FAO Reference Centre for Bivalve Mollusc Sanitation Proficiency Testing Scheme

Enumeration of *Escherichia coli* and the detection of *Salmonella* spp. in bivalve molluscan shellfish (PT 105)

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Cefas

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Version	Author	Date	Comment
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Contents

This scheme is intended to provide proficiency testing (PT) samples for laboratories undertaking examination of live bivalve molluscs for bacteriological determinands.

The scheme is organised by Cefas, the FAO Reference Centre (FAO RC) for Bivalve Mollusc Sanitation. The scheme is intended to complement the Cefas/UK HSA Shellfish External Quality Assessment (EQA) Scheme through assessing elements of the procedure (initial sample preparation and preparation of initial dilutions) not covered by the Shellfish Scheme Proficiency testing for food, water and environmental microbiology - GOV.UK (www.gov.uk) and to provide additional data to laboratories for ISO 17025 (Anon, 2017a) accreditation purposes.

A scoring system is used to help assess participants' performance. Details of this system are included as Appendix 2 of this report. The purpose of scoring is to help identify incorrect or outlying results. Further information on the use of scoring in PT and on recommended procedures for following up on poor performance can be accessed via the Cefas website (FAO Reference Centre for Bivalve Mollusc Sanitation - Cefas (Centre for Environment, Fisheries and Aquaculture Science)).

If you are experiencing problems with any aspects of these distributions, please contact Cefas (contact details below), or alternately refer to the troubleshooting guide included as Appendix 3 of this report.

Further advice on microbiological testing of bivalve shellfish can be obtained via the Cefas website (FAO Reference Centre for Bivalve Mollusc Sanitation - Cefas (Centre for Environment, Fisheries and Aquaculture Science)).

Due to the nature of this scheme repeat samples are not available.

1. Sample preparation

A single batch of 1240 Pacific oysters (*Magallana gigas*) was collected from a UK commercial harvesting area on the 12th November 2025. The oysters were evenly spread across 12 trays and immersed into 2 small-scale depuration units that had been partially filled with 500 litres of filtered (50 micron filter) seawater and maintained at a temperature of 16 °C. Seawater was re-circulated at 25 litres per min (with UV treatment) for 3 days to allow the shellfish to acclimatise and remove any bacterial content.

1.1. Sample 1

Following acclimatisation, the oyster trays were removed from the tank on the 16th November 2025 and 100 ml of inoculum containing known levels of *E. coli* (~5.1 x 10^6 cfu/100 ml), *Klebsiella pneumoniae* (~8.6 x 10^4 cfu/100 ml) and *Salmonella* Bristol (~1.9 x 10^6 cfu/100 ml) were added to the tank and thoroughly mixed. The oysters were then re-immersed in the tank and were left for approximately 4 hours with constant re-circulation (without UV). After 4 hours of exposure the oysters were removed from the tank and samples of 22 oysters were randomly selected and placed into individual bags. The sample bags were then placed in the fridge at 3±2 °C prior to dispatch on the 17th November 2025.

1.2. Sample 2 – Pacific oysters

Following acclimatisation, the oyster trays were removed from the tank on the 16th November 2025 and 22 oysters were randomly selected from the 6 trays and placed into individual bags. The sample bags were then placed in the fridge at 3±2 °C prior to dispatch on the 17th November 2025.

2. Sample distribution and examination

Each sample was packed in accordance with the Cefas protocol for packaging shellfish for transportation. Samples were dispatched at 10:00 on the 17th November 2025 to 22 participating laboratories using courier company Global 28. Participants were requested to analyse the samples immediately on receipt using their routine methods.

The samples for Laboratory 12 arrived on the 19th November but reception did not recognise the name of the receiver so requested the samples to be picked up and destroyed by the courier. The shipments for laboratories 48, 57, 189 and 235 experienced issues at customs causing the material to either be late arriving at their required destination or be delayed at customs. The sample intended for Laboratory 48 was destroyed at customs; the results reported by the other laboratories that experienced delays have not been included in the assessment of participants' results.

2.1. Sample temperature

Participants were requested to record the internal sample temperature on arrival. Temperatures recorded by participants are shown in Appendix 1.

3. Results

3.1. Reference results – *E. coli*

Six randomly selected samples were analysed for *E. coli* in duplicate on the 18th November 2025 under repeatability conditions using Cefas SOP No. 1175 based on ISO 16649-3 (Anon 2015) (Table 1). Sample homogeneity was assessed following the procedure described in ISO 22117 (Anon, 2019). Where no *E. coli* was detected (<18 MPN/100g, <20 MPN/100g or <10 cfu/100g) results were scored at 17, 19 or 9 respectively for statistical evaluation. The sample material as distributed was considered sufficiently homogenous.

Table 1 - *E. coli* MPN/100g reference results

Sample No.	Range	Median	Geomean	Median ±3*SD
Sample 1	$2.3 \times 10^3 – 1.3 \times 10^4$	4.9×10^3	4.8×10^3	$9.3 \times 10^2 – 2.6 \times 10^4$
Sample 2	$<1.8 \times 10^1 – 2.0 \times 10^1$	$<1.8 \times 10^1$	1.7×10^1	$3.2 \times 10^0 – 8.9 \times 10^1$

3.2. Reference results – *Salmonella* spp.

Six randomly selected samples were analysed for *Salmonella* spp. on the 18th November 2025 under repeatability conditions using Cefas SOP No. 1176 based on ISO 6579-1 (Anon 2017b) (Table 2).

Table 2 – *Salmonella* spp. reference results

Sample No.	*Salmonella* spp.	No. of replicates giving the expected results
Sample 1	Present in 25g	6
Sample 2	Absent in 25g	6

3.3. Participants' results

Performance assessment was carried out according to the procedures described in the Cefas/UK HSA shellfish EQA scheme for a single distribution, with minor modifications (Appendix 2). Reference values were excluded from the calculation of the participants' median. Participants' results and scores allocated for PT 105 are shown in Tables 3, 4, 5, 6, Figure 1 and Appendix 3.

3.3.1. *E. coli* results

Table 3 – *E. coli* MPN/100g Participants' results

Sample No.	Range	Median	Geomean	Median±3*SD	Median±5*SD
Sample 1 [a]	$1.3 \times 10^3 – 5.4 \times 10^4$	7.5×10^3	7.4×10^3	$1.4 \times 10^3 – 3.9 \times 10^4$	$4.7 \times 10^2 – 1.2 \times 10^5$
Sample 2 [a]	$<1.0 \times 10^1 – 2.0 \times 10^1$	$<1.8 \times 10^1$	1.7×10^1	$3.2 \times 10^0 – 8.9 \times 10^1$	$1.1 \times 10^0 – 2.7 \times 10^2$

Key: a = Results reported from laboratories experiencing transport issues have been omitted from these calculations.

Note: The median and upper and lower limits (±3 SD and ±5 SD) were calculated from participants' results. SD calculations were based on the inherent variability of the 5 x 3 MPN method (0.24 $\log_{10}$). Reference values were excluded from the calculation of participants' median.

Table 4 – Participants' results and allocated scores (MPN/100g)

Lab ID.	Sample 1 – Pacific oysters			Sample 2 – Pacific oysters		
	Rep 1	Rep 2	Score	Rep 1	Rep 2	Score
3	24000	13000	12	<18	20	12
10	3400	7900	12	<18	<18	12
12 [a]						
31	13000	13000	12	20	<18	12
41	4600	3300	12	20	<18	12
48 [a]						
57 [b]	700	700	-	<20	<20	-
70	4900	4600	12	<18	<18	12
72	17000	6300	12	<18	20	12
113	1300	2400	9	<18	<18	12
120	17000	7900	12	<18	<18	12
125	4900	7000	12	<18	<18	12
129	13000	9400	12	<18	<18	12
131	11000	13000	12	<18	<18	12
142	4600	4600	12	<18	<18	12
189 [b]	330	450	-	<18	<18	-
214	15000	11000	12	<10	<10	12
235 [b c]	780	170	-	<18	<18	-
286	2300	3300	12	<18	<18	12
290	7000	7900	12	<18	<18	12
315	54000	24000	9	<18	<18	12
366	4900	3300	12	<18	20	12

Key: a = Samples destroyed; b = Extended transport times, results included in the graph but not assessed; c = An MPN value was reported to be inconsistent with tube combination.

Table 5 – Summary statistics of participants' results

E. coli	Sample 1	Sample 2
Participants reported duplicate results for *E. coli* MPN	19	19
Participants reported a single result for *E. coli* MPN	0	0
Participants reported both replicate MPN results within expected range [1]	15	19
Participants reported a single MPN result within expected range [1]	0	0
Participants reported one replicate MPN result outside expected range [1]	1	0
Participants reported both replicate MPN results outside expected range [1, 2]	3	0
Participants reporting MPN result inconsistent with tube combination [3, 2]	1	0

[1] Expected range = participants' median ± theoretical 3SD; [2] Transport issues, samples were not scored; [3] Tube combination inconsistent with MPN value according to the MPN calculators and/or the MPN tables in the FAORC generic protocol.

3.3.2. *Salmonella* spp. results

Table 6 - Participants' results and allocated scores (*Salmonella* spp. in 25g)

Lab ID.	Sample 1 Rep 1	Score	Sample 2 Rep 1	Score
3	Present	2	Present	NS
10	Present	2	Not Detected	NS
12 [a]	-	-	-	-
31	Present	2	Not Detected	NS
41	Present	2	Not Detected	NS
48 [a]	-	-	-	-
57 [b]	Present	-	Not Detected	-
70	Present	2	Not Detected	NS
72	Present	2	Not Detected	NS
113 [d]	Present	2	Present	-
120	Present	2	Not Detected	NS
125	Present	2	Not detected	NS
129	NE	-	NE	-
131	Present	2	Present	NS
142	Present	2	Present	NS
189 [b]	Present	-	Not Detected	-
214	Present	2	Not Detected	NS
235 [b]	Present	-	Not Detected	-
286	Present	2	Not Detected	NS
290	Present	2	Not Detected	NS
315	Present	2	Present	NS
366	Present	2	Not Detected	NS

Key: a = Samples destroyed; b = Extended transport times, results included in the graph but not assessed; NS = sample not scored (see 4.2.2); NE – Not examined.

4. Comments

4.1. General comments

- Twenty out of 22 participating laboratories received the samples with 15 laboratories receiving the material within 48 hours of dispatch as recommended by the FAO RC. Fifteen laboratories analysed the samples on the day of arrival.
- All laboratories recorded an arrival temperature of <10°C.
- Laboratories 57, 189 and 235 experienced significant delays in receiving PT 105 material (samples arrived after 5 days in transit). Due to the extended length of transit time, the results reported from these laboratories were omitted from the performance assessment but have been included in the graph.
- Laboratory 48 did not receive its samples as they were destroyed by customs.
- Laboratory 12 was unable to analyse the samples due to the receptionist not recognising the

contact information on the box and arranging for the box to be picked up by the courier Global28. Following collection, the courier arranged for the material to be destroyed.

4.2. Result comments

4.2.1. Sample 1

***E. coli* –** Fifteen laboratories returned duplicate *E. coli* MPN/100g results between ±3 SD of the participants' median for Sample 1 (Figure 1) and obtained a maximum score of 12. Laboratories 113 and 315 reported one replicate result between ±3 SD of the participants' median and one replicate result between ±3 and ±5 SD of the participants' median and received a score of 9.

***Salmonella spp.* –** All laboratories that returned a result for *Salmonella* spp. and that were subject to performance scoring (16) correctly reported the presence of *Salmonella* spp. in Sample 1. All of these laboratories received a maximum score of 2.

4.2.2. Sample 2

***E. coli* –** Seventeen laboratories returned duplicate *E. coli* MPN/100g results between ±3 SD of the participants' median for Sample 2 and obtained a maximum score of 12 (due to the large number of <18 results for this sample a figure showing participant results is not included).

***Salmonella* spp. –** Eleven laboratories that returned a result for *Salmonella* spp. and that were subject to performance scoring correctly reported the absence of *Salmonella* spp. as intended according to the reference results. Laboratories 3, 113, 131, 142 and 315 detected the presence of *Salmonella* spp.. During the FAO RC reference testing bacteria producing somewhat *Salmonella*-like colonies on xylose lysine deoxycholate (XLD) agar were isolated; these were identified as *Citrobacter* spp. using the API miniaturised biochemical gallery method used for confirmation by the FAO RC.

Due to the high frequency of putative false positive results reported by participants, and the presence of *Citrobacter* spp. in the sample, which could possibly lead to positive results depending on the confirmation method used by the participant lab, it was decided not to score this sample for *Salmonella* identification. Those laboratories reporting positive results should consider whether their methods are suitable for discrimination between *Salmonella* and *Citrobacter* spp. however.

4.2.3. Results summary

Those laboratories who have lost marks for the enumeration of *E. coli* and/or *Salmonella* spp. detection should in the first instance refer to the troubleshooting guide included as Appendix 4. Laboratories are reminded that the MPN calculators and/or the MPN tables provided by the FAO RC should be used for MPN determination.

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.

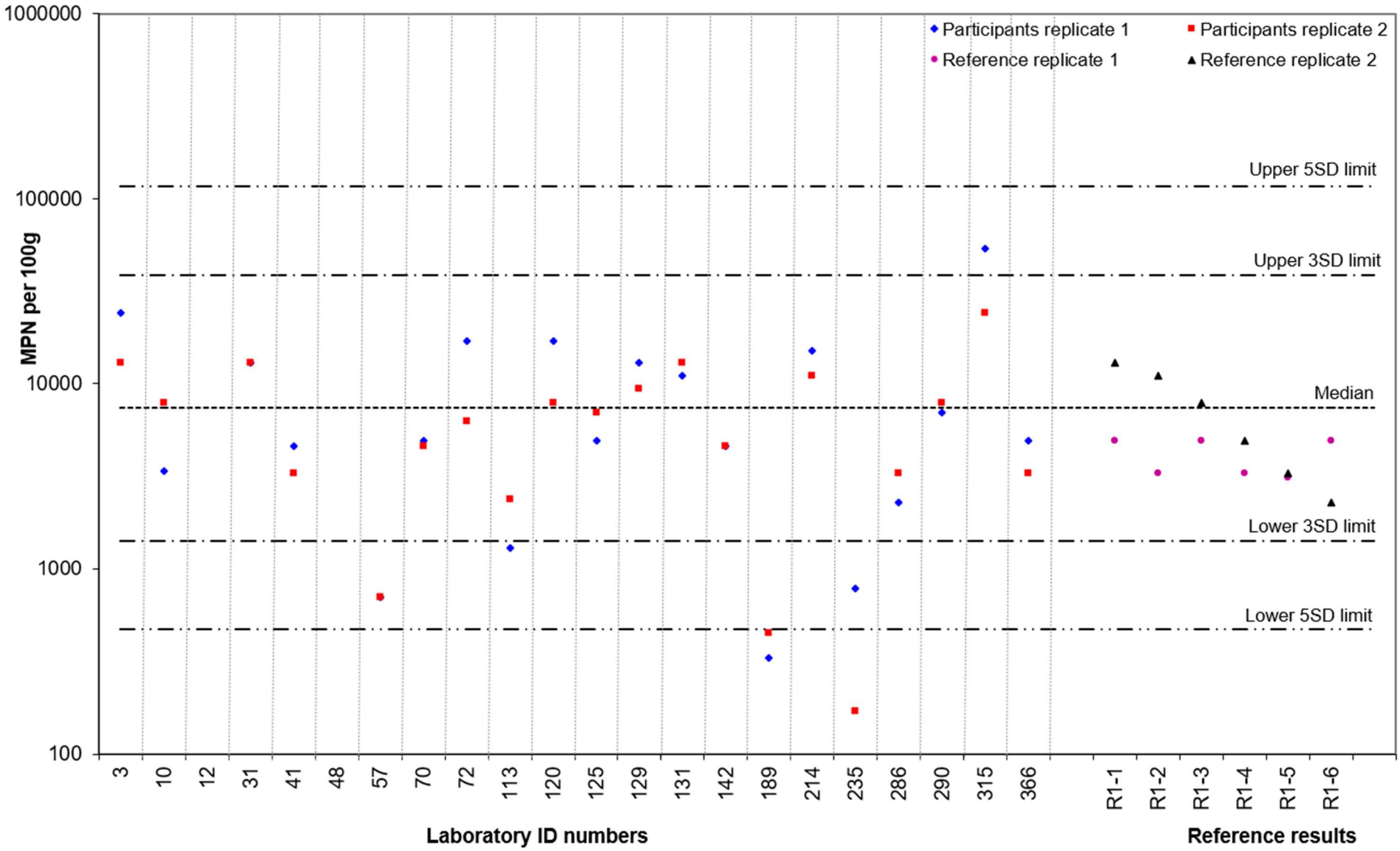
Anon, 2017a. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.

Anon, 2017b. ISO 6579-1. Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp.

Anon, 2019. ISO 22117. Microbiology of the food chain – Specific requirements and guidance for proficiency testing by interlaboratory comparison.

Anon, 2024. ISO 7218. Microbiology of the food chain – General requirements and guidance for microbiology examinations.

Figure 1 - Sample 1 – Pacific oysters - Participants' and FAO reference *E. coli* MPN results plotted against the participants' median



6. Appendices

6.1. Appendix 1 – Participants' sample information

Table 7 – Sample arrival and temperature

Lab ID.	Participants' records Date	Time	Internal temp. (°C)	Storage (°C)	Date analysed
3	19.11.25	12:20	2.7	4	19.11.25
10	18.11.25	15:00	1	-	18.11.25
12	19.11.25	-	-	-	-
31	18.11.25	15:45	2.6	5.0	19.11.25
41	19.11.25	11:00	2.1	3±2	19.11.25
48	-	-	-	-	-
57	01.12.25	12:00	chilled	4	03.12.25
70	18.11.25	9:20	2.2	3.4	18.11.25
72	18.11.25	8:45	4.6	3.9	18.11.25
113	26.11.25	-	-	-	-
120	18.11.25	12:00	3.8	4.0	19.11.25
125	18.11.25	14:10	5.3	4.0	18.11.25
129	19.11.25	15:45	4.35	-	19.11.25
131	18.11.25	9:50	3.6	3.9	18.11.25
142	18.11.25	9:05	4.9	-	18.11.25
189	27.11.25	12:00	15	5	27.11.25
214	19.11.25	11:00	4	6	21.11.25
235	21.11.25	unknown	unknown	5	21.11.25
286	18.11.25	10:00	2.8	3.1	18.11.25
290	20.11.25	15:30	5.1	4	21.11.25
315	18.11.25	10:05	5	-	18.11.25
366	18.11.25	13:45	5	-	18.11.25

6.2. Appendix 2 – Proficiency Testing scoring

Table 8 - *E. coli* MPN scores allocated to participants returning 2 replicate results

Result	Returning of results	Score allocated		Total score
		Rep. 1	Rep. 2	
Both replicate MPN results are within the expected range.	2	5	5	12
One replicate MPN result is outside the expected range and falls between the median ±3SD and median ±5SD values.	2	5	2	9
Both replicate MPN results are outside the expected range and fall between the median ±3SD and median ±5SD values.	2	2	2	6
One replicate MPN result is outside the median ±5SD value.	2	5	0	7
Both replicate MPN results are outside the expected range. The first falls between the median ±3SD and median ±5SD value and the second falls outside the median ±5SD values.	2	2	0	4
Both replicate MPN results reported are outside the median ±5SD value.	2	0	0	2

Table 9 – *E. coli* MPN scores allocated to participants returning 1 single replicate result

Result	Returning of results	Score allocated	Total score
Single replicate MPN result is within the expected range.	2	5	7
Single replicate MPN result is outside the expected range and falls between the median ±3SD and median ±5SD values.	2	2	4
Single replicate MPN result reported is outside the median ±5SD value.	2	0	2

Table 10 – *E. coli* score deductions

Result	Scores deducted	
	Rep. 1	Rep. 2
Tube combination inconsistent with MPN reported according to MPN calculators or MPN tables provided by the FAO RC and / or tube combination selected not consistent with rules given in ISO 7218	2	2
High censored result where fewer than 4 dilutions used (e.g. MPN = >18000 per 100g)	2	2
Sample not examined or results returned late - no explanation received	12	

Table 11 – *Salmonella* spp. scoring

Result	Scores allocated
Fully correct results	2
Misleading result, e.g. failure to isolate Salmonella	0

6.3. Appendix 3 – Participants' *E. coli* results including MPN tube combinations

Lab ID.	Sample 1 – Pacific oysters				Sample 1 – Pacific oysters			
	Tube combo	MPN/ 100g	Tube combo	MPN/ 100g	Tube combo	MPN/ 100g	Tube combo	MPN/ 100g
3	5 5 5 0	24000	5 5 4 0	13000	0 0 0 0	<18	1 0 0 0	20
10	5 4 4 0	3400	5 5 3 0	7900	0 0 0 0	<18	0 0 0 0	<18
12								
31	5 5 4 0	13000	5 5 4 0	13000	1 0 0 0	20	0 0 0 0	<18
41	5 5 1 1	4600	5 5 1 0	3300	1 0 0 0	20	0 0 0 0	<18
48								
57	5 2 1	700	5 2 1	700	0 0 0	<20	0 0 0	<20
70	5 5 2 0	4900	5 5 1 1	4600	0 0 0 0	<18	0 0 0 0	<18
72	5 5 4 1	17000	5 5 1 2	6300	0 0 0 0	<18	1 0 0 0	20
113		1300		2400		<18		<18
120	5 5 4 1	17000	5 5 3 0	7900	0 0 0 0	<18	0 0 0 0	<18
125	5 5 2 0	4900	5 5 2 1	7000	0 0 0 0	<18	0 0 0 0	<18
129	5 5 4 0	13000	5 5 2 2	9400	0 0 0 0	<18	0 0 0 0	<18
131	5 5 3 1	11000	5 5 4 0	13000	0 0 0 0	<18	0 0 0 0	<18
142	5 5 1 1	4600	5 5 1 1	4600	0 0 0 0	<18	0 0 0 0	<18
189	5 1 0 0	330	5 1 1 0	450	0 0 0 0	<18	0 0 0 0	<18
214		15000		11000		<10		<10
235 [a]	5 3 0	780	4 1 0	170	0 0 0	<18	0 0 0	<18
286	5 5 0 0	2300	5 5 1 0	3300	0 0 0 0	<18	0 0 0 0	<18
290	5 5 2 1	7000	5 5 3 0	7900	0 0 0 0	<18	0 0 0 0	<18
315	5 5 5 2	54000	5 5 5 0	24000	0 0 0 0	<18	0 0 0 0	<18
366	5 5 2 0	4900	5 5 1 0	3300	0 0 0 0	<18	1 0 0 0	20

Key: Yellow shading denotes MPN value not consistent with tube combo.

6.4. Trouble shooting advice

1. **Methods – Methods**

   Check that the method used is appropriate for the examination of the sample.

   a. Check that that any dilutions have been calculated correctly.
   b. Check that the dilutions analysed are as specified on the report form.
   c. Check the MPN tables (if used) are interpreted correctly.

2. **Interpretation of MPN tables**

   Record the number of TBX positives for each dilution to give a three or four figure tube combination number. Use the MPN calculator for 100 g test portions referenced in ISO 7218 (Anon, 2024) (ISO Standards Maintenance Portal), one of the MPN calculators available from the Cefas FAO Reference Centre website (Method Guidance and Calculation Spreadsheets - Cefas (Centre for Environment, Fisheries and Aquaculture Science)) or the MPN tables in the FAO Reference Centre *E. coli* generic protocol (Generic Protocols - Cefas (Centre for Environment, Fisheries and Aquaculture Science)) to calculate results expressed as MPN per 100 g.

   Note: In all cases the MPN must be calculated using the number of positive tubes counted at **ALL** tested dilutions, even if lower dilutions are completely negative. For example, if a four-dilution combination of 5,3,0,0 is obtained the result should be reported as 780 (MPN result for a combination of 5,3,0,0) rather than 790 (MPN result for a combination of 5,3,0).

   Note: Only category 1 or 2 tube combinations should be reported – category 3 combinations should be recorded / reported as 'Void'. The MPN tables in the FAO Reference Centre generic *E. coli* protocol only include category 1 or 2 tube combinations: any tube combination that does not appear in these MPN tables are an unacceptable (category 3) combination.

3. **Culture media**

   Check the quality control data for media to ensure that they are within specifications and performing adequately.

4. **Equipment**

   Check that the equipment used for the procedures (incubators, refrigerators, measuring instruments) are calibrated and performing adequately.

5. **Staff training**

   Check that the staff performing the tests are fully trained and familiar with all the procedural steps.

6. **Clerical procedures**

   Check that the sample labeling, laboratory numbering and clerical procedures are adequate and that you have procedures for ensuring that test results are reported accurately and on time.

7. **Accreditation**

   Check that quality procedures are documented and adhered to at all times.

8. **Internal quality controls (IQC)**

   Ensure adequate controls are in place and follow-up procedures are in place to deal with IQC failures.

Further advice can be obtained from the FAO RC on request.

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