Food and Agriculture Organization of the United Nations

Cefas INTERNATIONAL CENTRES OF EXCELLENCE

Centre for Environment Fisheries & Aquaculture Science

FAO Reference Centre for Bivalve Mollusc Sanitation

Fifth proficiency testing distribution for the detection of *Escherichia coli* in shellfish and the detection of faecal coliforms in water (PT 111)

Author(s): Louise Stockley

Date: September 2026



© Crown copyright 2026

This information is licensed under the Open Government Licence v3.0. To view this licence, visit

www.nationalarchives.gov.uk/doc/open-government-licence/

Cefas Document Control

Submitted to:	Participants of the FAO Reference Centre Bivalve Sanitation – Fifth proficiency testing for sanitary indicators
Date submitted:	02.10.26
Project Manager:	Sam Course
Report compiled by:	Louise Stockley
Quality control by:	James Lowther
Executive sign-off (approval for submission) by:	Michelle Price-Hayward
Version:	V2
Recommended citation for this report:	Stockley, L. (2026). FAO Reference Centre for Bivalve Mollusc Sanitation; Fifth proficiency testing distribution for the detection of *Escherichia coli* in shellfish and the detection of faecal coliforms in water (PT 111). Cefas report.

Version control history

Version	Author	Date	Comment
0.01	L. Stockley	15/09/26	Sent to JL for QA
0.02	J. Lowther	18/09/25	Returned to LS with comments
0.03	L. Stockley	22/09/26	Sent to MPH for final review
V1	L. Stockley	02/10/26	Sent to participants for review
V1.001	L. Stockley	05/10/26	Sent to JL to review changes following participant comment
V2	L. Stockley	06/10/26	Sent to participants for review
V2	L. Stockley	23.10.26	No changes required. Report finalised.

Contents

1. Introduction 5

2. Samples 5

2.1. *E. coli* in shellfish 5

2.2. Faecal coliforms in water 6

2.3. Distribution 6

2.4. Quality Control 6

2.4.1. *E. coli* in shellfish 7

2.4.2. Faecal coliforms in water 7

3. Analysis of participants' results 7

3.1. *E. coli* in shellfish - Sample 1 and 2 7

3.1.1. *E. coli* in shellfish - Sample 1 8

3.1.2. *E. coli* in shellfish - Sample 2 8

3.2. Faecal coliforms in water – Samples 1 and 2 11

3.2.1. Faecal coliforms in water – Sample 1 11

3.2.2. Faecal coliforms in water – Sample 2 12

4. Analysis of results 16

4.1. General comments 16

4.2. Sample analyses against the participants' median 17

4.2.1. *E. coli* in shellfish 17

4.2.2. Faecal coliforms in water 17

5. References 18

1. Introduction

In 2019, Cefas (the Centre for Environment, Fisheries and Aquaculture Science) was designated as the Food and Agriculture Organization (FAO) Reference Centre (RC) for Bivalve Mollusc Sanitation. The aim of the FAO RC is to provide support to FAO member countries in the development of bivalve shellfish production.

Part of the FAO RC work programme is to organise a proficiency testing (PT) distribution to help support existing or new bivalve programmes and help the capability of laboratories to test for indicators of faecal contamination which may be used wherever bivalves are commercially produced and traded.

Proficiency testing (PT), also known as comparative testing, involves multiple laboratories testing identical samples and comparing results. The results of PT can help demonstrate good performance, assist in the implementation of new methods, support laboratory quality accreditations, identify opportunities for continuous improvement and help build supportive networks of laboratories with similar goals, for example the development of networks of laboratories in countries with an interest in growing safe bivalve mollusc programmes.

2. Sample selection

The Fifth PT distribution contained Lenticule™ discs originating from the UK Health Security Agency (UK HSA) External Quality Assurance (EQA) schemes (the Shellfish scheme and the Recreational and Surface Water Scheme). Each Lenticule™ disc contains known levels of fully characterised microorganisms in a solid water-soluble matrix. The discs are very stable. Short periods at ambient temperature, such as during shipment, are not an issue for product stability. Participants were requested to store all samples at -20°C on arrival until testing begun.

2.1. *E. coli* in shellfish

Participants were requested to examine each Lenticule™ sample using their laboratory's in-house method for the enumeration of *E. coli* in shellfish and/or use the FAO RC generic protocol [Generic Protocols] for the enumeration of *E. coli* in shellfish based upon ISO 16649-3, Microbiology of the food chain – Horizontal method for the enumeration of β-

glucuronidase-positive *Escherichia coli* Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide.

ISO 16649-3 is an internationally recognised method for the enumeration of *E. coli* in bivalve shellfish and is the stipulated European Union reference method. The level of *E. coli* in the Lenticule™ sample should be reported per 100 g of flesh.

Note: These samples are designed for laboratories testing raw bivalve molluscs from harvesting beds for classification or end product testing.

2.2. Faecal coliforms in water

Participants were requested to examine each Lenticule™ sample using their own laboratory's in-house method for enumeration of faecal coliforms (FC) in water and/or the FAO RC generic protocol [Generic Protocols] for the enumeration of FC in water based upon the approach set out in US FDA BAM Chapter 4.

Note: These samples are designed for laboratories wishing to test water from bivalve mollusc growing areas.

2.3. Distribution

Samples were packaged according to IATA regulations, UN3373 as diagnostic specimens, division 6.2 under the packing instruction code 650 and distributed using the courier Worldnet on the 11th May 2026 to 18 participants. Relevant transport documentation, examination request forms and instructions on handling and sample reconstitution accompanied the samples. Laboratories were asked to test the *E. coli* samples in duplicate and obtain a single result for FC, returning results on completion. Those participants that returned results have been included in this report.

2.4. Quality Control (QC)

The Lenticules™ samples used for this PT distribution were certified according to ISO / IEC 17025 and produced under reproducible conditions compliant with ISO 17034 by the supplier (UK HSA).

2.4.1. Quality control of *E. coli* in shellfish samples

To obtain the reference results the UK HSA examined three randomly selected Lenticules™ before dispatch (Pre) and a further three during dispatch (Post) in duplicate under repeatability conditions. The method used was based on ISO 16649-3 (Anon, 2015). Results are given in Figure 1 and Table 2.

2.4.2. Quality control of Faecal coliforms in water samples

To obtain the reference results the UK HSA examined three randomly selected Lenticules before dispatch (Pre) and further three during dispatch (Post) under repeatability conditions. The method used was based on ISO 9308-1 (Anon, 2014) and the Standing Committee of Analysts – The Microbiology of drinking water, Part 4 (Anon 2016). Results are given in Figures 2 and 3, and Tables 4 and 6.

3. Analysis of participants' results

3.1. *E. coli* in shellfish participants results - Sample 1 and 2

For sample 1 (negative for *E. coli*) each participant's results were checked for incorrect reporting of the presence of *E. coli*. For sample 2 each participant's *E. coli* values (*E. coli* / 100g) reported were compared against the median of all participants' results, reference results were omitted from the calculation. The median is used rather than the mean as it is less affected by outlying results. The acceptability limits for participants were calculated as the participants' median ±2.68 theoretical standard deviations (SD_T) and ±4 SD_T following the recommended approach described by the UK HSA for PT performance assessments of MPN tests and Lenticule discs (FEPTU562.13.pdf (publishing.service.gov.uk). Reported *E. coli* values were $\log_{10}$ transformed before being compiled into a charts as shown in Figure 1.

Note: The median and upper and lower limits (±2.68 SD_T and ±4 SD_T) were calculated from participants' results. The value for SD_T used in these calculations is based on the inherent variability of a 5 x 3 MPN method (0.24 $\log_{10}$). Reference values were excluded from the calculation of the participants' median.

Note: To enable reported results to be statically analysed, results reported as <18 were treated as 17.

3.1.1. *E. coli* in shellfish - Sample 1

- **Sample contents**

Klebsiella pneumoniae (14) (wild strain), *Serratia fontícola* (14) (wild strain), *Staphylococcus saprophyticus* (3.4 x 10^2) (NCTC 7292) per Lenticule™ disc.

- **Sample results**

Table 1: Participants' results

Lab ID.	*E. coli* (per 100 g)	
	Replicate 1	Replicate 2
40	≤18	≤18
60	<18	<18
136	<18	<18
285	<18	<18
290	<18	<18
293	0	0
329	0	0
372	<18	<18
468	<18	<18
492	0	-
540	<3	<3

3.1.2. *E. coli* in shellfish - Sample 2

- **Sample contents**

Escherichia coli (3.3 x 10^2 – 2.2 x 10^3) (wild strain), *Salmonella* London 3,{10}{15};l,v;1,6 (1.3 x 10^2 per disc or 32 per 25g) (wild strain), *Enterobacter cloacae* (10) (wild strain), *Pseudomonas aeruginosa* (2.0 x 10^2) (wild strain) per Lenticule™ disc.

- **Sample results**

Table 2: Participants' and reference result dataset characteristics

Results	Range	Median	GM	Median ±2.68*SD_T	Median ±4*SD_T
Participants'	<1.8 x 10^1 – 9.3 x 10^4	7.8 x 10^2	9.2 x 10^2	1.8 x 10^2 – 3.4 x 10^3	8.6 x 10^1 – 7.1 x 10^3
Reference	3.3 x 10^2 – 2.2 x 10^3	7.8 x 10^2	7.9 x 10^2	-	-

Note: Results are in *E. coli* per 100 g. GM - geometric mean, SD_T - theoretical standard deviation (0.24 log_{10})

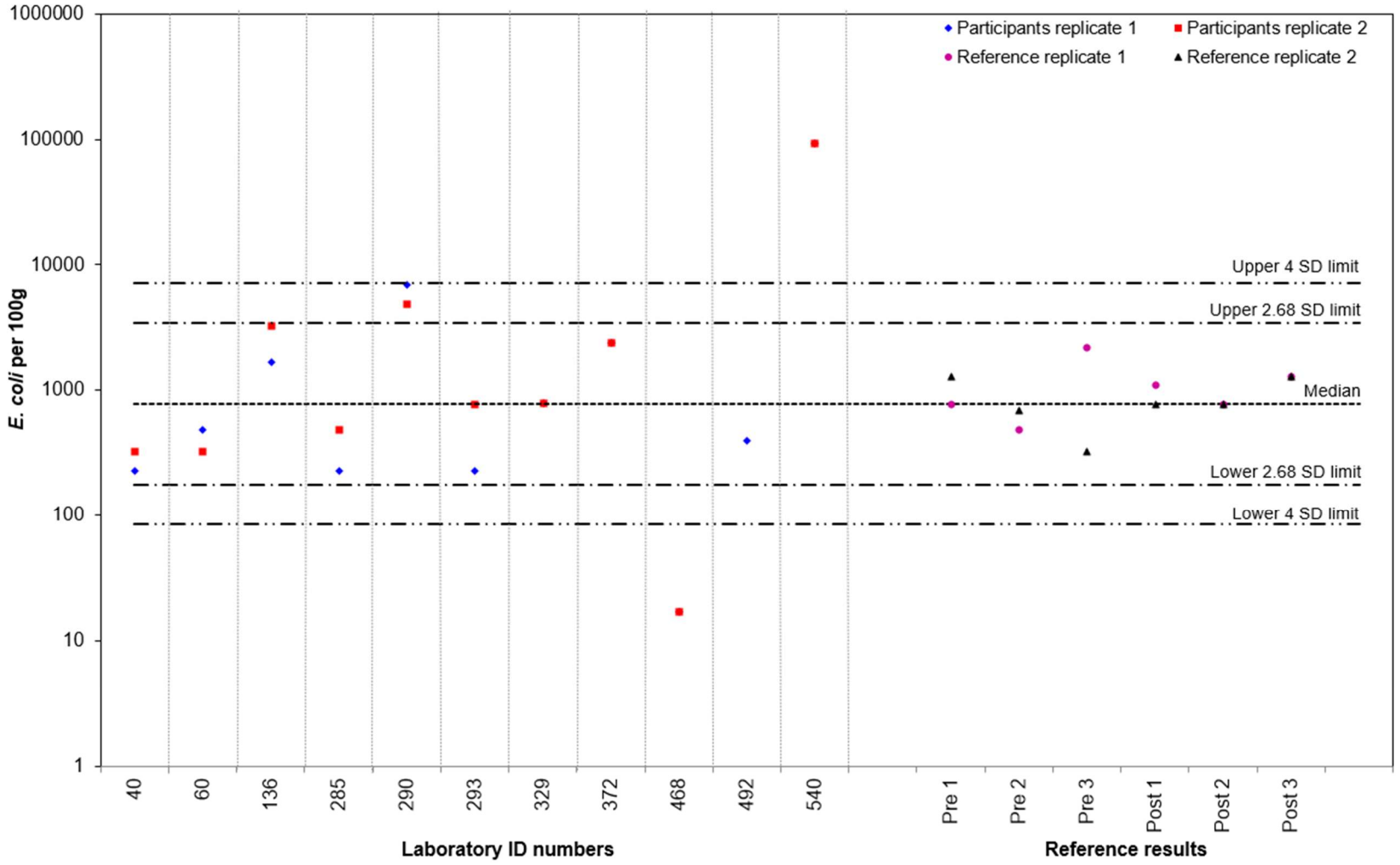
Table 3: Participants' results

Lab ID.	*E. coli* (per 100g)	
	Replicate 1	Replicate 2
40	230	330
60	490	330
136	1700	3300
285	230	490
290	7000	4900
293	230	780
329	790	790
372	2400	2400
468 [a]	<18	<18
492	400	-
540	93000	93000

Key: a; results reported as <18 treated as 17 for statistical analysis.

Figure 1: *E. coli* in shellfish - Sample 2

Note: Median value calculated using all participants' results



3.2. Faecal coliforms in water participants results – Samples 1 and 2

Each participant's reported result for FC was compared against the median of all participants' results; reference results were omitted from the calculation. The median is used rather than the mean as it is less affected by outlying results. The acceptability limits for participants were calculated as the participants' median ±2.68 SD_T and ±4 SD_T following the recommended approach described by the UK HSA for PT performance assessments of MPN tests and Lenticule™ discs (FEPTU562.13.pdf (publishing.service.gov.uk). Reported results were log_{10} transformed before being compiled into charts as shown in Figures 2 and 3.

Note: The median and upper and lower limits (±2.68 SD_T and ±4 SD_T) were calculated from participants' results. The value for SD_T used in these calculations is based on the inherent variability of a 5 x 3 MPN method (0.24 log_{10}). Reference values were excluded from the calculation of the participants' median.

Note: To enable reported results to be statically analysed, those results reported as zero were not included in statistical analysis.

3.2.1. Faecal coliforms in water – Sample 1

- **Sample content**

Escherichia coli (75) (wild strain), *Enterococcus faecalis* (42) (wild strain), *Salmonella* London 3,10:l,v:1,6 (85 per disc) (wild strain), *Kocuria salsicia* (68) (wild strain) per Lenticule™ disc.

- **Sample results**

Table 4: Participants' and reference result dataset characteristics

Results	Range	Median	GM	Median ±2.68*SD_T	Median ±4*SD_T
Participants'	$3.3 \times 10^1 – 3.6 \times 10^3$	6.9×10^1	1.3×10^2	$1.6 \times 10^1 – 3.0 \times 10^2$	$7.6 \times 10^0 – 6.3 \times 10^2$
Reference	5.9×10^1 - 9.8×10^1	6.9×10^1	7.4×10^1	-	-

Note: Results are in FC per 100 ml. GM - geometric mean, SD_T - theoretical standard deviation (0.24 log_{10})

Table 5: Participants' results

Lab ID	Faecal coliforms per 100ml
40	1200
60	33
136	33
285	50
290	33
293	130
295	148
468	490
492	3600
494	79
516	59
540	59

3.2.2. Faecal coliforms in water – Sample 2

- **Sample content**

Escherichia coli (1.2 x 10^2) (wild strain), *Enterococcus faecium* (1.1 x 10^2) (wild strain), *Staphylococcus intermedius* (3.3 x 10^3) (NCTC 11048) per Lenticule™ disc.

- **Sample results**

Table 6: Participants' and reference result dataset characteristics

Results	Range	Median	GM	Median ±2.68*SD_T	Median ±4*SD_T
Participants'	0 – 2.9 x 10^3	7.0 x 10^1	1.1 x 10^2	1.6 x 10^1 - 3.1 x 10^2	7.7 x 10^0 – 6.4 x 10^2
Reference	9.5 x 10^1 – 1.6 x 10^2	1.1 x 10^2	1.2 x 10^2	-	-

Note: Results are in FC per 100 ml. GM - geometric mean, SD_T - theoretical standard deviation (0.24 log_{10})

Table 7: Participants' results reported

Lab ID	Faecal coliforms per 100ml
40	230
60	17
136	23
285	35
290	350
293	70
295	0
468	460
492	2900
494	49
516	65
540	70

Figure 2: Faecal coliforms in water - Sample 1

Note: Median value calculated using all participants' results

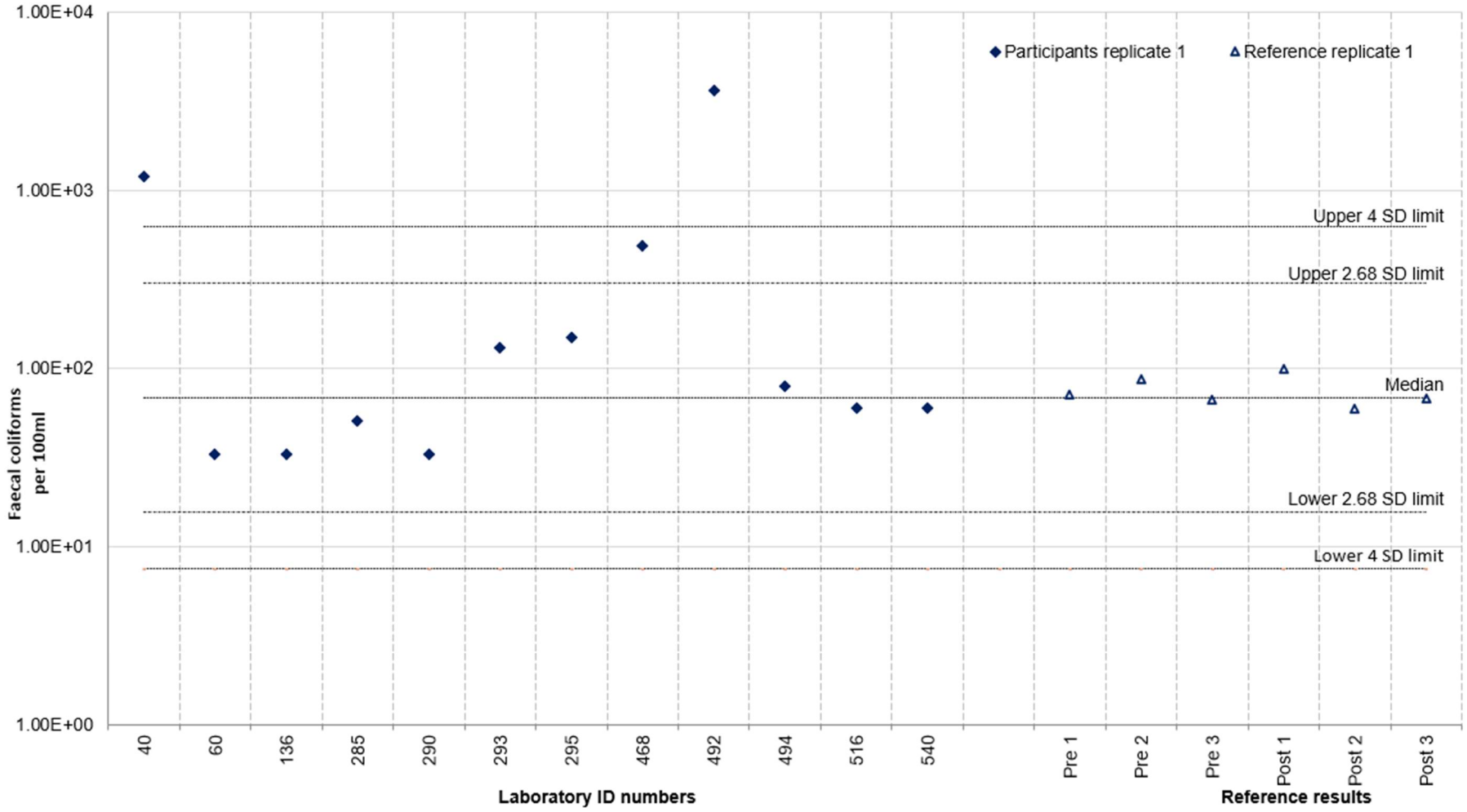
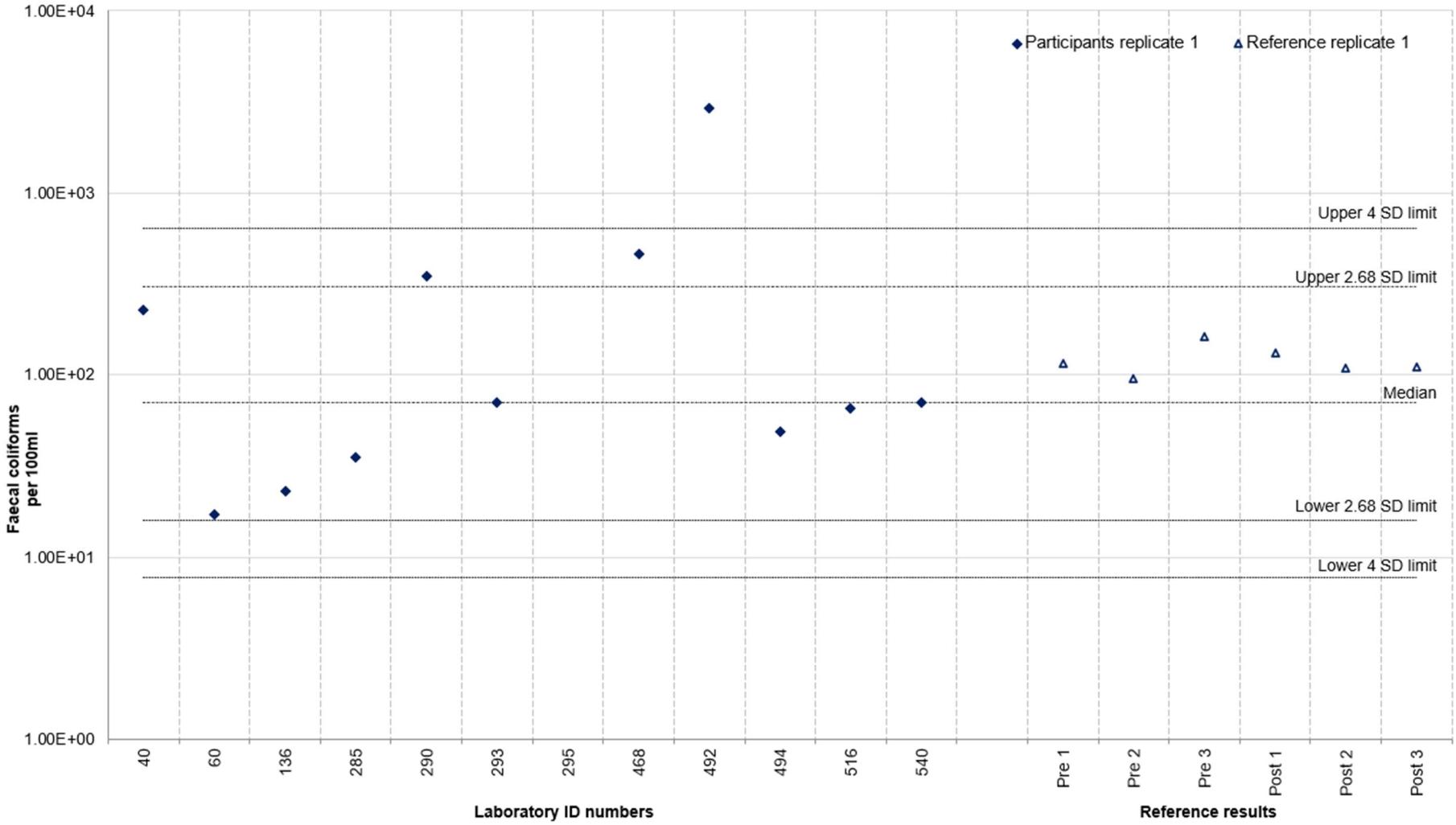


Figure 3: Faecal coliforms in water - Sample 2

Note: Median value calculated using all participants' results



4. Analysis of results

4.1. General comments

Eighteen laboratories were sent material, with 14 laboratories returning results (11 returned results for *E. coli* in shellfish, 12 returned results for faecal coliforms in water). Two sets of samples were returned to Cefas due to issues at customs. The methods used to test the samples are shown in Tables 8 and 9 with the number of laboratories citing each method.

Table 8: Method used for the enumeration of *E. coli* in shellfish

Method reference	No. of Labs
ISO 16649-3 (MPN) (FAO RC Generic protocol)	5
ISO 16649-2	1
NF EN ISO 7251	1
NCH 3056 : 2007	1
MPN 5-tube	3

Table 9: Method used for the enumeration of faecal coliforms in water

Method reference	No. of labs
Determination of faecal coliform bacteria in seawater by the most probable number (MPN) technique (Based on Bacteriological Analytical Manual – BAM) (FAO RC Generic Protocol)	2
Standard Methods for the examination of water and wastewater 23rd Edition 2023.	1
Standard Methods 9221 APHA (MPN)	2
NF EN ISO 9308-2	1
ISO 9308-1: 2014 Membrane filtration	1
Standard Methods for the Analysis of Water and Wastewater (SM 9222 Membrane filter technique)	2
MPN 5-Tube	3

4.2. Sample analyses against the participants' median

Fourteen laboratories returned results for the fifth FAO RC Lenticule™ PT distribution. The analyses given in section 4.2.1 and 4.2.2 shows participants' performance against the participants' median.

4.2.1. *E. coli* in shellfish participant assessment

Ten laboratories reported duplicate results for both samples and 1 laboratory reported a single result for both samples.

4.2.1.1. Sample 1

All eleven laboratories returned results as *E. coli* not detected in this sample.

4.2.1.2. Sample 2

Eight laboratories returned *E. coli* / 100 g results falling within ±2.68 SD_T of the participants' median (Figure 1). Laboratory 290 reported both replicates >2.68 SD_T but <4 SD_T different from the participants' median, and laboratories 468 and 540 reported both results outside ±4 SD_T of the participants' median.

4.2.2. Faecal coliforms in water participant assessment

Twelve laboratories reported results for both samples.

4.2.2.1. Sample 1

Nine laboratories returned a result (FC per 100 ml) that fell between ±2.68 SD_T of the participants' median (Figure 2). Laboratory 468 reported a result >2.68 SD_T but <4 SD_T different from the participants' median and laboratories 40 and 492 reported a result that fell outside ±4 SD_T of the participants' median.

4.2.2.2. Sample 2

Eight laboratories returned a result (FC per 100 ml) that fell between ±2.68 SD_T of the participants' median (Figure 3). Laboratories 290 and 468 reported a result >2.68 SD_T but <4 SD_T different from the participants' median. Laboratory 492 reported a result that fell

outside ±4 SD_T of the participants' median and Laboratory 295 reported a result of zero faecal coliforms in this sample.

5. References

Anon, 2015 - ISO 16649-3, Microbiology of the food chain – Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide.

Anon, 2014 - ISO 9308-1, Water quality – Enumeration of *Escherichia coli* and coliform bacteria: Part 1: Membrane filtration method for waters with low background flora.

Anon, 2016 - The Standing Committee of Analysts – The Microbiology of Drinking Water – Part 4 – Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli* (including *E. coli* O157:H7).

Tackling global challenges through innovative science solutions

Cefas, the Centre for Environment, Fisheries and Aquaculture Science, is an Executive Agency of Defra (the UK Government's Department for Environment, Food and Rural Affairs).

Through innovative solutions and world-leading applied science we work to ensure a sustainable future for our rivers, seas and the ocean, supporting healthy and productive marine and freshwater ecosystems.

Centre for Environment, Fisheries & Aquaculture Science

Cefas

Pakefield Road, Lowestoft, Suffolk, NR33 0HT, UK

The Nothe, Barrack Road, Weymouth, DT4 8UB, UK

www.cefas.co.uk | +44 (0) 1502 562244

OGL

LRQA CERTIFIED ISO 9001

LRQA CERTIFIED ISO 45001

disability confident LEADER

© Crown copyright 2026