UK Health Security Agency

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

External Quality Assessment for Food Microbiology

Distribution Number: **STX13**
Sample Numbers: **STX025 & STX026**

Distribution Date:	**January 2023**
Results Due:	**24 February 2023**
Report Date:	**8 March 2023**
Samples prepared and quality control tested by:	**Zak Prior**
Data analysed by:	**Joanna Donn** **Nita Patel**
Report compiled by:	**Joanna Donn** **Nita Patel**
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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
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:

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 30 participants were sent this distribution, of which 28 examined the samples, two 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: STX025

Sample type: Simulated food

Request: Examine sample for STEC

Contents: *Escherichia coli* O26:H11 *stx*1, *stx*2, *stx*1/2 and *eae* detected (>1.0x10^5) (NCTC 13733), *Citrobacter braaki* (1.0x10^3) (wild strain) and *Enterococcus mundtii* (1.0x10^3) (NCIMB 700582)

All levels presented are colony forming units per mL

A summary of the results returned by 28 laboratories is shown in the table below:

Examination	Expected result	Total participants reporting	Total participants reporting correctly	Percentage of correct results
***stx* 1**	Detected	16	15	94
***stx* 2**	Detected	17	15	88
***stx* 1 and 2**	Detected	15	15	100
eae	Detected	27	27	100
Serogroup	*E. coli* O157 – not detected	22	21	95
	E. coli O26 – detected	20	19	95
	E. coli O103 – not detected*	19	18	95
	E. coli O104 – not detected	10	10	100
	E. coli O111 – not detected	18	18	100
	E. coli O145 – not detected*	19	18	95
Serotype	H11 – detected	-	-	-

*One laboratory reported a detected result for this combination of serogroups O103/O145.

Your results reported

Examination	Expected result	Your result	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			

Sample: STX026

Sample type: Simulated food

Request: Examine sample for STEC

Contents: *Escherichia coli* O157:H& *stx*1, *stx*2, *stx*1/2 and *eae* detected (>1.0x10^5) (NCTC 12079), *Enterobacter asburiae* (1.0x10^3) (wild strain) and *Moraxella catarrhalis* (1.0x10^3) (wild strain)

All levels presented are colony forming units per mL

A summary of the results returned by 28 laboratories is shown in the table below:

Examination	Expected result	Total participants reporting	Total participants reporting correctly	Percentage of correct results
***stx* 1**	Detected	16	15	94
***stx* 2**	Detected	17	16	94
***stx* 1 and 2**	Detected	15	15	100
eae	Detected	27	27	100
Serogroup	*E. coli* O157 – detected	22	22	100
	E. coli O26 – not detected	20	20	100
	E. coli O103 – not detected	19	19	100
	E. coli O104 – not detected	10	10	100
	E. coli O111 – not detected	19	19	100
	E. coli O145 – not detected	19	19	100
Serology	H7 - detected	10	10	100

Your results reported

Examination	Expected result	Your result	UKHSA score	Z-score
***stx* 1**	Detected			
***stx* 2**	Detected			
stx* 1 and *2	Detected			
eae	Detected			
Serogroup	*E. coli* O157 – detected			
	E. coli O26 – not detected			
	E. coli O103 – not detected			
	E. coli O104 – not detected			
	E. coli O111 – not detected			
	E. coli O145 – not detected			
Serology	H7 – detected			

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'.

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

Summary of participants results STX025 (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**	
FEPTU	Detected						Detected		O26		O157 O145, O26, O103, O111	O104			Bio-Rad iQ-Check® VirX lysis Reagent Bio-Rad iQ-Check STEC SerO; Bio-Rad iQ-Check® STEC VirX Qiagen Rotor-Gene Q

Summary of participants results STX026 (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**	
FEPTU	Detected						Detected		O157		O26, O103, O111, O145	O104			Bio-Rad iQ-Check® VirX lysis Reagent Bio-Rad iQ-Check STEC SerO; Bio-Rad iQ-Check® STEC VirX Qiagen Rotor-Gene Q

Interpretation of results for sample STX025 and STX026 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 STX025	Comments by FEPTU (based on results obtained for STX025)	Interpretation by laboratory for STX026	Comments by FEPTU (based on results obtained for STX026)

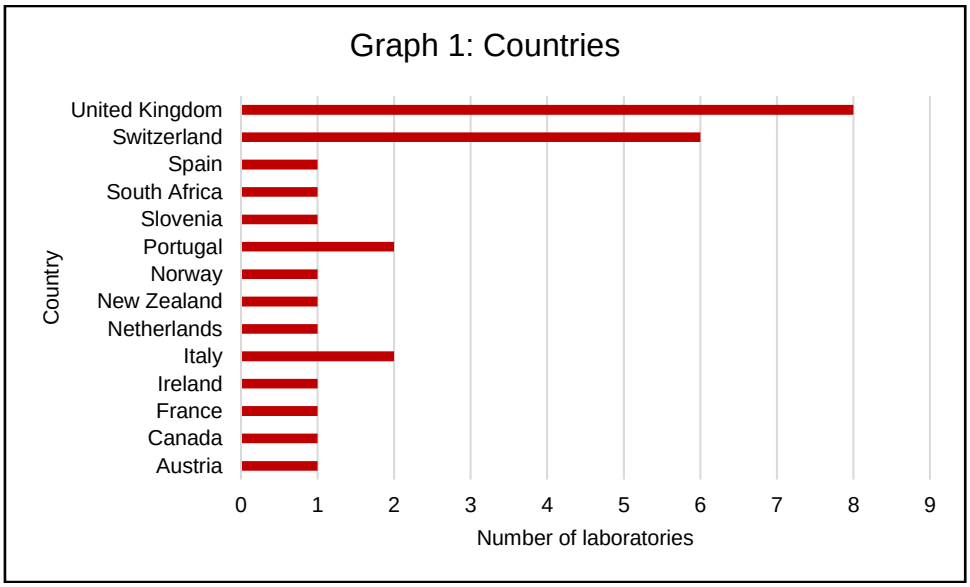
Questionnaire results:

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.

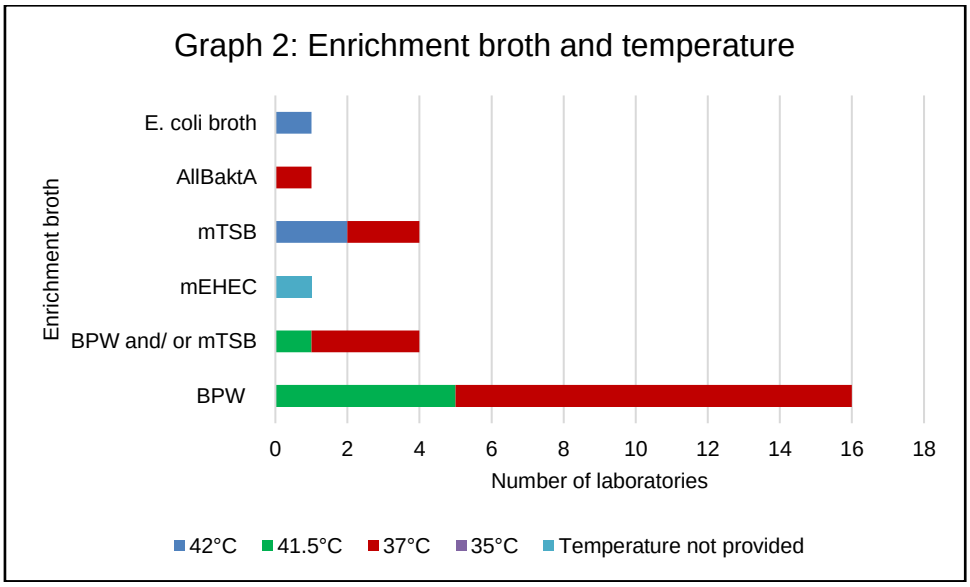
28 laboratories returning a result provided information additional information. However the total numbers will not always correspond to 28 as some laboratories did not provide information on all the questions and some questions allowed for more than one option to be selected.

Graph 1 shows the countries taking part in this PT exercise (n=28).



1. **The use of ISO/TS 13136:2012[ii]**
   - 13/28 (46%) 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 enriching viable STEC organisms (Graph 2)(n=27)



[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=28). Some laboratories use more than one kit.

Extraction assay used	Number of laboratories
3M™ Molecular Detection Assay 2 - STEC Gene Screen	1
Applied Biosystems™, PrepSEQ™ Rapid Spin Sample Preparation Kit	2
Bio-Rad iQ-Check® VirX lysis Kit	3
Bio-Rad InstaGene™ Matrix	1
BIOTECON foodproof® StarPrep Three Kit	2
CONGEN Biotechnologie GmbH SureFast® PREP	1
Pall Corporation Extraction Pack Food 1	1
Promega Maxwell® RSC PureFood Pathogen Kit	1
Qiagen DNeasy Blood & Tissue Kits	2
Qiagen QIAamp DNA mini kit	1
Qiagen EZ1® DNA Tissue Kit	2
Qiagen QIAsymphony mericon Bacteria Kit	3
Thermo Scientific™ SureTect™ *Escherichia coli* O157:H7	3
Thermo Scientific™ SureTect™ STEC screening PCR assay	3
Thermo Scientific™ SureTect™ STEC ID PCR assay	1
Thermo ScientificTM KingFisher Plant DNA Kit	1

4. Type of molecular test (n=27)

- 22/27 (81%) reported using a Real-time RT-PCR
- 2/27 (7%) reported using conventional PCR
- 2/27 (7%) used both a conventional and Real-time RT-PCR
- 1/27 (4%) used a molecular detection system

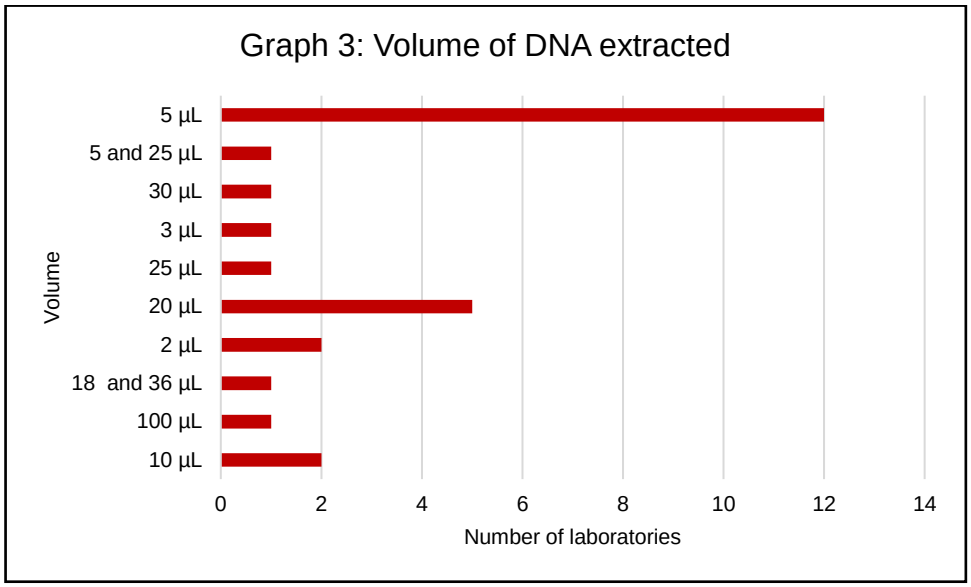
5. Primer / probe assays used by participants

- 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 Lyokit
    - Microsynth AllColi
    - BioRad iQ-Check STEC SerO
    - Bio-Rad iQ-Check® STEC VirX
    - ThermoFisher™ SureTect™ *E.coli* O157:H7 PCR Assay

6. Volume of extracted DNA used in assays

- Participants used between 3 - 1000 µL of extracted DNA (Graph 3, n=27)
- The majority used 5 µL

[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.



7. Amplification platform used is shown in the table below (n=24), to note some laboratories use multiple platforms

Amplification platforms used	Number of laboratories
Agilent Technologies Stratagene Mx3005P qPCR System	1
Applied Biosystems® QuantStudioTM 6 Flex Real-Time PCR System	1
Applied Biosystems® 7500 Fast Real-Time Selected System	1
Applied Biosystems® QuantStudio 5 Real Time PCR system	5
bioMérieux GENE-UP®	1
Bio-Rad CFX96 Touch Deep Well RT-PCR Detection System	8
Pall Corporation GeneDisc® Cycler	1
Qiagen Rotor-Gene 6000	1
Qiagen Rotor-Gene Q	3
Roche Diagnostics LightCycler® 480	1
Roche Diagnostics LightCycler® 2.0	1

8. PCR cycle information

a) Initial denaturation temperature and time (n=19)

- 1/19 used a denaturation temperature of 94 °C
- 18/19 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=19): one laboratory stated cycle parameters defined by the system used.
    - 1/19 (5%) used 30 cycles
    - 2/19 (11%) used 35 cycles
    - 6/19 (32%) used 40 cycles
    - 1/19 (5%) used 44 cycles
    - 4/19 (21%) used 45 cycles
    - 5/19 (26%) used 50 cycles

19 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
9	95	00:00:03	60	00:00:30				
66	95	00:00:05	60	00:00:30				
67	95	00:00:05	60	00:00:05				
166	37	00:10:00	95	00:05:00	10	00:02:00	4	
264	95	00:00:05	60	00:00:30				
329	95	00:00:02	58	00:00:15	72	00:00:08		
330	95	00:00:05	60	00:00:30				
336	95	00:00:15	58	00:00:20	72	00:00:30		
345	95	00:00:05	60	00:01:00				
419	95	00:00:15	60	00:00:15	72	00:00:10		
566	95	00:00:15	58	00:00:32	72	00:00:30		
569	95	00:05:00	95	00:00:05	60	00:00:30		
576	95	00:00:10	60	00:00:30	72	00:00:01		
677	95	00:00:03	60	00:00:30				
1093	95	00:00:15	58	00:00:20	72	00:00:30		
2132	94	00:01:00	60	00:01:00	72	00:01:30		
2456	95	00:02:00	60	00:02:00	72	00:02:30		
2457	95	00:00:05	60	00:00:10				
2669	95	00:00:05	60	00:00:30				
2720	95	00:00:05	60	00:00:45				

End of report.