Rad InstaGene™ Matrix	1
BIOTECON foodproof® StarPrep Kit	2
BIOTECON foodproof® StarPrep Three Kit	1
CONGEN SureFast STEC 4plex ONE	2
CONGEN Biotechnologie GmbH SureFast® PREP	1
Hygiena StarPrep II kit	1
In-house	1
KYLT® DNA Extraction-Mix II	1
Promega Maxwell® Pure Food Pathogen Kit	2
Promega Maxwell® 16 Cell DNA purification kit	1
Qiagen DNeasy Blood & Tissue Kits	1
Qiagen EZ1® DNA Tissue Kit	1
Qiagen QIAsymphony mericon Bacteria Kit	2
Qiagen QIAamp DNA kit	1
Roche Diagnostics MagNA Pure Compact Nucleic Acid Isolation Kit	1
Thermo Scientific™ SureTect™ *Escherichia coli* O157:H7	6
Thermo Scientific™ SureTect™ STEC screening PCR assay	6

4. Type of molecular test (n=34)

- 31/34 (91.1%) reported using a Real-time RT-PCR
- 1/34 (2.9%) used both a conventional and Real-time RT-PCR
- 2/34 (5.9%) used a conventional

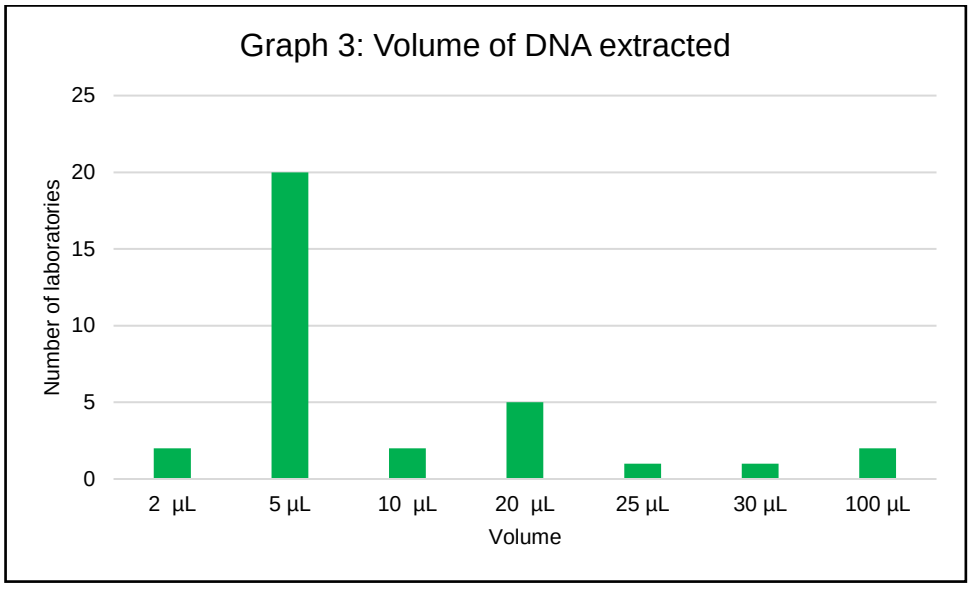
5. Primer / probe assays used by participants

- Some participants used more than one assay as part of their testing procedures.
- The majority of participants used a commercial assay.
- There was a large variation in commercial assays used by participants the most commonly used assays were:
    - BIOTECON diagnostics Foodproof® STEC Screening and identification Lyokit
    - Microsynth AllColi
    - BioRad iQ-Check STEC SerO and STEC VirX
    - ThermoFisher™ SureTect™ *E.coli* O157:H7 PCR Assay

[iii] 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.

6. Volume of extracted DNA used in assays

- Participants used between 2 - 100 µL of extracted DNA (Graph 3, n=33)
- The majority used 5 µL



7. Amplification platform used is shown in the table below (n=33) – some laboratories used two platforms

Amplification platforms used	Number of laboratories
Agilent Technologies Stratagene Mx3005P qPCR System	1
Applied Biosystems® QuantStudio™ 6 Flex Real-Time PCR System	1
Applied Biosystems® QuantStudio 5 Real Time PCR system	8
Applied Biosystems® StepOnePlus Real-Time PCR System	1
Applied Biosystems® GeneAmp® PCR System 9700	1
Bio-Rad CFX96 Touch Deep Well RT-PCR Detection System	12
Qiagen Rotor-Gene 6000	2
Qiagen Rotor-Gene Q	2
Roche Diagnostics LightCycler® 480	2
Roche diagnostic LightCycler® 96 system	3

8. PCR cycle information

a) Initial denaturation temperature and time (n=18)

- 24/25 (96.0%) used a denaturation temperature of 95 °C
- The times of these varied from 2 to 15 minutes.

b) Cycling

- Participants used between x30 - 50 cycles (n=28):
  - 2/28 (7.1%) used 30 cycles
  - 1/28 (3.6%) used 35 cycles
  - 1/28 (3.6%) used 39 cycles
  - 4/28 (14.3%) used 40 cycles
  - 2/28 (7.1%) used 44 cycles
  - 10/28 (35.7%) used 45 cycles
  - 8/28 (28.6%) used 50 cycles

28 laboratories provided more information on their cycles, this is shown in the table below.

Lab ID	Step 1 temp (ºC)	Step 1 hold	Step 2 temp (ºC)	Step 2 hold	Step 3 temp (ºC)	Step 3 hold	Step 4 temp (ºC)	Step 4 hold
	95	00:00:03	60	00:00:30				
	95	00:00:05	60	00:00:30				
	95	00:00:15	60	00:00:30				
	95	00:00:05	60	00:00:45				
	95	00:00:05	60	00:00:45				
	95	00:00:05	60	00:00:30				
	95	00:00:15	60	00:00:30				
	95	00:00:02	58	00:00:15	72	00:00:08		
	95	00:00:05	60	00:00:30				
	95	00:00:15	58	00:00:20	72	00:00:30		
	95	00:00:05	60	00:01:00				
	95	00:07:00	95	00:00:05	60	00:00:45		
	95	00:00:15	60	00:00:15	72	00:00:10		
	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	00:00:05	60	00:00:30				
	95	00:00:10	60	00:00:30	72	00:00:01		
	95	00:00:15	58	00:00:20	72	00:00:30		
	95	00:00:05	60	00:01:00				
	95	00:10:00	95	00:00:02	58	00:00:15	72	00:00:08
	94	00:01:00	60	00:01:00	72	00:01:30		
	95	00:00:30	52	00:01:00	72	00:01:00	4	00:05:00
	95	00:00:15	60	00:01:00				
	95	00:00:02	58	00:00:15	72	00:00:08		
	95	00:00:05	60	00:00:10				
	95	00:00:05	60	00:00:30				
	95	00:00:02	58	00:00:15	72	00:00:08		
	95	00:00:15	60	00:01:00				

Information highlighted in green should be reviewed by the laboratory

End of report.