UK Health Security Agency

Summary of Results

External Quality Assessment of Water Microbiology

Pilot Wastewater Scheme

Distribution Number: WW00P **Sample Numbers: WW00PA & WW00PB**

Distribution Date:	**July 2024**
Results Due:	**30 August 2024**
Report Date:	**24 October 2024**
Samples prepared and quality control tested by:	**Divya George** **Nafeesa Hussain** **Cansev Katar** **Sabine Naujokat** **Jake Videlefsky**
Data analysed by:	**Nita Patel** **Zak Prior**
Report compiled by:	**Nita Patel** **Zak Prior**
Authorised by:	**Nita Patel**

UK Health Security Agency
Food and Environmental Proficiency Testing Unit (FEPTU)
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London
NW9 5EQ

Overview:
Wastewater harbors a complex and dynamic bacterial community. By elucidating the roles of both harmful and beneficial microbes, we can optimise wastewater treatment strategies for cleaner water and improved public health. The implementation of rigorous quality control measures, exemplified by the use of proficiency testing samples, ensures the accuracy and reliability of wastewater analysis, a cornerstone of effective environmental protection.

Guide to Scoring and Statistics:
There were not enough quantified results (<10) to score or produce meaningful statistics.

FEPTU Quality Control:
To demonstrate homogeneity of the sample, a minimum of 10 LENTICULE® discs, selected randomly from a batch, are tested in duplicate for parameters requiring enumeration.

To demonstrate stability of the sample, a minimum of six LENTICULE discs, selected randomly from a batch, are examined throughout the distribution period for enumeration parameters.

UKHSA uses methods stipulated in the Environmental Agency Standing Committee of Analysts series of documents: Microbiology of Water and Associated Materials (2017) and methods stipulated in the Microbiology of Recreational and Environmental Waters (2000) and the DWI's series of documents: The Microbiology of Drinking Water (2002) - Methods for the Examination of Waters and Associated Materials.

The FEPTU results are used for guidance in the preliminary intended results.

Guidelines and general advice:

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

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	
Microbiological advice	Zak Prior or Nita Patel	**E-mail: foodeqa@ukhsa.gov.uk**
General comments and complaints	Zak Prior or Nita Patel	
Scheme Advisor	N/A	
Scheme Co-ordinator	Nita Patel	

Accreditation:

This non-accredited pilot distribution was produced using principles and practices within ISO/IEC 17043:2010.

Sample: WW00PA

Contents: *Escherichia coli* ($8.9x10^6$) (wild strain), *Citrobacter koseri* ($1.0x10^7$) (wild strain), *Enterococcus faecalis* ($8.5x10^5$) (wild strain) and *Salmonella* Enteritidis 1,9,12;g,m;- ($1.4x10^4$) (wild strain)

Expected results:

All counts are expressed as colony forming units (cfu) per 100 mL

Parameter	*Escherichia coli*	Total coliforms	Faecal coliforms	Enterococci	*Salmonella* spp.	SARS-CoV-2
FEPTU median	$8.9x10^6$ (6.94 log_{10})	$1.8x10^7$ (7.26 log_{10})	$8.9x10^6$ (6.94 log_{10})	$8.5x10^5$ (5.93 log_{10})	Detected	Not detected
No. results returned	11	9	8	11	7	2
Assigned value (Participants median all results)	Not determined due to insufficient data				Detected	Not detected
Minimum and maximum values	$7.2x10^6$ – $1.9x10^7$	$1.5x10^6$ – $3.3x10^7$	$1.0x10^5$ – $9.0x10^6$	$6.3x10^5$ – $1.7x10^6$	N/A	N/A
Standard deviation* (log_{10})	Not determined due to insufficient data				N/A	N/A
Total number of censored values (greater than)	5	5	4	5	N/A	N/A
False Positives	N/A	N/A	N/A	N/A	N/A	N/A
False Negatives	1	0	0	1	0	0
Not examined	2	4	4	2	6	11

*Robust *S** based on median absolute deviation about the participants' median *(MADe)* and is based on logged data

Total sent samples	21
Non returns	8

Sample: WW00PB

Contents: *Escherichia coli* ($9.7x10^6$) (wild strain), *Pantoea agglomerans* ($3.5x10^5$) (wild strain), *Pseudomonas aeruginosa* ($1.5x10^4$) (wild strain) and SARS-CoV-2 Omicron (B.1.1.529) (level not determined) (inactivated wild strain)

Expected results:

All counts are expressed as colony forming units (cfu) per 100 mL

Parameter	*Escherichia coli*	Total coliforms	Faecal coliforms	Enterococci	*Salmonella* spp.	SARS-CoV-2
FEPTU median	$8.8x10^6$ (6.94 log_{10})	$8.8x10^6$ (6.94 log_{10})	$8.8x10^6$ (6.94 log_{10})	$5.7x10^6$ (6.75 log_{10})	Not detected	Detected
No. results returned	11	9	8	11	7	2
Assigned value (Participants median all results)	Not determined due to insufficient data				Not detected	Detected
Minimum and maximum values	$4.5x10^6$ – $2.0x10^7$	$7.9x10^6$ – $2.1x10^7$	$2.7x10^6$ – $8.1x10^6$	$3.6x10^6$ – $2.1x10^7$	N/A	N/A
Standard deviation* (log_{10})	Not determined due to insufficient data				N/A	N/A
Total number of censored values (greater than)	5	5	5	5	N/A	N/A
False Positives	N/A	N/A	N/A	N/A	N/A	N/A
False Negatives	0	0	0	0	0	0
Not examined	2	4	4	2	6	11

*Robust *S** based on median absolute deviation about the participants' median *(MADe)* and is based on logged data

Total sent samples	21
Non returns	8

WW00PA Summary of participants results: results shown in red are considered incorrect

E. coli

Lab ID	*E. coli* result cfu/100 mL	Log_{10}
9	$>2.0x10^4$	
145	$7.2x01^6$	6.86
1041	$9.2x10^6$	6.96
1159	$>2.4x10^5$	
1242	$>2.4x10^3$	
1459	$>1.0x10^4$	
1519	0	
1748	$1.9x10^7$	7.27
1784	$>1.0x10^5$	
2332	$8.2x10^6$	6.91
2668	$8.0x10^6$	6.90

Coliforms

Lab ID	Coliform result cfu/100 mL	Log_{10}
9	$>2.0x10^4$	
145	$9.0x10^6$	6.95
1041	$1.6x10^7$	7.20
1159	$>2.4x10^5$	
1242	$>2.4x10^3$	
1459	$>1.0x10^4$	
1748	$3.3x10^7$	7.51
1784	$>1.0x10^5$	
2332	$1.5x10^6$	6.19

Faecal coliforms

Lab ID	Faecal coliforms result cfu/100 mL	Log_{10}
145	$9.0x10^6$	6.95
1041	$3.9x10^6$	6.59
1159	$>2.4x10^5$	
1242	$>2.4x10^3$	
1459	$>1.0x10^4$	
1748	$1.0x10^5$	5.00
1784	$>1.0x10^5$	
2332	$7.3x10^6$	6.86

Enterococci

Lab ID	Enterococci result cfu/100 mL	Log_{10}
9	$>1.0x10^5$	
145	$8.1x10^5$	5.91
1041	$7.3x10^5$	5.86
1159	$>1.0x10^4$	
1242	$>2.4x10^3$	
1459	$>1.0x10^4$	
1519	0	
1748	$1.7x10^6$	6.24
1784	$>1.0x10^5$	
2332	$6.3x10^5$	5.80
2668	$6.9x10^5$	5.84

Salmonella spp.

Lab ID	*Salmonella* spp. result
9	Detected
145	Detected
1041	Detected
1159	Detected
1459	Detected
1519	Detected
1784	Detected

SARS-CoV-2

Lab ID	Sars-Cov-2 result	CT value	Genomic units
1853	Not Detected		
2332	Not Detected	N/A	<7 copies/mL

WW00PB Summary of participants results:

E. coli

Lab ID	*E. coli* result cfu/100 mL	Log_{10}
9	$>2.0x10^4$	
145	$4.9x10^6$	6.69
1041	$1.4x10^7$	7.15
1159	$>2.4x10^5$	
1242	$>2.4x10^3$	
1459	$>1.0x10^4$	
1519	$1.6x10^7$	7.20
1748	$2.0x10^7$	7.30
1784	$>1.0x10^5$	
2332	$4.5x10^6$	6.65
2668	$6.9x10^6$	6.84

Coliforms

Lab ID	Coliform result cfu/100 mL	Log_{10}
9	$>2.0x10^4$	
145	$1.3x10^7$	7.10
1041	$1.6x10^7$	7.20
1159	$>2.4x10^5$	
1242	$>2.4x10^3$	
1459	$>1.0x10^4$	
1748	$2.1x10^7$	7.33
1784	$>1.0x10^5$	
2332	$7.9x10^6$	6.90

Faecal coliforms

Lab ID	Faecal coliforms result cfu/100 mL	Log_{10}
145	$8.1x10^6$	6.91
1041	$2.7x10^6$	6.43
1159	$>2.4x10^5$	
1242	$>2.4x10^3$	
1459	$>1.0x10^4$	
1748	$> 2.4x10^6$	
1784	$>1.0x10^5$	
2332	$3.5x10^6$	6.54

Enterococci

Lab ID	Enterococci result cfu/100 mL	Log_{10}
9	$>1.0x10^5$	
145	$5.7x10^6$	6.67
1041	$4.1x10^6$	6.61
1159	$>1.0x10^4$	
1242	$>2.4x10^3$	
1459	$>1.0x10^4$	
1519	$6.0x10^6$	6.78
1748	$2.1x10^7$	7.33
1784	$>1.0x10^5$	
2332	$3.6x10^6$	6.56
2668	$4.3x10^6$	6.63

Salmonella spp.

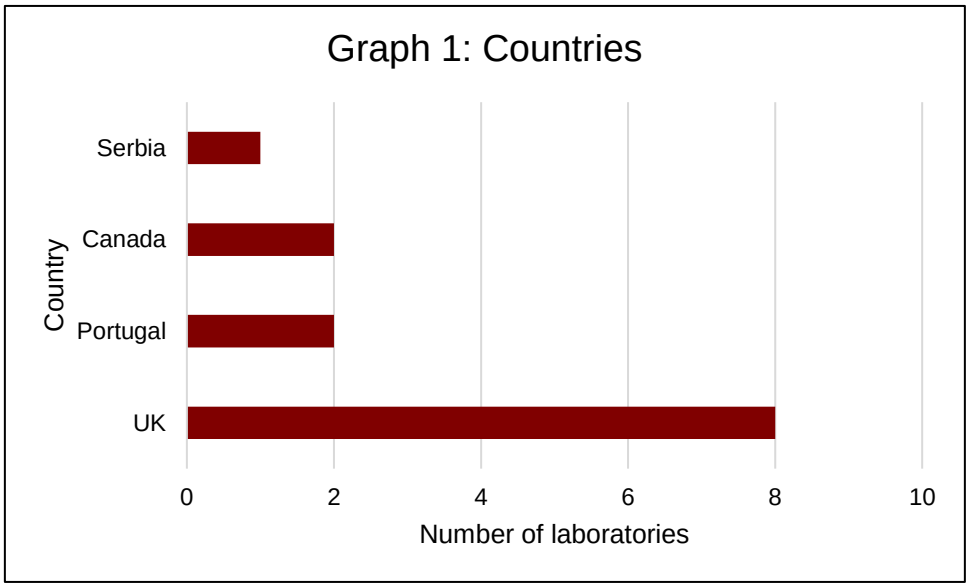
Lab ID	*Salmonella* spp. result
9	Not Detected
145	Not Detected
1041	Not Detected
1159	Not Detected
1459	Not Detected
1519	Not Detected
1784	Not Detected

SARS-CoV-2

Lab ID	SARS-CoV-2 result	CT value	Genomic units
1853	Detected	32.68	$1.3x10^4$ GU/mL
2332	Detected	26.60	$6.9x10^3$ copies/mL

Participation

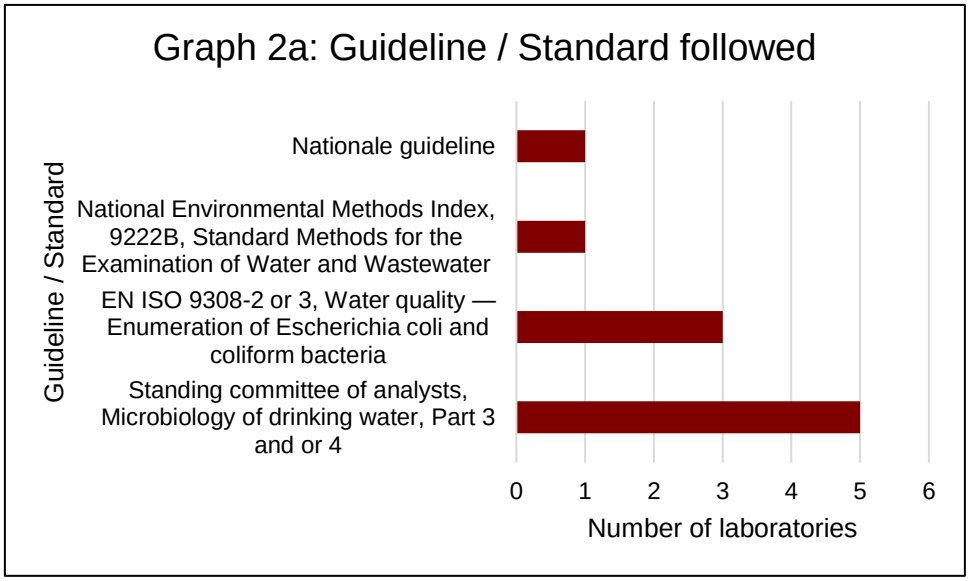
A total of four countries participated in this pilot distribution (graph 1). The majority of which were in the UK (8/13).



Questionnaire comments:

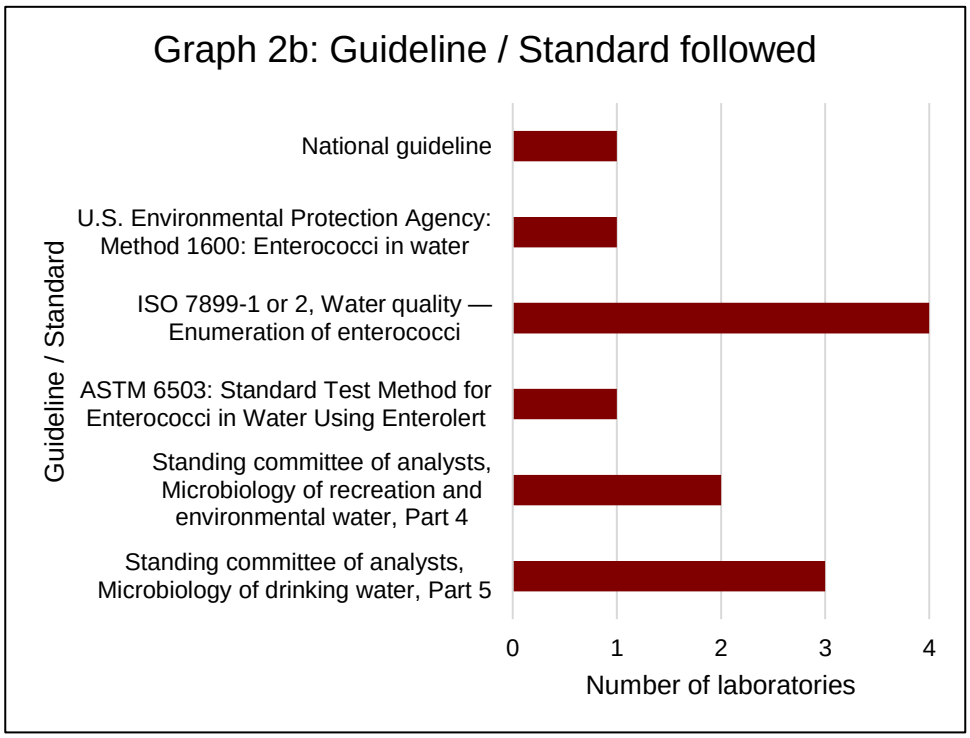
1. **Standard and or guideline used for the sample examination: for *E. coli*, coliform and faecal coliform.**

   Of the ten responses received, the majority used Standing committee of analysts, microbiology of drinking water part 3 and/or 4 (Graph 2a). Some laboratories used multiple guidelines or standards.



- For Enterococci

  Of the ten responses received, the majority used ISO 7899-1:1998 or ISO 7899-2:2000 Water quality – Detection and enumeration of intestinal enterococci (Graph 2b). Some laboratories used multiple guidelines or standards.



- For *Salmonella* spp.

  Of the six responses three laboratories used Standing committee of analysts, Microbiology of Recreational and environmental waters Part 8 and three used ISO 19250:2010 Water quality - Detection of *Salmonella* spp.

- For SARS-CoV-2

  Of the two responding laboratories, neither used a published standard or guideline.

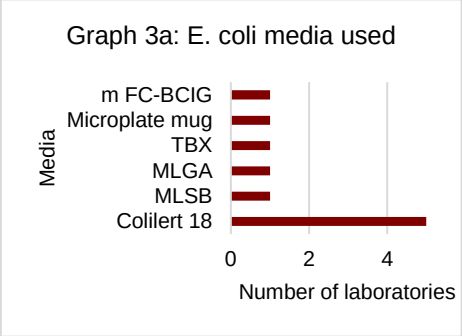
2. Methodology

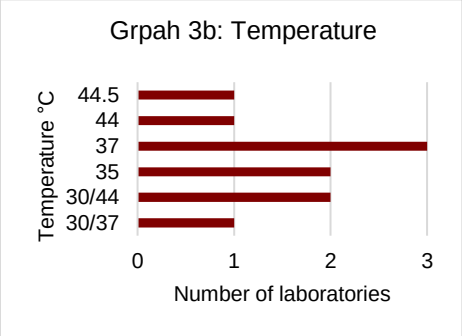
Quantitative bacteriology

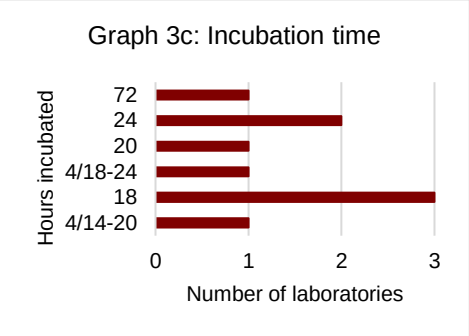
(Please see Appendix (I) for media definitions)

E. coli

- Media, temperature and incubation times are shown in graphs 3a – 3c for *E. coli*:





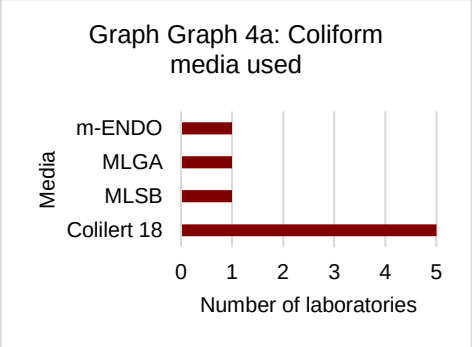


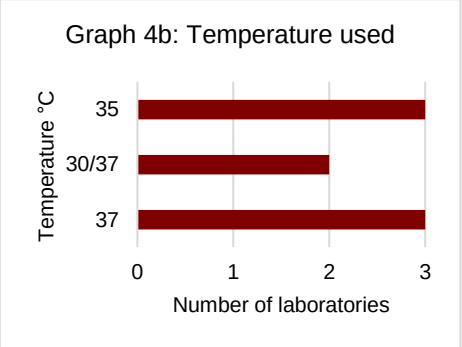
- Confirmation:

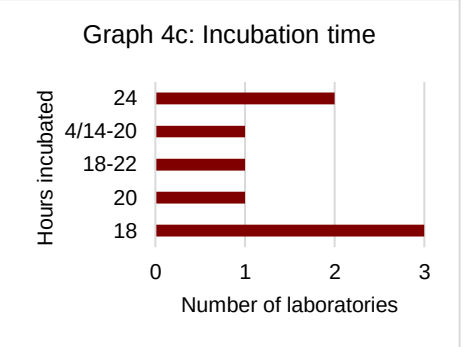
  Of the six participants responding 4/6 do not do confirmation tests. Biochemical or MALDIToF are used by the other two laboratories that do confirmation tests.

Coliforms

- Media, temperature, and incubation times are shown in graphs 4a – 4c for coliforms:





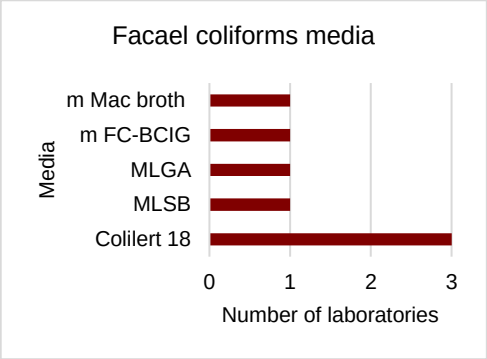


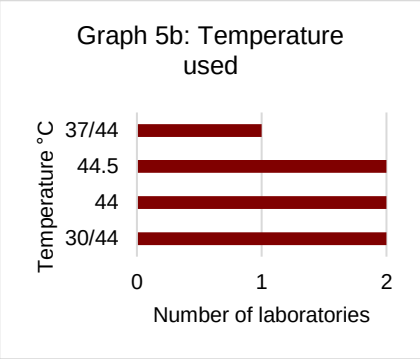
- Confirmation:

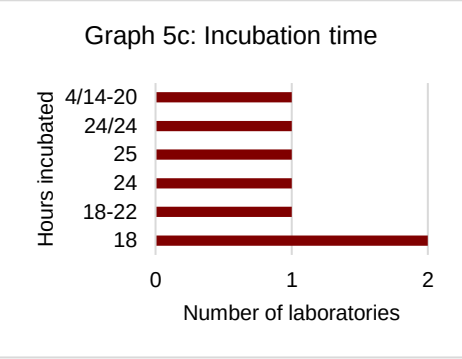
  Of the six participants responding 4/6 do not do confirmation tests. Biochemical or MALDIToF are used by the other two laboratories that do confirmation tests.

Faecal coliforms

- Media, temperature and incubation times are shown in graphs 5a – 5c for faecal coliforms:





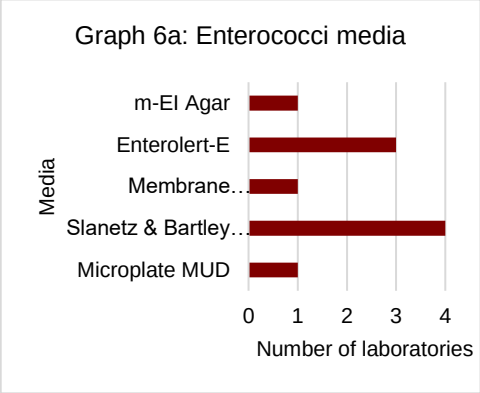


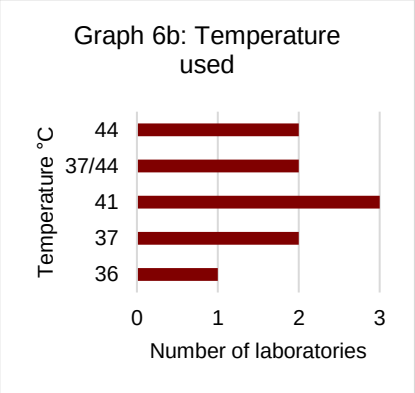
- Confirmation:

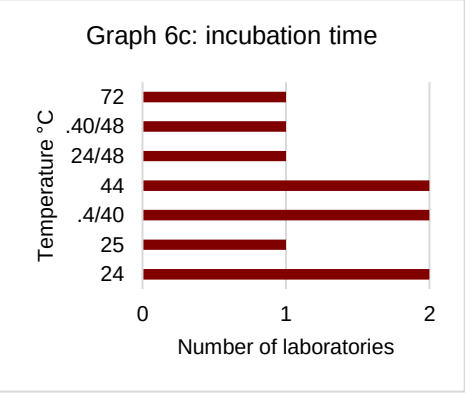
  Of the four participants responding 3/4 do not do confirmation tests. MALDIToF is used by the other laboratory that does confirmation tests.

Enterococci

- Media, temperature and incubation times are shown in graphs 6a – 6c for enterococci:







- Confirmation:

  6/7 of the participants perform a confirmation tests. Five laboratories used aesculin hydrolysis and one by MALDIToF.

Salmonella spp.:

- Media

  All six participants responding used a combination of buffered peptone water (BPW), Rappaport Vassiliadis (RVS), Muller-Kauffmann Tetrathionate Novobiocin (MKTTn), Xylose-Lysine Deoxycholate Agar (XLD), Brilliant Green Agar (BGA) and or chromogenic media.

- Incubation temperature

  Four our of six participants used 37 °C. The other two laboratories used 36 °C or 37 °C and 41.5 °C.

- Incubation time

  All six participants used 24 to 48 hour incubation time.

- Confirmation tests

  All labs perform a confirmation test on presumptive *Salmonella* spp. colonies. See table below of confirmations that individual laboratories performed.

Lab ID	Serology & PCR
9	Serology & PCR
145	Nutrient Agar, MacConkey, serology & PCR
1041	Agglutination & API 20E
1159	Enteropluri test kit
1459	MALDIToF
1519	Urease test, Lysine Decarboxylase test, Triple sugar iron, & Agglutination

- Comments or conclusions and other tests

  One out of six laboratories provide comments or conclusions on the results for *Salmonella* testing. Similarly, one out of six laboratories performed another test on this water type of Shiga toxin producing *Escherichia coli*.

Membrane pore size:

- All six participants responding used a membrane filtration process using a 0.45 µm pore size.

SARS-CoV-2

1. What volume of the wastewater sample is normally tested and concentrated?

   - The laboratories responded: 1.2 mL and 50 mL

2. Please provide details of your concentration method(s)

   Below are the responses from the laboratories:

   a) CeresNanotrap

   b) Raw wastewater is homogenized extensively by inversion. All downstream manipulations performed inside biological safety cabinets. The 50 ml volume is vacuum-filtered through a 0.45 micron mixed-ester cellulose filter. Volume is measured at exactly 50 ml using volumetric funnels. Using sterile tools, filters are cut into 8 pieces of roughly equal size and transferred into sterile microcentrifuge tubes. Filters are typically submitted to extraction immediately but may be frozen at -80°C if extraction must proceed on another day.

3. Please give details of primers (e.g. in house / commercial kit)

   - Both laboratories responding used in house 'N' gene targeting assays.

4. Describe your RNA extraction method (including volumes)

   Below are the responses from the laboratories:

   a) Microbiome kit

   b) We use the Qiagen AllPrep PowerViral DNA/RNA kit for extraction, applying a protocol very similar to manufacturer's instructions.

5. What volume of the extracted RNA is used in your PCR?

   - Both labs used 5 µL

6. Please provide details of master mix and volume used etc Below are the responses from the laboratories:

   a) Promega Enviro 2x Mastermix 10 µL

   b) Final reaction volume is 25 microlitres. Recipe as follows. PCR grade water 7 µL; TaqMan Fast Virus 1-step Master Mix 5 µL; 10 uM primer stock mixes (contains forward and reverse) 0.5 µL for each of three targets; 10 uM probe stocks 0.25 µL each target; RNA extract 5 µL. 9: 5 min at 50°C; 20 s at 95°C; 40 cycles of 3 s at 95°C, 40 s at 60°C".

7. Please provide details of your cycle run. Below are the responses from the laboratories:

   a) Reverse Transcription 55 °C 10 mins; Heat Inactivation 95 °C 2 mins; 45 cycles; Denaturation 95 °C 15 secs; Annealing and Extension 60 °C 1 min.

   b) 5 min at 50°C; 20 s at 95°C; 40 cycles of 3 s at 95°C, 40 s at 60°C

8. What amplification platform do you use? Below are the responses from the laboratories:

   a) Techne PrimePro 48.

   b) Bio-Rad CFX Opus.

9. What is your limit of detection? Below are the responses from the laboratories:

   a) CT 40, 49 copies per µL

   b) 13 gene copies per reaction for target N1. Corresponds to 7 gene copies per ml in usual assay conditions.

10. What is your limit of quantification? Below are the responses from the laboratories:

    a) CT 40, 49 copies per µL

    b) 22 gene copies per reaction for target N1. Corresponds to 12 gene copies per ml in usual assay conditions.

11. Does your laboratory routinely carry out both detection and quantification tests on samples?

- Both laboratories responded “yes”.

12. Do you test for any other viruses in wastewaters or other water types?

    a) Influenza A, Influenza B, NorovirusG1, Norovirus G2, RSV, Adenovirus and mpox

    b) At present, we do not test other viral targets than SARS-CoV-2 in water, but we do study other potential targets, and may offer that as a service in the future.

13. Is your laboratory currently participating in any other SARS-CoV-2 PCR scheme?

    One laboratory responded “yes” and the other “no”.

Questionnaire General comments

- **What other water sample types do you test?**

a) Surface, sediments, sludge, sewage, slurry, manure, marine and fluvial waters.

- **What other tests are performed on these water types?**

a) *Legionella*, microbial source tracking

b) As a laboratory for the Ministry of the Environment, we receive samples taken to check whether there has been a discharge of wastewater or other faecal matter into the environment (spillage, overflow, etc.). We do verify for the presence of 'traditional' fecal indicators.

c) We also have projects for microbial source tracking: we then perform qPCR with Bacteroidales markers found in literature (still in development)

- **Further comment/s made that will help FEPTU to ensure the scheme is appropriate when launched:**

a) Please provide the freeze dried pellet in a larger vial. Whilst adding the 3.5ml the pellet popped to the top of the vial and spilled. I lost part of sample A. A larger vial would have prevented this.

b) Rehydration of the original sample requires a larger container as once the diluent was added the sample was almost overflowing the container

c) For *Salmonella*, you could indicate that a presence/absence analysis is ok for the assay. For your information, we didn't perform the test since we thought a quantification value was expected. We do perform Salmonella analysis with an home made method based on Method 1682: Salmonella in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium, from USEPA.

d) A guide to recommended dilutions for the round, like that in the *Legionella* Isolation Scheme would be helpful - We analysed 1:10 and 1:100 dilutions (as we do for other WW PT's) but the intended target appears too high to obtain a countable range for us with just those dilutions.

e) Specifying and enforcing minimal methodological requirements would probably go a long way in ensuring the relevance and usefulness of the PT. Make sure those requirements are well documented. Pass/fail criteria should be transparent. Do not hesitate to reach out and interact with participants in the initial stages of the PT scheme to ensure information, instructions and requirements are properly understood. Collaborating with labs to identifying sources of variability would help make the PT scheme relevant, by helping advance the field.

Appendix (I)

Abbreviation	Definition
MLSB	Membrane Lauryl Sulphate Broth
m EI	modified Enterococcus enrichment broth
m FC	Modified faecal coliform
m FC-BCIG	Modified faecal coliform 5-bromo-6-chloro-3-indoyl-beta-D-glucuronide
m Mac broth	Modified MacConkey broth with andrade 0,5% lactose peptone
m-ENDO	Modified Endo Agar Lactose Eosin and methylene blue (LES)
Microplate MUD	Microplate modified universal detection medium
Microplate mug	Microplate 4-methylumbelliferyl-β-D-glucuronide agar
MLGA	Membrane Lactose Glucuronide Agar
SB	Slanetz and Bartley Medium
TBX	Tryptone Bile X-glucuronide agar

End of report